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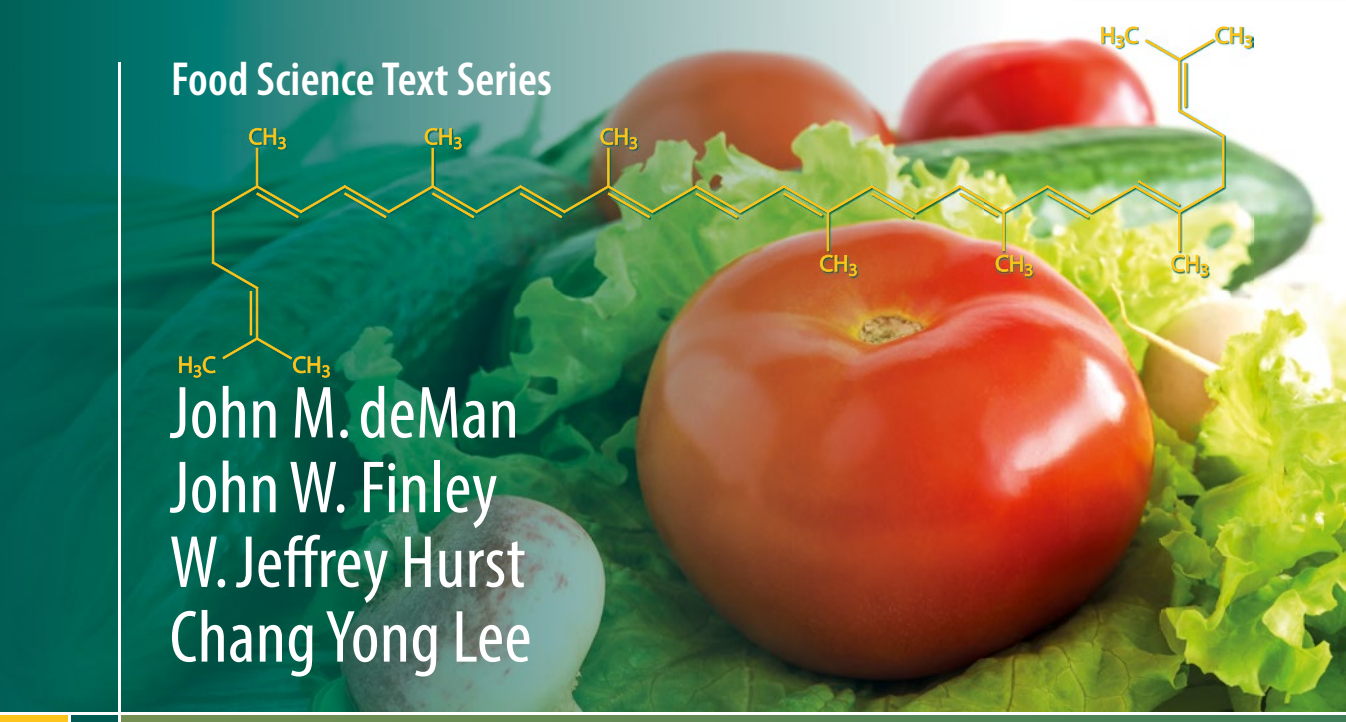

John M. deMan
John W. Finley
W. Jeffrey Hurst
Chang Yong Lee

Principles of Food Chemistry

Fourth Edition

Springer

Food Science Text Series

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W. Jeffrey Hurst • Chang Yong Lee

Principles of Food Chemistry

Fourth Edition

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ISSN 1572-0330 ISSN 2214-7799 (electronic)
Food Science Text Series
ISBN 978-3-319-63605-4 ISBN 978-3-319-63607-8 (eBook)
https://doi.org/10.1007/978-3-319-63607-8

Library of Congress Control Number: 2017956195

1st edition: © AVI 1980
2nd edition: © AVI 1989

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

This book was designed to serve as an introductory text for courses in food chemistry as part of food science programs meeting the Institute of Food Technologists standards. The original concept for the preparation of this book was to present basic information on the composition of foods and the chemical and physical characteristics they undergo during processing, storage, and handling. The basic principles of food chemistry remain the same, but much additional research carried out in recent years has expanded and in some cases refined our knowledge. As with the third edition we have refined and expanded the material in all chapters. Because of the rapidly growing interest we have added chapters on transgenic crops as well as a chapter on beer and wine production. We felt the transgenic crop chapter was important so that students have a basic understanding of the technology and how it has evolved over the last 10,000 years. The chapter on beer and wine production is included to help the students appreciate the science behind fermented beverages. This knowledge will be valuable because the opportunities for food scientists in those areas are growing exponentially. In the area of water as a food component, the issue of the glass transition has received much attention. This demonstrates the important role of water in food properties. Carbohydrates and lipids are of major sources of food energy and are of major interest for their functional and nutritional properties in obesity and diabetes. Understanding how to the chemistry of these ingredients will help food scientists better formulate new nutritionally superior foods in the future. Our understanding of the functionality of proteins expands with increasing knowledge about their composition and structure. Carbohydrates serve many functions in foods, and the non-caloric dietary fiber has assumed an important role.

Color, flavor, and texture are important attributes of food quality, and in these areas, especially those of flavor and texture, great advances have been made in recent years. There is concern among consumers about the safety of additives including colors and flavors. We have also included a section on natural toxicants as well as ingredients that can cause adverse effects. It is important to realize that many components in foods can be harmful or safe depending on the concentrations in the foods. Enzymes are playing an ever increasing part in the production and transformation of foods. Modern methods of biotechnology have produced a gamut of enzymes with new and improved properties.

In the literature, information is found using different systems of units: metric, SI, and the English system. Quotations from the literature are

presented in their original form. It would be difficult to change all these units in the book to one system. To assist the reader in converting these units, an appendix is provided with conversion factors for all units found in the text.

It is hoped that this fourth edition will continue to fulfill the need for a concise and relevant text for the teaching of food chemistry. We hope that this edition will serve as a memorial to the enormous contributions of John deMan and continue to provide teaching and reference material of value.

Guelph, ON — John M. deMan
Lakewood Ranch, FL — John W. Finley
Hershey, PA — W. Jeffrey Hurst
Ithaca, NY — Chang Yong Lee

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About the Editors

John M. deMan (1926–2010) was a University Professor Emeritus in the Department of Food Science at the University of Guelph, Ontario, Canada. He was the Chairman of the Department and Past President of the Canadian Institute of Food Science and Technology. He published over 250 papers and book chapters on multiple aspects of food science and technology. He received many professional awards, including the Dairy Research Award of the American Dairy Science Association, the Institute Award of the Canadian Institute of Food Science and Technology, the Alton E. Bailey Award of the American Oil Chemist Society, the Stephen S. Chang Award of the Institute of Food Technologists, and the Kaufmann Memorial Award of the International Society of Fat Research. He was a Fellow of the Institute of Food Technologists, the Canadian Institute of Food Science and Technology, and the Malaysian Oil Science and Technology Association.

John W. Finley received a B.S. in Chemistry from LeMoyne College and a Ph.D. from Cornell University. Dr. Finley retired from Louisiana State University where he was head of the Food Science program from 2007 to 2014. His laboratory studied low calorie ingredients, anti-inflammatory compounds in the diet, modified nutritional lipids, and edible fiber. Previously he headed Fundamental Science at Nabisco, was a Fellow at Kraft Food, served as chief technology officer of A.M. Todd Co. and the leader of the Food Science program at Monsanto, and Research Scientist with the USDA Regional Research Center.

Dr. Finley is a Fellow of the American Chemical Society, Fellow of Agricultural and Food Division of the American Chemical Society, Fellow of the Royal Society of Chemistry, Fellow of the Institute of Food Technologists, and Certified Food Technologist by Fellow Institute of Food Technologists. He was recognized as an Outstanding Alumnus of Michigan State University. Other awards include Harris Distinguished lecturer at the Ohio State University and a Leadership Award at Nabisco, and his memberships include Sigma Xi at Michigan State University and Phi Kappa Phi at Cornell University.

Dr. Finley has edited 8 books, holds 70 patents, and 135 publications.

W. Jeffrey Hurst retired from the Hershey Company as Principal Scientist after being with the corporation for over 39 years. His research focused on monitoring new developments in measurement technology as they apply to food systems and the review of new and emerging compounds important to

the food industry. He is a member of the American Chemical Society, the Institute of Food Technologist. He is a member of the American Society of Mass Spectrometry and Fellow of the American Institute of Chemists (FAIC). Furthermore, he was named a Fellow of the AOAC, a Pioneer in Laboratory Robotics, and is a Diplomate of the American Association Integrated Medicine. Dr. Hurst was a member of the US Air Force and retired as a Major. He also serves as a member of the External Advisory Board of the University of Illinois at Chicago NIH bv Botanical Center. This book will be the tenth one that he has edited or written. He was founding editor of Lab Robotics Automation and Seminars in Food Analysis. He has numerous patents with over 300 papers and presentations.

Chang Yong Lee received a B.S. in Chemistry from Chung-Ang University in Seoul, Korea, and a Ph.D. from Utah State University. He has been working as a faculty member at Cornell University since 1969. Professor Lee has been teaching food chemistry for a number of years in the Department of Food Science. His research interests have been on biochemical aspects of plant foods. Recently his laboratory has been working on the bioactivity of phytochemicals that is related to health benefits. He served as Chair of the Department of Food Science and Technology and Co-director of Cornell Institute of Food Science (2002–2008). Dr. Lee has held visiting professor appointments at several institutions, including Korea Institute of Science and Technology; Inter-American Institute of Agricultural Science at EMBRAPA, Brazil; Institut National de la Recherche Agronomique, Avignon, France; Beijing Vegetable Research Center, China; Ecole Nationale Superieure des Industries Agricoles et Alimentaire, France; Graduate School of Biotechnology, Korea University; and Kyung Hee University, Korea.

Professor Lee has authored more than 300 research articles. He was a recipient of Platinum Award on his edited books on polyphenols from the American Chemical Society's Division of Agricultural and Food Chemistry. *Journal of Agricultural and Food Chemistry* and the Institute for Scientific Information (ISI) acknowledged Professor Lee as one of the Highly Cited Researchers (HCR) in 2004. Thomson Reuters selected him as one of 112 scientists in the world in the field of Agricultural Science during 2002–2012 who published the greatest number of highly cited papers ranked in the top 1% by citations. Again in 2015, Thomson Reuters listed Lee as one of the World's Most Influential Scientific Minds in Agricultural Science. Professor Lee was awarded USDA Secretary's Honor Award for Excellence in Research in 2001 and 2004, and Babcock-Hart Award from the International Life Science Institute and the Institute of Food Technologists in 2003. He is elected Fellow of the American Chemical Society's Division of Agriculture and Food Chemistry (1991), the Institute of Food Technologists (1996), the Korean Academy of Science and Technology (1998), and International Academy of Food Science and Technology (2006). He was appointed as International Scholar (2011–2014) at Kyung Hee University in Korea and recently (2014–present) he has been serving as Adjunct Distinguished Professor at King Abdulaziz University, Saudi Arabia.

Water

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Yrjo H. Roos, John W. Finley, and John M. deMan

Water in Foods

Water has a chemical formula of H_2O which represents two hydrogen atoms covalently bound to one oxygen atom. Water is an odorless, tasteless and transparent liquid at room temperature. It appears colorless in small quantities although in larger bodies there is an inherent blue hue. Ice and water vapor are also colorless, although ice under pressure as in glaciers exerts a range of blue colors.

Water is the most abundant molecule in food and is an essential ingredient to support life and since all foods come from living organisms, water is an essential component of foods. In many foods both the intracellular water and interstitial water are essential. The ability of water to dissolve a wide variety of materials makes it a nearly universal solvent. Water functions to determine the physical attributes of meat, vegetable and fruit products. For food polymers, water serves as a structural component and a plasticizer contributing to the attributes of proteins, starch and food fibers. Water also serves as solvent or dispersing medium in wide variety of foods including milk, juices and other beverages. Water can be dispersed in emulsions in products like butter or margarine or be the continuous phase of emulsions such as mayonnaise. The water content of foods is extremely variable. Table 1.1 contains the water, energy, protein, lipid, ash, carbohydrate and fiber contents of a range of foods.

Water determines the physical, chemical and microbiological stability of foods. When water freezes and thaws the nature of the food can change dramatically. Many food processes involve the addition or removal of water which changes the stability or nature of the food. Frequently the process used to remove water has a significant effect on the physical nature of the food and the ability to rehydrate. For example, drum dried milk powder is much denser and more difficult to rehydrate than spray dried milk powder.

Physical Properties of Water and Ice

Some of the physical properties of water and ice which are considerations in foods are presented in Tables 1.2, 1.3, and 1.4. Much of this information was obtained from Landolt et al. (1923) and Perry (1963). The physicochemical properties of water are important considerations in understanding and showing how water contributes to food processing. The exceptionally high values of the thermodynamic parameters (energy to thaw ice and convert water to steam) of water are of importance for food processes and operations such as freezing and drying. The considerable expansion of water during freezing may contribute to structural damages to foods when they are frozen.

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_1

Table 1.1 Typical water, energy and macronutrient content in 100 g of selected foods

Food	Water (g)	Energy (kcal)	Protein (g)	Lipids (g)	Ash (g)	Carbo-hydrates (g)	Fiber (g)
Butter, with salt	15.9	717	0.9	81.1	2.1	0.1	0
Cheese, mozzarella	50.0	300	22.2	22.4	3.3	2.2	0
Cheese, parmesan, hard	29.2	392	35.8	25.8	6.0	3.2	0
Milk, whole, 3.25% milkfat	88.1	61	3.2	3.3	0.7	4.8	0
Yogurt, plain, whole milk	87.9	61	3.5	3.3	0.7	4.7	0
Egg, WHL, RAW, FRSH	76.2	143	12.6	9.5	1.1	0.7	0
Yogurt, greek, plain, nonfat	85.1	59	10.2	0.4	0.7	3.6	0
Oil, soybean, salad or cooking	0	884	0	100	0	0	0
shortening	0	884	0	100	0.0	0	0
Chicken, broilers or fryers	65.4	237	16.7	18.3	0.8	0	0
Turkey, whole, meat only, raw	75.4	112	22.6	1.9	1.0	0.1	0
Salami, cooked, beef&pork	45.2	336	21.9	25.9	4.7	2.4	0
Frankfurter	53.9	302	10.7	26.4	3.8	5.2	0
Oatmeal	83.6	71	2.5	1.5	0.3	12.0	1.7
Cheerios	5.1	376	12.1	6.7	2.8	73.2	9.4
Apples, raw, with skin	85.6	52	0.3	0.2	0.2	13.8	2.4
Bananas, raw	74.9	89	1.1	0.3	0.8	22.8	2.6
Blueberries, raw	84.2	57	0.7	0.3	0.2	14.5	2.4
Orange juice, raw	88.3	45	0.7	0.2	0.4	10.4	0.2
Raw ham	62.5	245	17.43	18.87	0.88	0	0
Pork chops, bone-in, LN, raw	73.62	127	21.99	3.71	1.01	0	0
Cured, ham, raw	66.09	173	22.45	9.17	2.7	0.21	0
Frozen pinton beans	55.8	170	9.8	0.5	1.4	32.5	5.7
Broccoli, raw	89.3	34	2.82	0.37	0.87	6.64	2.6
Carrots, raw	88.29	41	0.93	0.24	0.97	9.58	2.8
Onions, raw	89.11	40	1.1	0.1	0.35	9.34	1.7
Potatoes, Flesh & SKN, raw	79.34	77	2.02	0.09	1.08	17.47	2.2
Squash, winter, acorn, raw	87.78	40	0.8	0.1	0.9	10.42	1.5
Tomatoes, red, ripe, raw	94.52	18	0.88	0.2	0.5	3.89	1.2
Almonds, dry RSTD, W/salt	2.41	598	20.96	52.54	3.07	21.01	10.9
Beef brisket	70.29	155	20.72	7.37	1.02	0	0
Sirloin, steak, broiled	60.33	212	29.33	9.67	1.26	0	0
Beef, ground, 70% lean	54.3	332	14.35	30	0.7	0	0
Cod, Atlantic, raw	81.22	82	17.81	0.67	1.16	0	0
Salmon, Atlantic, wild, raw	68.5	142	19.84	6.34	2.54	0	0
Tuna, fresh, Yellowfin, raw	74.03	109	24.4	0.49	1.64	0	0
Peanut butter	1.47	591	25.72	50.81	3.25	18.75	5.6
Beer	92.77	41	0.36	0	0.11	2.97	0
Red table wine	86.49	85	0.07	0	0.28	2.61	0

USDA Nutrient Composition Files: http://www.ars.usda.gov/Services/docs.htm

Freezing of water in foods occurs over a wide temperature range, resulting in stresses in frozen foods. Structural damage may result from fluctuating temperatures, even if the fluctuations remain below the freezing point as ice crystals grow and causes stresses to cellular structures. This is an important quality attribute of frozen foods. An example is that frozen strawberries loose much of their texture after a freezing and thawing cycle because of loss of cellular integrity. Table 1.2 contains the basic physico-chemical properties of water.

Table 1.2 Physical–chemical properties of water

Property	Value
Molar mass (g/mol)	18.015
Molar volume (mol/L)	55.5
Boiling point (BP, °C at 1 bar)	100 °C
Freezing point (FP, °C at 1 bar)	0 °C
Triple point	0 °C, 6.1 mbar
Surface tension (20 °C)	73 dynes at 20 °C
Vapor pressure (20 °C)	0.0212 atm at 20 °C
ΔH of vaporization	40.63 kJ/mol
ΔH of fusion	6.013 kJ/mol
Heat capacity (Cp)	4.22 kJ/kg
Viscosity (Pas, 20 °C)	1.002 centipoise at 20 °C
Density (kg/m^3)	1 g/mL
Maximum density (kg/m^3)	4 °C

The physical properties of water and ice are presented in Tables 1.3 and 1.4 according to data of Landolt et al. (1923) and Perry and Green 9:2007. There is a significant variation in the properties of liquid water and ice with temperature. The influence of temperature is presented in Tables 1.3 and 1.4. In comparison to other liquids higher amounts of heat are required to convert water from solid ice to liquid water and to water vapor.

Within the three physical states of water (ice, liquid and steam) the physical properties have major impacts on food processing. The effects of heat on water drive processing conditions particularly freezing products, or water removal such as concentration or drying. Figure 1.1 illustrates vapor pressure on the surface of water. As external pressure increases it becomes increasingly difficult for water molecules to escape from a liquid, thus raising the boiling point of the water. The vapor pressure of water is affected by temperature and therefore, if external pressure is lower water boils at lower temperatures. Such phenomenon occurs for example at higher altitudes (Fig. 1.2). It is well known that water boils at 100 °C at sea level but at 95 °C at 1500 m above sea level. Therefore, cooking times of foods must be extended at higher altitudes to achieve equivalent results such as making a hardboiled egg or hydrating pasta. At very high elevations cooking times must be adjusted to obtain desired physical changes such as gelation of starch, and to assure sufficient heat and time for microbial safety.

This allows calculation of the pressure of the vapor phase at a given temperature when the temperature and pressure at another point, and the enthalpy of vaporization are known. The boiling point is the temperature at which the vapor pressure at equilibrium exceeds atmospheric pressure. In other words, Knowing the boiling point of a substance at 1 bar (the normal boiling point) with the heat of evaporation, ΔH_{vap} for that substance, the boiling point, T_2 at another pressure with values for P_1 and T_1 (the normal boiling point), P_2, and ΔH_{vap}, (also called the latent heat) can be solved. The liquid–vapor transition follows a very specific curve on the pressure–temperature plane of a PVT diagram. This is given by the Clausius–Clapeyron relation, which at temperatures and pressures that are not close to the critical point, can be approximated as:

Table 1.3 provides typical physical properties of water which are important to foods and food processing. The vapor pressure of water is defined as the pressure at which air over the water is saturated. If the pressure is increased the water will condense, if the pressure decreases more water will evaporate into the atmosphere. It is important to recognize the changes in vapor pressure with changes in temperature. This is an equilibrium where there is no net change although individual atoms migrate between liquid and vapor phase. Density refers to the mass of a material divided by its volume. It can be seen that as the temperature of water increases the increased molecular motion from the heat results in a decline in density.

Table 1.4 illustrates vapor pressure of water in ice as a function of temperature. This becomes important in frozen foods as protecting the surface from water loss is required and conversely maximising the rate of water removal during freeze drying. The changes in the coefficient of expansion are important in maintaining quality of frozen foods. If the temperature of a frozen food stored at −30 °C increased to −5 °C there would be a large increase in the size of the ice crystals potentially damaging product quality and texture.

When ice and water are mixed together, then the temperature of the solution will be 0 °C as long as both liquid and solid phases coexist. Thus 0 °C is the freezing point for water or the

Table 1.3 Physical properties of water at various temperatures

	Temperature (°C)					
Water	0	20	40	60	80	100
Vapor pressure (mbar)	6.11	23.37	73.75	199.18	473.56	1013.25
Density (kg/m^3)	0.9998	0.9982	0.9922	0.9832	0.9718	0.9583
Specific heat (kJ/kg °C)	4.215	4.179	4.176	4.181	4.194	4.213
Heat of vaporization (kJ/kg)	2499	2452	2405	2357	2306	2255
Thermal conductance (kcal/m^2 h °C)	0.565	0.599	0.628	0.652	0.670	0.680
Surface tension (mN/m)	75.62	72.75	69.55	66.17	62.60	58.84
Viscosity (mPa s)	1.792	1.002	0.653	0.466	0.355	0.282
Refractive index	1.3338	1.3330	1.3306	1.3272	1.3230	1.3180
Dielectric constant	88.0	80.4	73.3	66.7	60.8	55.3
Coefficient of thermal expansion × 10^{-4}	–	2.07	3.87	5.38	6.57	–

Table 1.4 Physical properties of ice at various temperatures

	Temperature (°C)						
Ice	0	−5	−10	−15	−20	−25	−30
Vapor pressure (mbar)	6.11	4.01	2.60	1.65	1.03	0.63	0.37
Heat of fusion (kJ/kg)	334	–	–	–	–	–	–
Heat of sublimation (kJ/kg)	2836	–	2813	–	2789	–	2771
Density (kg/m^3)	0.9168	0.9171	0.9175	0.9178	0.9182	0.9185	0.9188
Specific heat (kJ/kg °C)	2.039	–	1.996	–	1944	–	1.884
Coefficient of thermal expansion × 10^{-5}	9.2	7.1	5.5	4.4	3.9	3.6	–

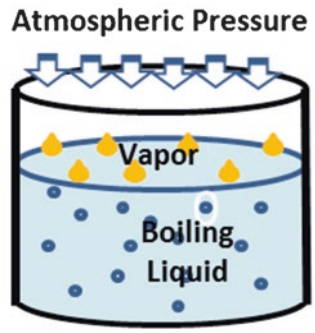


Fig. 1.1 At the boiling temperature of a liquid: vapor pressure = atmospheric pressure

melting point for ice. This is called the equilibrium point. If water is cooled to 0 °C it does not freeze; it must be cooled to below 0 °C before freezing can occur. Likewise, ice has to be heated slightly above 0 °C before melting occurs. Unlike freezing, however, melting will begin as soon as the temperature rises to above 0 °C. To initiate ice formation water must be cooled to temperatures substantially below the freezing point. The difference is due to the need for nucleation to occur before an ice crystal growth is initiated. Nucleation is the process by which a minimum number of water molecules form an embryo that can grow to form a crystal, after which ice crystal growth results in continued expansion of the crystals.

Structure of the Water Molecule

The unique properties of water are a result of the structure of the water molecule (Fig. 1.3) and its dielectric properties which allow it to form hydrogen bonds. Water molecules consist of two hydrogen atoms joined to an oxygen atom by covalent bonds. Oxygen is more electronegative than hydrogen. That is, the high electronegativity causes the oxygen atom to pull the shared pairs of electrons more towards the oxygen atom. As a result, the O–H bond acquires polarity.

Oxygen atoms have six electrons ($1s^2\ 2s^2\ 2p^4$) in its outermost shell. The ‘s’ and ‘p’ orbitals of

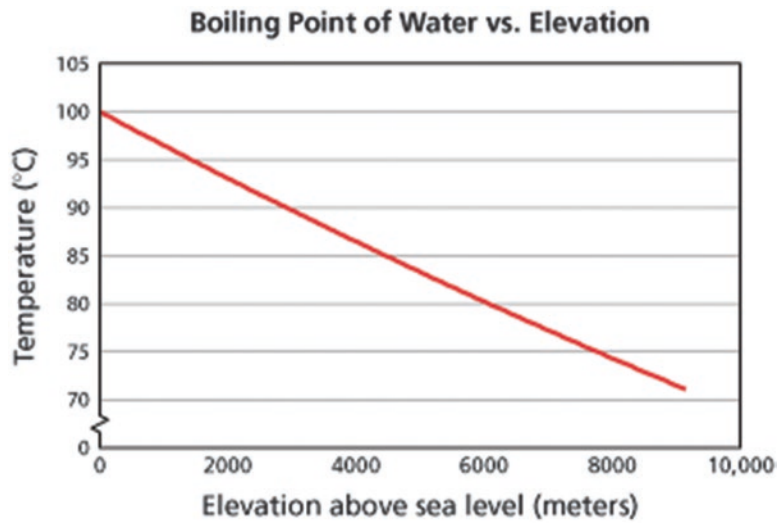


Fig. 1.2 Boiling point of water at various altitudes

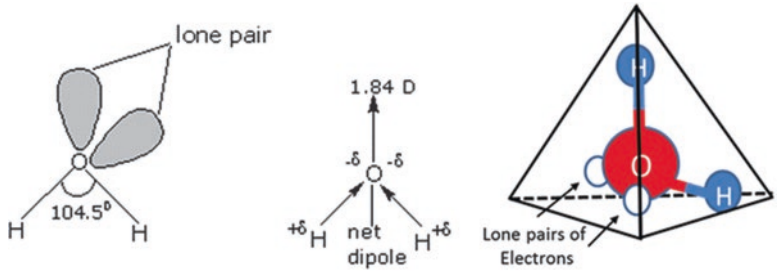


Fig. 1.3 Angles of H atoms, dipoles of lone pairs of electrons leading to the tetrahedral arrangement of water

the valence shell are sp^3 hybridized to form four sp^3 hybrid orbitals oriented tetrahedrally around the oxygen atom. Two of the hybrid orbitals are singly occupied with the half-filled orbital of the hydrogen atoms. Lone pairs of electrons occupy the other two. Therefore, oxygen is bonded to the two hydrogen atoms by two O–H covalent bonds, and there are two lone-pairs of electrons on the oxygen atom. The H–O–H bond angle is 104.5°, which is slightly less than the tetrahedral angle of 109°28′. Therefore, the structure of water molecule is an angular or bent structure. Figure 1.3 illustrates the tetrahedral structure of water. In the water molecule the atoms are arranged at an angle of 104.5°, and the distance between the nuclei of hydrogen and oxygen is 0.0957 nm. The water molecule can be considered a spherical quadrupole with a diameter of 0.276 nm, where the oxygen nucleus forms the center of the quadrupole. The two negative and two positive charges form the angles of a regular tetrahedron. Because of the separation of charges in a water molecule, the attraction between neighboring molecules is higher than is normal with van der Waals' forces.

In the frozen state each water molecule accepts two hydrogen bonds from two other water molecules and donates two hydrogen atoms to form hydrogen bonds with two more water molecules, producing an open, cage like structure (Fig. 1.4). The structure of liquid water is very similar, but in the liquid, the hydrogen bonds are continually broken and formed because of rapid molecular motion.

In the liquid state water molecules are held together by intermolecular hydrogen bonds. Each oxygen atom can form two hydrogen bonds utilizing both the lone pairs of electrons the oxygen atom. Liquid water contains aggregates of varying number of water molecules held together by hydrogen bonds and 'free' water molecules in

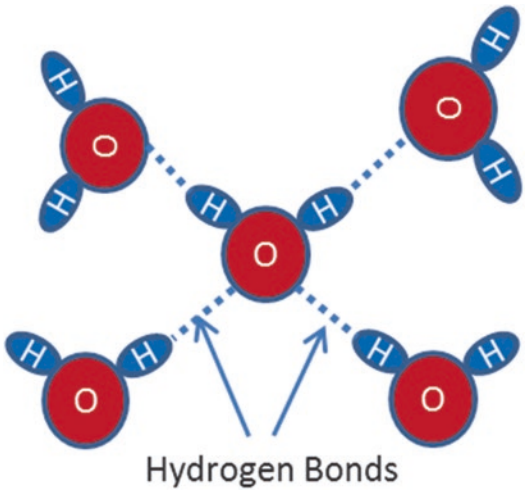


Fig. 1.4 Hydrogen bonds in water

equilibrium. These intermolecular aggregates are continually forming, collapsing at various rate depending on temperature.

Hydrogen bonding has very major influence on the properties of water.

In ice crystals a hexagonal matrix is formed with tetrahedral structure of water molecules surrounding each oxygen atom. One hydrogen atom exists between each pair of oxygen atoms. Thus, each and every hydrogen atom is covalently bonded to one oxygen atom and hydrogen bonded to another oxygen atom. This packing results in large open spaces between water molecules in the ice resulting in the lower density of ice compared to liquid water.

When ice melts some of the hydrogen bonds are broken and the water molecules become more closely packed. This results in an increase in the density of water above its melting points 0 °C. Density of water attains a maximum value of 1 g/mL at 4 °C; above 4 °C, the density decreases due to the normal temperature effects.

In ice, every H_2O molecule is bound by four bridges to each neighbor. The binding energy of the hydrogen bond in ice amounts to 20.9/mol (Meryman and Pauling 1960). Similar strong interactions occur between OH and NH and between small, strongly electronegative atoms such as O and N. This is the reason for the strong association in alcohols, fatty acids, and amines and their great affinity to water. A comparison of

Table 1.5 Physical properties of some hydrides at normal atmospheric conditions

Compound	Melting point (°C)	Boiling point (°C)	Molar heat of vaporization (kJ)
CH_4	−184	−161	8.2
NH_3	−78	−33	23.3
HF	−92	+19	7.49
H_2O	0	+100	40.7

the properties of water with those of the hydrides of elements near oxygen in the Periodic Table (CH_4, NH_3, HF, DH_3, H_2S, HCl) indicates that water has unusually high values for various physical constants, such as melting point, boiling point, heat capacity, latent heat of fusion, latent heat of vaporization, surface tension, and dielectric constant. Some of these values are listed in Table 1.5.

Structure of Ice

In ice an individual water molecule connects to four others in a tetrahedral arrangement. This arrangement results in the hexagonal crystal lattice in regular ice, as shown in Fig. 1.5. The lattice is loosely built and has relatively large hollow spaces. These hollow spaces in typical ice structure result in expansion during freezing and cause the high specific volume of ice. This is why ice floats on the surface of water. In the hydrogen bonds, the hydrogen atom is 0.1 nm from one oxygen atom and 0.176 nm from another hydrogen atom. When ice melts, the hydrogen bonds are broken and the water molecules pack together more compactly in a liquid state (http://www1.lsbu.ac.uk/water/hexagonal_ice.html; http://www.uwgb.edu/dutchs/petrology/Ice%20Structure.HTM; Franks 2000).

Each oxygen atom inside the ice lattice is surrounded by four other oxygen atoms in a tetrahedral arrangement. The distance between oxygen atoms is approximately 2.75 Å. The hydrogen atoms in ice are arranged following the Bernal-Fowler rules: (1) two protons are close (about 0.98 Å) to each oxygen atom, much like in a free water molecule; (2) each H_2O molecule is oriented so that the two protons point toward two adjacent oxygen atoms; (3) there is only one

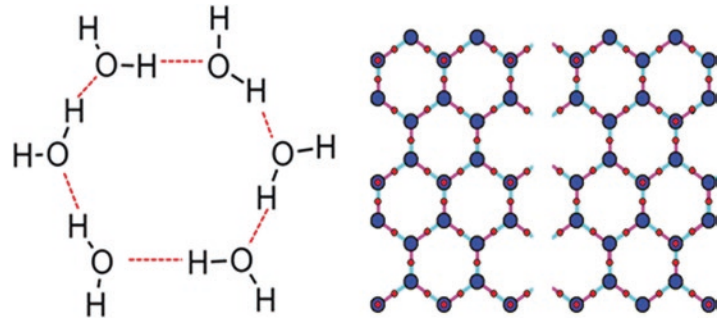


Fig. 1.5 Hydrogen bonding among water molecules leading to hexagonal arrangement of water molecules in ice

proton between two adjacent oxygen atoms; (4) under ordinary conditions any of the large number of possible configurations is equally probable (http://www.its.caltech.edu/~atomic/snowcrystals/ice/ice.htm).

When there is a change of state from ice to water, rigidity is lost, but water still retains a large number of ice-like clusters. The term *ice-like cluster* does not imply an arrangement identical to that of crystalline ice. The HOH bond angle of water is several degrees less than that of ice, and the average distance between oxygen atoms is 0.31 nm in water and 0.276 nm in ice. Research has not yet determined whether the ice-like clusters of water exist in a tetrahedral arrangement, as they do in ice. Since the average intermolecular distance is greater than in ice, it follows that the greater density of water must be achieved by each molecule having some neighbors. A cubic structure with each HOH molecule surrounded by six others has been suggested.

At 0 °C, water contains ice-like clusters averaging 90 molecules per cluster. With increasing temperature, clusters become smaller and more numerous. At 0 °C, approximately half of the hydrogen bonds present remain unbroken, and even at 100 °C approximately one-third are still present. All hydrogen bonds are broken when water changes into vapor at 100 °C. This explains the large heat of vaporization of water.

Growth of Ice Crystals

The crystal structure of ice is such that it does not allow the inclusion of impurities, except within defects in the crystal structure. When an ice nucleus begins to grow, any solutes which are present in the liquid will be excluded from this growing ice front. If the rate of crystal growth is faster than the rate at which diffusion of the particular solutes can carry them away a concentration gradient will form in the liquid which surrounds the ice crystal. The concentrated solute will lower the freezing point of the solution. The solution at the interface will have a freezing point equal to the temperature of the interface; at this point, ice growth will be limited by diffusion of the solute away from the crystal. When this occurs solution away from the ice crystal is supercooled (a temperature below the melting point). Eventually diffusion will ensure that the system goes to equilibrium, however a situation of instability is created when this occurs.

Latent Heat of Fusion

When water freezes, heat is liberated by the process. This latent heat of fusion is due to the energy released from hydrogen bonds in the crystal. In ice a water molecule is hydrogen bonded to four other neighboring water molecules, each bond having an energy between 10 and 40 kJ/mol. This equals an energy of 80 cal/g (335 J/g). The energy released in the transformation of 1 g of water at 0 °C to ice at the same temperature is enough energy to raise the temperature of 1 g of the water from 0 to 80 °C!

The speed of crystallization—that is, the progress of the ice front in centimeters per second—is determined by the removal of the heat of fusion from the area of crystallization. The speed of crystallization is low at a high degree of super-cooling (Meryman 1966). This affects the size of crystals in the ice. When large water

masses are cooled slowly, there is sufficient time for heterogeneous nucleation in the area of the ice crystal growth. At that point the crystallization speed is very rapid so that a few nuclei grow to a large size, resulting in a large crystalline structure. At greater cooling speed, high supercooling occurs; this results in more nucleation and growth of smaller ice crystals.

Upon freezing, H–O–H molecules associate in an orderly manner to form a rigid structure that is more open (less dense) than the liquid form. There still remains considerable movement of individual atoms and molecules in ice, particularly just below the freezing point. At 10 °C an H–O–H molecule vibrates with an amplitude of approximately 0.044 nm, nearly one-sixth the distance between adjacent HOH molecules. Hydrogen atoms may wander from one oxygen atom to another.

The production of heat from crystallization also interferes with crystal growth. The heat is created at the crystal surface and must either be diffused in the crystal or throughout the liquid. The removal of this heat occurs by conduction and can only occur through the liquid if it is supercooled when nucleation occurs. If the latent heat of fusion is conducted away through the ice the growing crystal will remain essentially smooth the part of the interface which grows beyond the planar front. The system will not be able to lose its heat as quickly as the ice on either side of it. If the heat is conducted away through the liquid, growth occurs preferentially along the a-axes compared with growth along the c-axis. This occurs because of the rise in temperature of the liquid surrounding the crystal. As the molecules become more energetic, they are less likely to join a planar surface where they can only hydrogen bond with a single neighbor. This is the phenomenon responsible for the hexagonal symmetry that we see in growing ice crystals.

Solidification Without Crystallization

If a liquid is cooled so quickly so that nucleation cannot occur, it is possible to avoid ice formation. This process is called vitrification and results in an amorphous solid, generally referred to as glass. The liquid remains in a supercooled liquid state until temperature is lowered to below the glass transition temperature (T_g) which is indicated by a decrease in heat capacity when measured in differential scanning calorimetry. Once a supercooled liquid reaches temperatures below T_g, the system is not a viscous liquid, but is a solid in a metastable state. Achieving vitrification with pure water requires in laboratory very small amounts of water and allowing an extremely fast cooling. When high concentrations of solutes are present, solutions can be vitrified more easily. (http://people.ucalgary.ca/~kmuldrew/cryo_course/cryo_chap6_2.html).

Crystal growth, in contrast to nucleation, occurs readily at temperatures close to the freezing point. It is more difficult to initiate crystallization than to continue it. The rate of ice crystal growth decreases with decreasing temperature. This is important in protecting quality of frozen foods where excessive crystal growth can damage the structure of the food. A schematic graphical representation of nucleation and crystal growth rates is given in Fig. 1.6. Solutes of many types at low concentrations will greatly slow ice crystal growth. The mechanism of this action is not known. The effect of membranes on ice crystal propagation was studied by Lusena and Cook (1953), who found that membranes can be either permeable, partly permeable, or impermeable to growing ice crystals. Permeability to ice crystal growth increases with porosity and is also affected

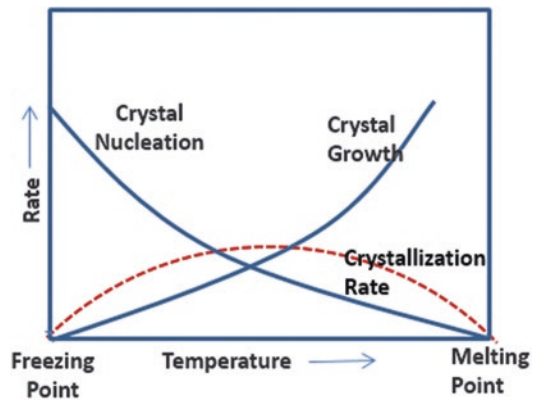


Fig. 1.6 Schematic representation of the rate of nucleation and crystal growth

by rate of cooling, membrane composition and membrane properties, and concentration of the solute(s) present in the aqueous phase. When ice crystal growth is retarded by solutes, the ice phase may become discontinuous either by the presence of a membrane or spontaneously.

Ice crystal size at the completion of freezing is related directly to the number of ice nuclei. The greater the number of nuclei, the smaller the size of the crystals. This is the reason that the cryo-freezing in food processing causes less damage to the cell structure and maintains better textural quality when the frozen products are thawed. In liquid systems nuclei can be added by a process called *seeding*. Practical applications of seeding include adding finely ground lactose to evaporated milk in the evaporator, and recirculating some portion of crystallized fat in a heat exchanger during manufacture of margarine. If the system is maintained at a temperature close to the freezing point (FP), where crystallization starts (Fig. 1.6), only a few nuclei form and each crystal grows extensively. The slow removal of heat energy produces an analogous situation, since the heat of crystallization released by the few growing crystals causes the temperature to remain near the melting point, where nucleation is unlikely. In tissue or unagitated fluid systems, slow removal of heat results in a continuous ice phase that slowly moves inward, with little if any nucleation. The effect of temperature on the linear crystallization velocity of water is given in Table 1.4.

When the temperature is lowered to below the FP (Fig. 1.6), increasing rate of supercooling is required before nucleation becomes the predominant factor and crystal growth appears thereafter. At low supercooling large crystals are formed; as supercooling increases, smaller crystals are formed. Control of crystal size is more difficult in tissues than in agitated liquids. Agitation promotes nucleation and, therefore, reduces crystal size. Lusena and Cook (1954) suggested that large ice crystals are formed when freezing takes place above the critical nucleation temperature (close to FP in Fig. 1.6). When freezing occurs at the critical nucleation temperature, small ice crystals form. The effect of solutes on nucleation and rate of ice crystal growth is a major factor controlling the pattern of propagation of the ice front. Lusena and Cook (1955) also found that solutes depressed the nucleation temperature to the same extent that they depressed the freezing point. Solutes retard ice growth at 10 °C supercooling, with organic compounds having a greater effect than inorganic ones. At low concentrations, some proteins are as effective as alcohols and sugars in retarding crystal growth.

Once formed, crystals have a tendency to enlarge. Recrystallization is particularly evident when storage temperatures are allowed to fluctuate. This is evident in foods stored in freezers where temperatures fluctuate. The larger crystals alter texture and can damage cellular structure in foods.

Slow freezing results in large ice crystals located exclusively in extracellular areas. Rapid freezing results in tiny ice crystals located both extra- and intracellularly. During the freezing of food, water is transformed to ice with a high degree of purity, and solute concentration in the unfrozen liquid is gradually increased. This is accompanied by changes in pH, ionic strength, viscosity, osmotic pressure, vapor pressure, and other properties.

When water freezes, it expands nearly 9%. The volume change of a food that is frozen will be determined by its water content and by solute concentration. Highly concentrated sucrose solutions do not show significant expansion (Table 1.6). Air spaces may partially accommodate expanding ice crystals. Volume changes in some fruit products upon freezing are shown in Table 1.7. The effect of air space is obvious.

Table 1.6 Volume change of water and sucrose solutions on freezing

Sucrose (%)	Volume increase during temperature change from 21 to −18 °C
0	8.6
10	8.7
20	8.2
30	6.2
40	5.1
50	3.9
60	None

Table 1.7 Expansion of fruit products during freezing

Product	Percent volume increase during temperature change from 21 to −18 °C
Apple juice	8.3
Orange juice	8.0
Whole raspberries	4.0
Crushed raspberries	6.3
Whole strawberries	3.0
Crushed strawberries	8.2

The expansion of water on freezing results in local stresses that undoubtedly produce mechanical damage in cellular materials. Freezing may cause changes in frozen foods that make the product unacceptable. Such changes may include destabilization of emulsions, flocculation of proteins, increase in toughness of fish flesh, loss of textural integrity, and increase in drip loss of meat. Ice formation can be influenced by the presence of carbohydrates. The effect of sucrose on the ice formation process has been described by Roos and Karel (1991a, b, c).

Surface Tension of Water

Water has a high surface tension which makes it sticky and as a result it tends to form droplets rather than spread out as a film. This surface tension in water accounts for water's ability to move in capillaries such as the roots of plants or in vessels in living bodies.

When we look at a drop of water it almost appears to have skin around it making it appear like a flattened sphere. This surface tension is because water molecules are attracted to one another. Conversely non-polar compounds like hexane do not form droplets because there is little intermolecular attraction. In water this attraction is because the two hydrogens line up one side of the oxygen atom and the hydrogens have a slightly positive charge and the oxygen has a slightly negative charge. This polarity causes water to be more cohesive and sticky.

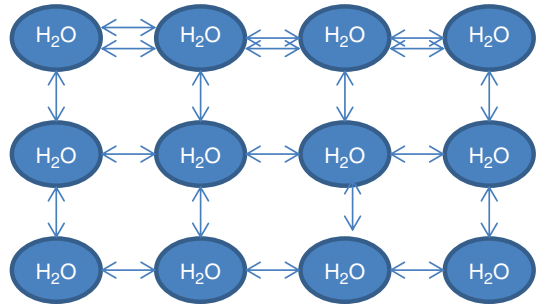


Fig. 1.7 Interaction of water molecules at surface increase surface tension

Water has greater molecular interaction at surface:

Water molecules tend to be attracted to one another. At the surface, however, there are no water molecules in the air above the liquid air above which results in stronger bonds between those molecules at the surface as shown in Fig. 1.7. This surface layer results in surface tension which creates a barrier between the atmosphere and the water. The hydrogen bonding binding between water molecules results in close alignment of water molecules at the water air interface. This interaction drives droplet formation and higher surface tension in liquid water.

In liquid, a water molecule will show net force because the forces by the neighboring molecules all cancel out (Fig. 1.7). At the surface there will be a net inward force because there are no forces acting from above. This inward net force causes the molecules on the surface to contract and to resist being stretched or broken. The surface tension of water is why small objects will "float" on the surface of water as long as the object cannot break through and separate the top layer of water molecules. The surface of the fluid, the surface under tension will behave like an elastic membrane.

Examples of Surface Tension

Insect on surface of water held by surface tension

Water has a high surface tension against air; therefore, small insects such as the water strider can walk on water because their weight is not enough to penetrate the surface of the water. When soaps and detergents are added to the water the surface tension is reduced which makes the water better able to wet other surfaces. Many food emulsifiers act like detergents to facilitate water contact with proteins or lipids in food systems.

When gases are formed in water the surface tension of the water provides the necessary tension at the surface that tends to force the bubbles to become spherical. When water droplets are formed the surface tension phenomenon causes the droplets to take on a spherical shape.

U.S. Department of the Interior | U.S. Geological Survey

URL: http://water.usgs.gov/edu/surface-tension.html

Colligative Properties of Aqueous Solutions

The colligative properties of the solution are those properties that are determined by the number of particles dissolved in the solution. The freezing point of a solution of water is lowered as a function of the dissolved particles in the solution and conversely, the boiling point is raised as a function of particles in solution. The colligative properties important in food chemistry are freezing depression, boiling point elevation, osmotic pressure and vapor pressure.

Freezing Point Depression

When solutes are present in solution the freezing point (defined as the highest temperature where last ice crystals dissolve in heating) is lowered relative to the freezing point of the pure solvent. The depression of freezing point of an aqueous solution is determined by the formula:

$$\Delta T_f = iK_f m$$

where:

ΔT_f = The depression in freezing point of the solution

i = the number of ions or particles per molecule (van 't Hoff factor)

K_f = freezing point depression constant for the solvent (1.86 °C kg/mol for water)

m = Molar concentration of the solution

For example:

1. A solution of sodium chloride containing 75 g NaCl/500 mL.
   The molecular weight of NaCl is 58.44
   m (molal) = 75 NaCl g/58.44/0.5 mL (500 mL /1000) = 100/58.55/0.5 = 2.57
   i = 2 (1 Na^+ and 1 Cl^-)
   K_f = Constant for water is 1.86 °C kg/mol

Therefore:

$\Delta T_f = iK_f m$

$\Delta T_f = 2 \times 1.86 \times 2.57 = 9.56$

The sodium chloride solution will start to freeze at −9.56 °C

2. A solution of 75 g glycerol/500 mL

The molecular weight of glycerol is 92.09

m = 75 g/92.09/0.5 L (500 mL/1000) = 75/92.09 /0.5 = 1.62

i = 1 (glycerol is one molecule)

K_f = freezing constant for water is 1.86 °C kg/mol

Therefore:

$\Delta T_f = iK_f m$

$\Delta T_f = 1 \times 1.86 \times 1.62 = 4.82$

The glycerol solution will show initial freezing at −4.82 °C

Boiling Point Elevation

When solutes are dissolved in a solution the boiling point will be raised. Like freezing point depression, the boiling point elevation is dependent on the number of particles in solution. The calculation for increase in boiling point is similar to the equation for calculating the depression in freezing point. The equation is:

$$\Delta T_b = iK_b m$$

ΔT_b = the increase in boiling point of the solution
i = the number of ions or particles per molecule (van 't Hoff factor)
K_b = boiling point increase constant for the solvent (0.52 °C kg/mol for water)
m = Molar concentration of the solution

Using the same two examples as above:

1. A solution of sodium chloride containing 75 g NaCl/500 mL.
   The molecular weight of NaCl is 58.44
   m (molal) = 75 NaCl g/58.44/0.5 mL (500 mL /1000) = 100/58.55/0.5 = 2.57
   i = 2 (1 Na^+ and 1 Cl^-)
   K_b = Boiling constant for water is 0.52 °C kg/mol
   Therefore:
   $\Delta T_b = iK_f m$
   $\Delta T_b = 2 \times 0.52 \times 2.57 = 2.67$ °C
   The sodium chloride solution will boil at 102.67 °C
2. A solution of 75 g glycerol/500 mL
   The molecular weight of glycerol is 92.09
   m = 75 g/92.09/0.5 L (500 mL/1000) = 75/92.09/0.5 = 1.62
   i = 1 (glycerol is one molecule)
   K_b = Boiling Constant for water is 0.52 °C kg/mol

Therefore:
$\Delta T_b = iK_b m$
$\Delta T_b = 1 \times 0.52 \times 1.62 = 0.84$
The glycerol solution will boil at 100.84 °C

Osmotic Pressure

Osmosis is the process whereby a solvent passes through a semipermeable membrane from one solution to another. Examples of semipermeable membranes are the cell membranes in cells of living things (plants and animals) or synthetic membranes for dialysis. Osmotic pressure drives solvent molecules through the semipermeable membrane from the low solute concentrations to the high solute concentrations. When equilibrium is reached the solute concentrations are equal on both sides of the membrane. Osmotic pressure is defined as the pressure applied to on the high concentration side to stop osmosis.

Osmotic pressure is expressed by the formula:

$$\Pi = \text{iMRT}$$

where
Π is the osmotic pressure in atm
i = van 't Hoff factor of the solute.
M = molar concentration in mol/L
R = universal gas constant = 0.08206 L atm/mol K
T = absolute temperature in K

An example of benefits in food from controlling osmotic pressure is dried fruit with added sugar. The fruit cells are dehydrated preserving the fruit. When the fruit is exposed to potential spoilage organisms the moisture migrates from the spoilage organism to the fruit stopping growth of the microbe.

Vapor Pressure Lowering

Vapor pressure for a liquid is defined as the equilibrium pressure of gas molecules from a liquid that exists above the liquid in a closed system. In a closed system, the vapor pressure above the liquid is dependent on the temperature. The vapor pressure of pure water at room temperature (20 °C) is about 2666 Pa, (which is about one forteith of the total atmospheric pressure at sea level). When an aqueous solution is compared to pure water the vapor pressure will be lowered proportional to the solute in solution. Raoult's law states that when a substance is added to a

solution, the vapor pressure of the solution will decrease. This change depends on *the mole fraction of the dissolved solute in solution and the ori*ginal vapor pressure of the solvent.

$$P_{solvent} = X_{solvent} P^{o}_{solvent}$$

$P_{solvent}$ = Vapor pressure of a solvent
$X_{solvent}$ = Mole fraction of solvent
$P^{o}_{solvent}$ = the vapor pressure of the pure solvent at a particular temperature.

At any temperature there is a pressure at which the vapor above the substance is in dynamic equilibrium with its liquid or solid form. This is defined as the **vapor pressure** of the substance at that temperature. At equilibrium, the rate at which the solid or liquid evaporates is equal to the rate that the gas is condensing back to the solid or liquid form. This pressure is constant regardless of how much of the substance is present. The law only works for ideal mixtures. In mixtures each component contributes to the total vapor pressure.

$$P_A = X_A \times P^{o}_A$$
$$P_B = X_B \times P^{o}_B$$
$$\text{Total Vapor Pressure} = P_A + P_B$$

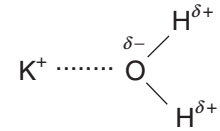
where:
X_A = Mole fraction of Component A (moles A/(moles A + moles B)
X_B = Mole fraction of Component B
P^{o}_A = Vapor pressure of pure component A at a specific temperature
P^{o}_B = Vapor pressure of pure component B at a specific temperature
P_A = Partial pressure for Component A
P_B = Partial Pressure for Component B

Ionic Interactions Are Attractions Between Oppositely Charged Ions

In compounds with ionic bonds, the bonded atoms are sufficiently different in electronegativity that the bonding electrons are never shared: these electrons are always found around the more electronegative atom. For example, in sodium chloride (NaCl), the bonding electron contributed by the sodium atom is completely transferred to the chlorine atom. Even in solid crystals of NaCl, the sodium and chlorine atoms are ionized, so it is more accurate to write the formula for the compound as Na^+Cl^-.

These bonds are called ionic bonds (or interactions) that result from the attraction of a positively charged ion—a cation—for a negatively charged ion—an anion. Unlike covalent or hydrogen bonds, ionic bonds do not have fixed or specific geometric orientations. The electrostatic field around an ion—its attraction for an opposite charge—is uniform in all directions. Crystals of salts such as Na^+Cl^- do have very regular structures because the specific crystalline structure is the energetically most favorable way of packing together positive and negative ions. The force that stabilizes ionic crystals is called the *lattice energy.*

In aqueous solutions, simple ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-, do not exist as free, isolated entities. Instead, each is surrounded by a tightly held shell of water molecules (Fig. 1.8). Ionic interaction occurs between the ion and the oppositely charged end of the water dipole, as shown below for the K^+ ion:



Sodium chloride is an example of an ionic solute that dissolves in water. In water sodium chloride separates into positively charged sodium ions and negatively charged chloride ions. Each of these ions is surrounded by water molecules breaking up the crystalline lattice of the salt and pulling the ions into solution. The negative side

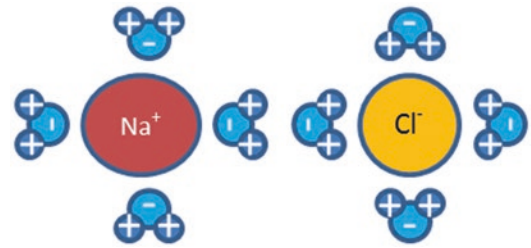


Fig. 1.8 Sodium and chloride ions surrounded by water molecules with polarity of water stabilizing the solution

of the water molecules is attracted to the positively charged sodium ion and the positively charged sides of the water molecule are attracted to the negatively charged chloride ion.

With non-ionic molecules that have small dipole moments such as sugars, carboxylic acids and amines the water molecules surround the partially charged portion of the molecule and pull it into solution.

Simple sugars like sucrose are dissolved in similar ways. The water breaks the weak bonds holding the sugar crystals together and the water molecules line up with the –OH groups on the sugar like they do with each other in solution. The solvation process requires energy because the hydrogen bonding in water and in the sugar crystals must be broken. Some energy is given off when the sugar molecules form intermolecular bonds with the water.

Molecular solids such as sucrose are dissolved when they are surrounded by water molecules. Ionic solids such as sodium chloride are dissolved as hydrates of sodium ions and chloride ions in solution.

When concentrations of ions become large enough a reverse reaction occurs and the sodium and chloride ions re-associate forming new solids. When the rate of precipitation and the rate of solution are in equilibrium the solution defined as saturated.

The dipole charge differences in water allow water molecules to be attracted to each other and to other polar molecules through hydrogen bonding. The hydrogen bond is an intermolecular force that exists when two partial electric charges of opposite polarity interact with one another. Chemically these bonds are much weaker than covalent or ionic bonds. Hydrogen bonds can be between molecules such as with water in solution or within a large macromolecule such as a protein where they help determine the three dimensional shape of the molecule. Hydrogen bonding in water determines the physical properties of water such as relatively high boiling point and when water freezes the structure is of lower density. Water like most other materials becomes denser before freezing but unlike other liquids becomes less dense when ice crystals are formed. The intermolecular hydrogen bonding is why liquid water has a higher specific heat capacity.

Inorganic salts are considered to be soluble if the solubility is at least 0.1 mol/L at room temperature, and not soluble if the solubility is less than 0.001 M/L at room temperature. Table 1.8 summarizes the water solubility's of some representative inorganic salts.

Water influences the conformation of macromolecules because it exerts effects on many of the non-covalent bonds that stabilize the conformation of the large molecules such as proteins, starches and fibers (Klotz 1965). Three types of non-covalent bonds exert forces on macromolecules: hydrogen bonds, ionic bonds, or hydrophobic interactions (apolar bonds). In proteins, water competes with the intermolecular amide hydrogen bonds forming water–amide hydrogen bonds. The greater the hydrogen bonding ability of the solvent, the weaker the C=O···H–N bond. In aqueous solvents the heat of formation or disruption of this bond is zero. This means that a

Table 1.8 Examples of solubility of inorganic salts

1. The Na^+, K^+, and NH_4^+ ions form *soluble salts*. Thus, NaCl, KNO_3, $(NH_4)_2SO_4$, Na_2S, and $(NH_4)_2CO_3$ are soluble
2. The nitrate (NO_3^-) ion forms *soluble salts*. Thus, $Cu(NO_3)_2$ and $Fe(NO_3)_3$ are soluble
3. The chloride (Cl^-), bromide (Br^-), and iodide (I^-) ions generally form *soluble salts*. Exceptions to this rule include salts of the Pb^{2+}, Hg_2^{2+}, Ag^+, and Cu^+ ions. $ZnCl_2$ is soluble, but CuBr is not
4. The sulfate (SO_4^{2-}) ion generally forms *soluble salts*. Exceptions include $BaSO_4$, $SrSO_4$, and $PbSO_4$, which are insoluble, and Ag_2SO_4, $CaSO_4$, and Hg_2SO_4, which are slightly soluble
Insoluble salts
1. Sulfides (S^{2-}) are usually *insoluble*. Exceptions include Na_2S, K_2S, $(NH_4)_2S$, MgS, CaS, SrS, and BaS
2. Oxides (O^{2-}) are usually *insoluble*. Exceptions include Na_2O, K_2O, SrO, and BaO, which are soluble, and CaO, which is slightly soluble
3. Hydroxides (OH^-) are usually *insoluble*. Exceptions include NaOH, KOH, $Sr(OH)_2$, and $Ba(OH)_2$, which are soluble, and $Ca(OH)_2$, which is slightly soluble
4. Chromates (CrO_4^{2-}) are usually *insoluble*. Exceptions include Na_2CrO_4, K_2CrO_4, $(NH_4)_2CrO_4$, and $MgCrO_4$
5. Phosphates (PO_4^{3-}) and carbonates (CO_3^{2-}) are usually *insoluble*. Exceptions include salts of the Na^+, K^+, and NH_4^+ ions

C=O···H–N hydrogen bond cannot provide stabilization in aqueous solutions. The competitive hydrogen bonding by H_2O lessens the thermodynamic stability of interamide hydrogen bonds.

Properties of Hydrogen Bonds

Hydrogen bonding of water molecules is of crucial importance because all life forms exist in an aqueous environment constituted about 70–80% intracellular water. Although the exact structure of liquid water is unknown, it appears that liquid water contains transient hydrogen-bonded networks. Water molecules are in rapid motion, constantly making and breaking hydrogen bonds with adjacent molecules. As the temperature of water increases toward 100 °C at atmospheric pressure, the kinetic energy of its molecules becomes greater than the energy of the hydrogen bonds connecting them, and the gaseous form of water appears.

Normally, a hydrogen atom forms a covalent bond with only one other atom as typically seen in hydrocarbon side chains. A hydrogen atom covalently bonded to a donor atom, D, may form an additional weak association, the hydrogen bond, with an acceptor atom, A:

$$D^{\delta-}-H^{\delta+} + :A^{\delta-} \rightleftharpoons D^{\delta-}-H^{\delta+}\cdots\cdots:A^{\delta-}$$

Hydrogen bond

For a hydrogen bond to form, the donor atom must be electronegative, so that the covalent D–H bond is polar. The acceptor atom also must be electronegative, and its outer shell must have at least one nonbonding pair of electrons that attracts the δ^+ charge of the hydrogen atom. In biological systems, both donors and acceptors are usually nitrogen or oxygen atoms, especially those atoms in amino ($-NH_2$) and hydroxyl (–OH) groups. Because all covalent N–H and O–H bonds are polar, their H atoms can participate in hydrogen bonds. By contrast, C–H bonds are nonpolar, so these H atoms are almost never involved in a hydrogen bond.

Liquid water molecules provide an excellent example of hydrogen bonding. The hydrogen atom in one water molecule is attracted to a pair of electrons in the outer shell of an oxygen atom in an adjacent molecule. Not only do water molecules hydrogen-bond with one another, they also form hydrogen bonds with other kinds of molecules as shown in Fig. 1.9. The presence of polar groups such as hydroxyl (–OH) or amino ($-NH_2$) groups enhances the solubility of molecules in

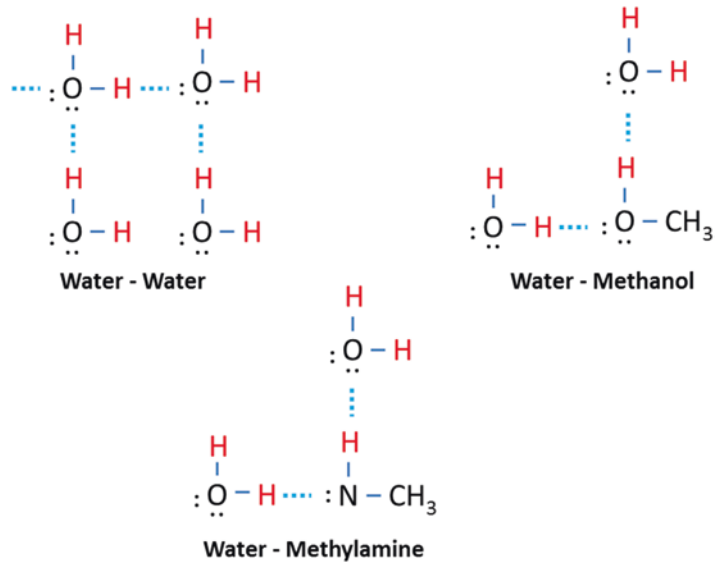


Fig. 1.9 Water readily forms hydrogen bonds with simple organic compounds

water. For instance, the hydroxyl group in methanol (CH_3OH) and the amino group in methylamine (CH_3NH_2) can form several hydrogen bonds with water, enabling the molecules to dissolve in water to high concentrations.

Most hydrogen bonds are 0.26 – 0.31 nm long, about twice the length of covalent bonds between the same atoms. The distance between the nuclei of the hydrogen and oxygen atoms of adjacent hydrogen-bonded molecules in water is approximately 0.27 nm, about twice the length of the covalent O–H bonds in water. The hydrogen atom is closer to the donor atom, D, to which it remains covalently bonded, than it is to the acceptor. The length of the covalent D–H bond is a bit longer than it would be if there were no hydrogen bonds, because the acceptor "pulls" the hydrogen away from the donor. The strength of a hydrogen bond in water (≈5 kcal/mol) is much weaker than a covalent O–H bond (≈110 kcal/mol) (Lodish et al. 2000) (http://www.ncbi.nlm.nih.gov/books/NBK21726/).

Hydrophobic Interactions

Water molecules surrounding an apolar or non-polar solutes are more ordered, leading to a loss in entropy. As a result, apolar groups in an aqueous environment tend to associate with each other rather than with the water molecules. This concept of a hydrophobic interaction has been schematically represented by Sa et al. (2013), as shown in Fig. 1.10. Under appropriate conditions apolar molecules can form crystalline hydrates, in which the compound is enclosed within the space formed by a polyhedron made up of water molecules. Such polyhedrons can form a large lattice, as indicated in Fig. 1.10. The polyhedrons may enclose apolar guest molecules to form apolar hydrates (Speedy 1984; Sa et al. 2013). These pentagonal polyhedra of water molecules are unstable and normally change to liquid water above 0 °C and to normal hexagonal ice below 0 °C. In some cases, the hydrates melt well above 30 °C. There is a remarkable similarity between the small apolar molecules that form these clathrate-like hydrates and the apolar side chains of proteins. Some of these are shown in Fig. 1.11. Because small molecules such as the ones shown in Fig. 1.11 can form stable water cages, it may be assumed that some of the apolar amino acid side chains in a polypeptide can do the same. The concentration of such side chains in proteins is high, and the combined effect of all these groups can be expected to result in the formation of a stabilized and ordered water region around the protein molecule. The stabilized water structure around a protein molecule are illustrated in Fig. 1.12.

The hydrophobic effect is responsible for the portioning in a mixture of oil and water, resulting

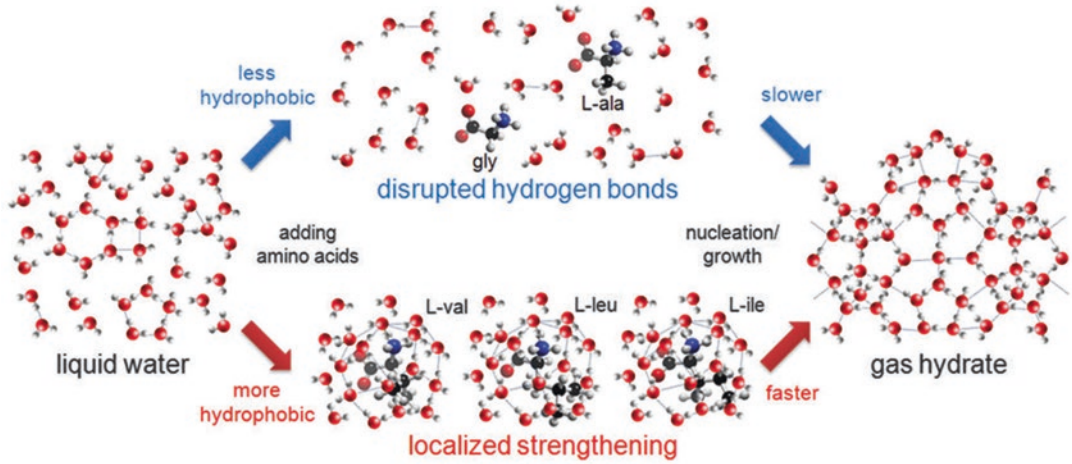


Fig. 1.10 Hydrophobic amino acids form different hydrates in water. http://www.nature.com/articles/srep02428#f5, Sa J-H, Kwak G-H, Lee BR, Park D-H, Han K, Lee K-H. (2013) Hydrophobic amino acids as a new class of kinetic inhibitors for gas hydrate formation. Scientific Reports 3: 2428

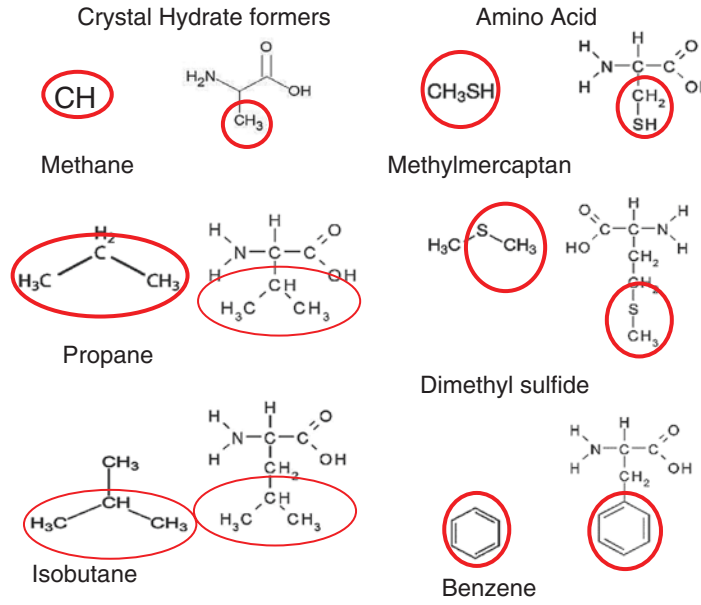


Fig. 1.11 Comparison of hydrate-forming molecules and amino acid apolar side chains

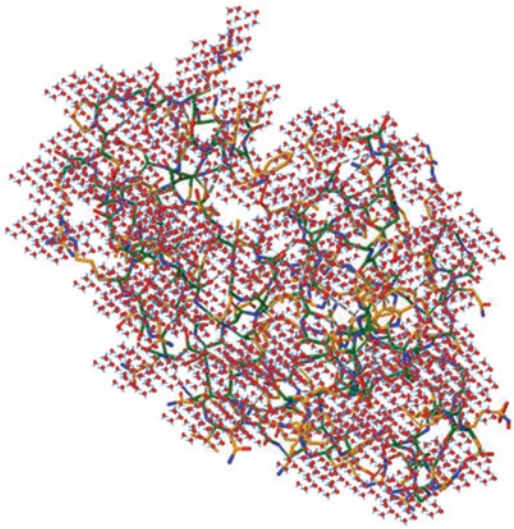


Fig. 1.12 Protein hydrated with water molecules stabilizing the three-dimensional structure of the protein

in separation into two distinct phases of oil and water. The hydrophobic effect is also seen in cell membranes providing the stability in the membrane network. It is also seen on the surface of milk fat globule membranes. Hydrophobic bonding in proteins facilitates protein folding as well as the insertion of membrane proteins into the nonpolar lipid environments (Kauzmann 1959; Charton and Charton 1982; Breiten et al. 2013; Lockett et al. 2013).

Water Activity and Sorption Phenomena

Water activity (a_w) is the partial vapor pressure of water in a substance divided by the partial vapor pressure of water at the same temperature. Essentially it is an expression of relative humidity surrounding the food system. The standard state vapor pressure is most often defined as the partial vapor pressure of pure water at the same temperature. The vapor pressure over pure distilled water has a water activity of exactly one. As temperature increases, a_w typically increases, except in some products with crystalline salt or sugar. **Water activity is a physical chemistry derived quantity. It applies to a system at equilibrium and it should always be referred to using a lower case a with subscript w, i.e., a_w.**

Controlling the water activity in foods is more critical to maintain quality and microbiological stability of the food. Low water activity inhibits microbial growth, provides textural characteristics such as crispness and crunchiness in products like snack foods and ready to eat breakfast cereals.

The sound produced by 'crunching' a breakfast cereal or a tortillas chip is lost if the a_w exceeds 0.65. Rates of chemical reactions increase hydrophobic regions but reduced in hydrophilic regions. The water activity must be carefully balanced to obtain optimum quality of dehydrated foods and intermediate moisture foods (Leake 2006). Foods with an $a_w > 0.95$ (equivalent to about 43% w/w sucrose) tend to be highly perishable and prone to microbial growth. Most bacteria growth is inhibited below about $a_w = 0.91$ (equivalent to about 57% w/w sucrose); similarly, most yeasts cease growing below $a_w = 0.87$ (equivalent to about 65% w/w sucrose) and most molds cease growing below $a_w > 0.80$ (equivalent to about 73% w/w sucrose). The lower limit of microbial growth is about $a_w = 0.6$.

Products with higher a_w tend to support more microbial growth. Bacteria usually require at least 0.91, and fungi at least 0.7. Water will migrate from areas of high a_w to areas of low a_w. For example, if a sugar syrup or honey has an $a_w \approx 0.6$ and the relative humidity of air above it is 0.85, the sugar solution will absorb moisture from the air. A consequence of this is that the surface could ultimately gain enough moisture for mold growth on the surface. Another example is that crackers which have an a_w of 0.3 are exposed to air with 80% relative humidity, the crackers will lose crispness and snap when eaten. If salami ($a_w \approx 0.87$) is exposed to dry air ($a_w w \approx 0.5$), the salami will lose moisture and could adversely impact texture or oxidative stability of the product.

Water activity does not correlate directly with water content of the food. It is related to the amount of soluble solids and their molecular size in the material thus sweetened condensed milk contains over 30% water and small size sugars but has the same a_w as flour or rice which contain around 12% moisture and starch and proteins as shown in Fig. 1.13.

Water activity influences many characteristics in foods that impact quality:

- a_w ~ 0.2–0.3 below this value reactions requiring water phase do not occur
- a_w ~ 0.2–0.3 below this rate of lipid oxidation increases
- a_w ~ 0.35–0.4 stickiness and caking are observed

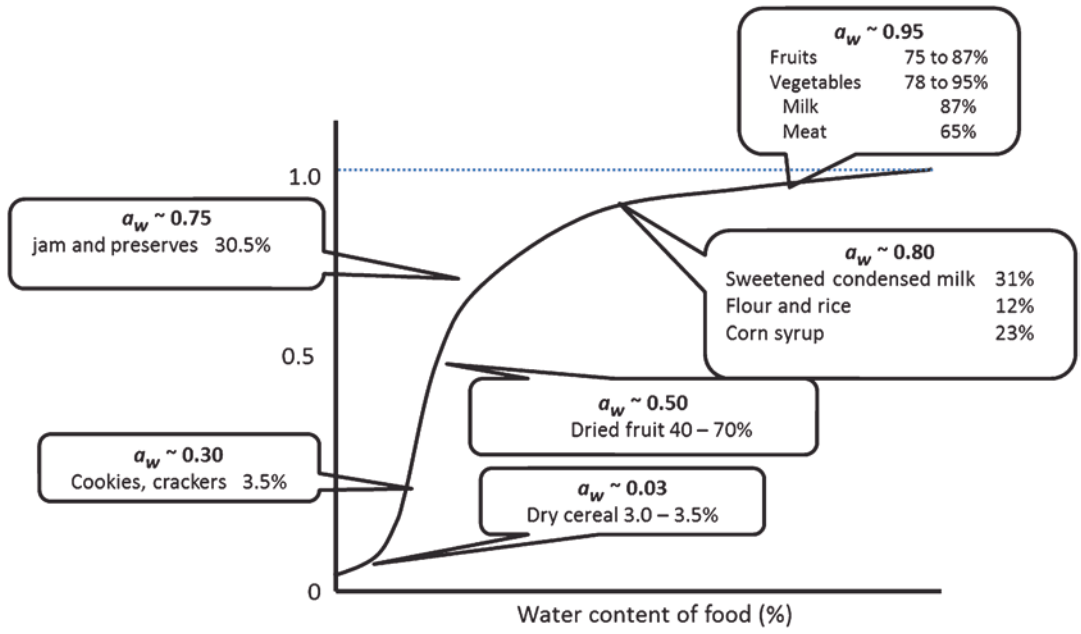


Fig. 1.13 Water activity in foods at different moisture contents

- a_w ~ 0.4–0.5 loss of crispness on gain of moisture
- a_w ~ 0.5 onset of hardening (e.g. raisins) on loss of moisture
- a_w ~ 0.6 onset of microbial growth
- a_w ~ 0.65–0.85 reaction rate in amorphous systems enzyme activity, lipid oxidation
- a_w ~ 0.85 onset of growth of bacterial pathogens.

Water activity (a_w) describes the effective water concentration for hydration of materials. Water activity of 1.0 indicates pure water and zero is the complete absence of water molecules. Mixing of solute lowers the water activity of the system when the system reaches equilibrium. Water activity has been reviewed for a variety of aqueous systems (Blandamer et al. 2005) and biological systems (Schiraldi et al. 2012). Water activity can be determined from the dew-point temperature of the atmosphere in equilibrium with the material (Mathlouthi 2001; Blandamer et al. 2005). A high a_w (>0.8) indicates a moist system and a low a_w (<0.7) indicates a 'dry' system. The nature of a hydrocolloid or protein polymer network can influence the water activity (Trombetta et al. 2005). The water activity of an aqueous solution in equilibrium with ice (a_w^i) is equal to the water vapor pressure over ice, to the water pressure over pure liquid water and does not depend on the solute's nature or concentration (Koop et al. 2000). Solutions with the same ice melting point therefore have the same water activity.

Figure 1.14 is a water activity isotherm displaying the hysteresis often encountered depending on whether the water is being added to the dry material or removed (drying) from the wet material. This hysteresis is due to non-reversible structural changes and non-equilibrium effects. There are many empirical equations but none predict with sufficient accuracy, therefore, water activity isotherm should be experimentally determined for each material.

Water activity is a property of aqueous solutions and materials that contain water from the liquid state to dry powders, a_w is defined as the ratio of the vapor pressures of pure water and a solution:

$$a_w = \frac{p}{p_o}$$

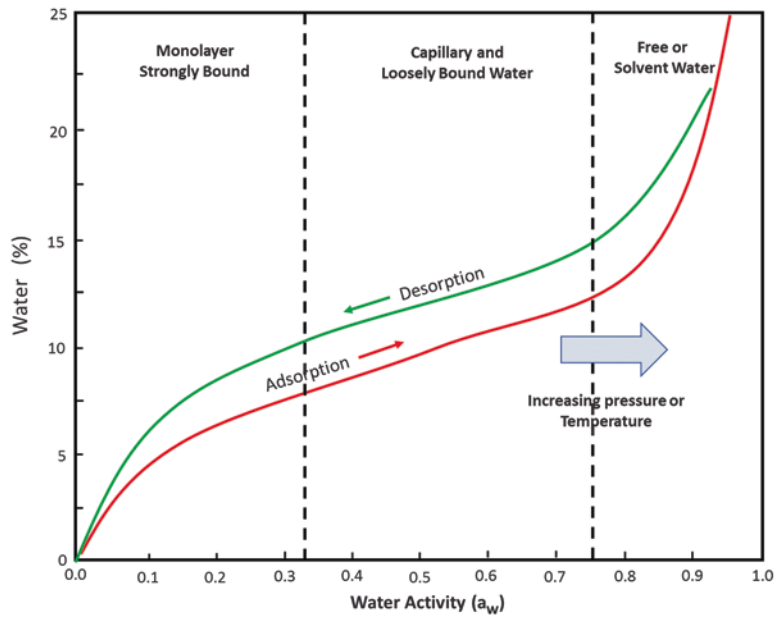


Fig. 1.14 Adsorption desorption isotherms as a function of water activity. http://www1.lsbu.ac.uk/water/water_activity.html

where a_w = water activity

p = partial pressure of water in a food

p_o = vapor pressure of water at the same temperature

According to Raoult's law, the lowering of the vapor pressure of a solution is proportional to the mole fraction of the solute: a_w can then be related to the molar concentrations of soute (n_1) and solvent (n_2):

$$a_w = \frac{p}{p_o} = \frac{n_1}{n_1 + n_2}$$

The extent to which a solute reduces a_w is a function of the chemical nature of the solute. The equilibrium relative humidity (ERH) in percentage is ERH/100. ERH is defined as:

$$ERH = \frac{p^{equ}}{p^{sat}}$$

where

p^{equ} = partial pressure of water vapor in equilibrium with the food at temperature T and 1 atmosphere total pressure

p^{sat} = the saturation partial pressure of water in air at the same temperature and pressure

At high moisture contents, when the amount of moisture in the product exceeds the soluble solids, the water activity is close to or equal to 1.0. When the moisture content is lower than the solids, water activity is lower than 1.0, as indicated in Fig. 1.13. Below moisture content of about 50% the water activity decreases rapidly and the relationship between water content and relative humidity is represented by the sorption isotherms. The adsorption and desorption processes are not fully reversible; therefore, a distinction can be made between the adsorption and desorption isotherms by determining whether a dry product's moisture levels are increasing, or whether the product's moisture is gradually lowering to reach equilibrium with its surroundings, implying that the product is being dried (Fig. 1.15). Generally, the adsorption isotherms are required for the observation of hygroscopic products, and the desorption isotherms are useful for investigation of the process of drying. A steeply sloping curve indicates that the material is hygroscopic (curve A, Fig. 1.15); a flat curve indicates a product that is not very sensitive to moisture (curve B, Fig. 1.15). Many foods show the type of curves given in Fig. 1.15C, where the first part of the curve is quite flat, indicating a low hygroscopicity, and the end of the curve is quite steep, indicating highly hygroscopic conditions. Such curves are typical for foods with high sugar or salt contents and low capillary adsorption. Such foods are hygroscopic. The reverse of this type of curve is rarely encountered. These curves show that a hygroscopic product or hygroscopic conditions can be defined as the case where a

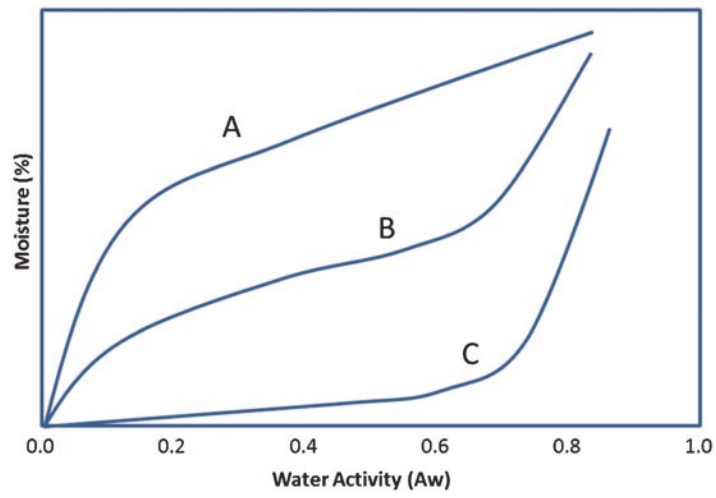


Fig. 1.15 Sorption isotherms of hygroscopic product (*A*) and non-hygroscopic product (*B*), high crystalline sugar or salt with low capillary absorption (*C*)

small increase in relative humidity causes a large increase in product moisture content.

Sorption isotherms usually have a sigmoid shape and can be divided into three areas that correspond to different conditions of the water present in the food (Fig. 1.16). The first part (A) of the isotherm, which is usually steep, corresponds to the adsorption of a monomolecular layer of water; the second, flatter part (B) corresponds to adsorption of additional layers of water; and the third part (C) relates to condensation of water in capillaries and pores of the material. There are no sharp divisions between these three regions, and no definite values of relative humidity exist to delineate these parts. Labuza (1968) has reviewed the various ways in which the isotherms can be explained. The kinetic approach is based on the Langmuir equation, which was initially developed for adsorption of gases and solids. This can be expressed in the following form:

$$\frac{a}{V} = \left[\frac{K}{bV_m}\right] + \frac{a}{V_m}$$

where

a = water activity

b = a constant

$K = 1/p_o$ and p_o = vapor pressure of water at T_o

V = volume adsorbed

V_m = monolayer value

When a/V is plotted versus a, the result is a straight line with a slope equal to $1/V_m$ and the monolayer value can be calculated. In this form, the equation has not been satisfactory for foods, because the heat of adsorption that enters into the constant b is not constant over the whole surface, because of interaction between adsorbed molecules, and because maximum adsorption is greater than only a monolayer.

A form of isotherm widely used for foods is the one described by Brunauer et al. (1938) and known as the BET isotherm or equation. A form of the BET equation given by Labuza (1968) is

$$\frac{a}{(1-a)V} = \frac{1}{V_m C} + \left[\frac{a(C-1)}{V_m C}\right]$$

where

C = constant related to the heat of adsorption

A plot of $a/(1 - a)V$ versus a gives a straight line, as indicated in Fig. 1.17 The monolayer coverage value can be calculated from the slope and the intercept of the line. The BET isotherm is only applicable for values of a from 0.1 to 0.5. In addition to monolayer coverage, the water surface area can be calculated by means of the following equation:

$$S_o = V_m \cdot \frac{1}{M_{H_2O}} \cdot N_o \cdot A_{H_2O}$$
$$= 3.5 \times 10^3 V_m$$

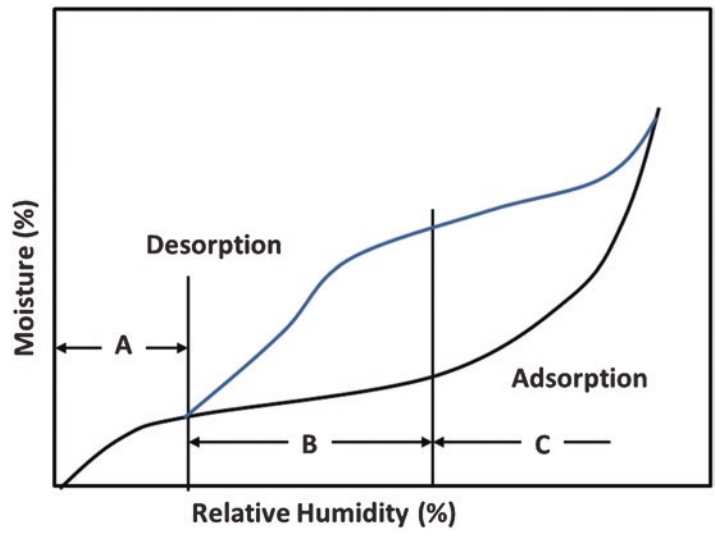


Fig. 1.16 Adsorption and desorption isotherms

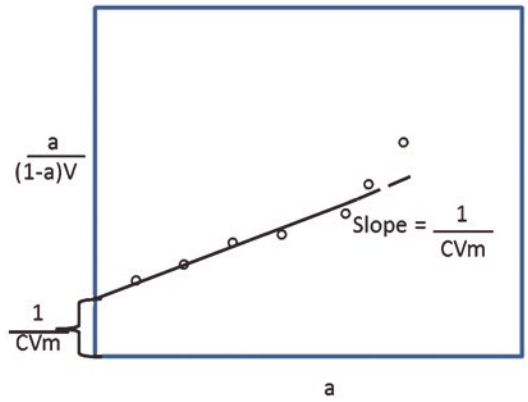


Fig. 1.17 BET monolayer plot. Adapted from Labuza (1968). A = a_w, V = Volume absorbed, C = heat of adsorption constant, V_m = monolayer value

where

S = surface area, m^2 / g solid

M_{H_2O} = molecular weight of water, 18

N_o = Avogadro's number, 6×10^{23}

A_{H_2O} = area of water molecule, 10.6×10^{20} m^2

The BET equation has been used in many cases to describe the sorption behavior of foods. For example, note the work of Saravacos (1967) on the sorption of dehydrated apple and potato. The form of BET equation used for calculation of the monolayer value was

$$\frac{p}{W\left(p_o - p\right)} = \frac{1}{W_1 C} + \frac{C-1}{W_1 C} \cdot \frac{P_o}{P}$$

where

W = water content (in percent)

p = vapor pressure of sample

p_o = vapor pressure of water at same temperature

C = heat of adsorption constant

W_1 = moisture consent corresponding to monolayer

Labuza (1968) described several approaches to analyze the sorption isotherms. The Langmuir isotherm as modified by Brunauer et al. (1938) has been most widely used with food products. The Langmuir isotherm assumes that there are a fixed number of sites where the sorption process can occur. The process continues until all the sites are filled. The BET theory assumes multiple layers will continue to form after the first monolayer is complete.

Another method to analyze the sorption isotherms is the GAB sorption model described by van den Berg and Bruin (1981) and used by Roos (1993) and Jouppila and Roos (1994). The use of the GAB model is described in Roos and Drusch (2015).

The best explanation for syneresis phenomenon appears to be the so-called ink bottle theory (Labuza 1968). It is assumed that the capillaries have narrow necks and large bodies, as represented schematically in Fig. 1.18. During adsorption the capillary does not fill completely until an activity is reached that corresponds to the large radius *R*. During desorption, the unfilling is controlled by the smaller radius *r*, thus lowering the water activity. Several other theories have been advanced to account for the hysteresis in sorption. These have been summarized by Kapsalis (1987).

The position of the sorption isotherms depends on temperature: the higher the temperature, the lower the position on the graph. This decrease in the amount adsorbed at higher temperatures follows the Clausius Clapeyron relationship,

$$\frac{d\left(\ln a\right)}{d\left(1/T\right)} = -\frac{Q_s}{R}$$

where

Q_s = heat of adsorption

R = gas constant

T = absolute temperature

By plotting the natural logarithm of activity versus the reciprocal of absolute temperature at constant moisture values, straight lines are obtained with a slope of $-Q_s/R$ (Fig. 1.19). The values of Q_s obtained in this way for foods having less than full monolayer coverage are between about 2000 and 10,000 cal/mol, demonstrating the strong binding of this water.

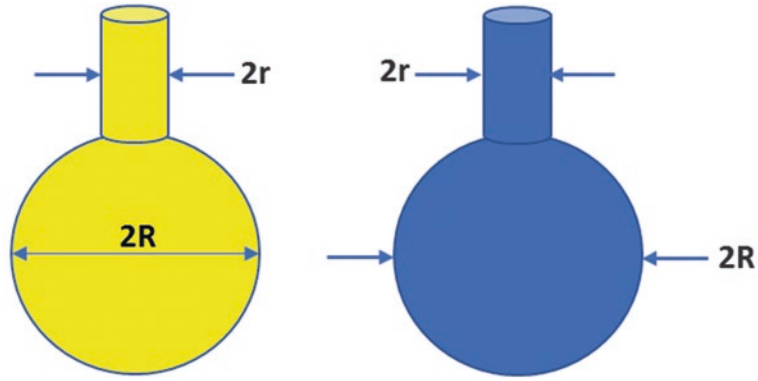


Fig. 1.18 Ink bottle theory of hysteresis in sorption. *Adapted from*: Labuza (1968)

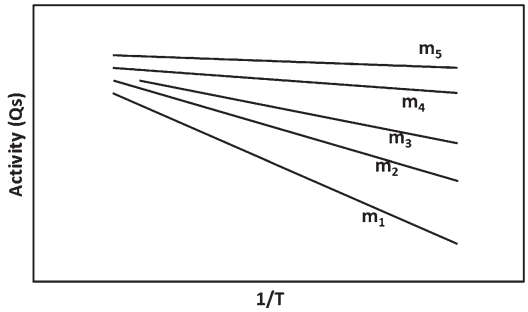


Fig. 1.19 Method for determination of heat of adsorption. Moisture content increases from M_1 to M_5. *Adapted from*: Labuza (1968)

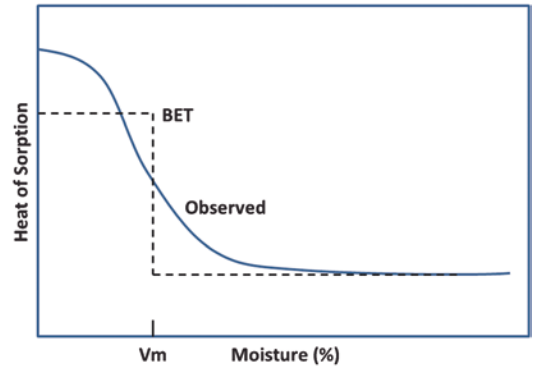


Fig. 1.20 Relationship of heat of sorption and moisture content as actually observed and according to BET Theory. Adapted from Labuza (1968)

According to the principle of BET isotherm, the heat of sorption Q_s should be constant up to monolayer coverage and then should suddenly decrease. Labuza (1968) has pointed out that the latent heat of vaporization ΔH_v, about 10.4 kcal/mol, should be added to obtain the total heat value. The plot representing BET conditions as well as actual findings are given in Fig. 1.20. The observed heat of sorption at low moisture contents is higher than theory indicates and falls off gradually, indicating the gradual change from Langmuir to capillary water.

The position of the sorption isotherms depends on temperature: the higher the temperature, the lower the position on the graph. This decrease in the amount adsorbed at higher temperatures follows the Clausius Clapeyron relationship,

$$\frac{d\left(\ln a\right)}{d\left(1/T\right)} = -\frac{Q_s}{R}$$

where
Q_s = heat of adsorption
R = gas constant
T = absolute temperature

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Types of Water

The sorption isotherm indicates that differently structured forms of water may be present in foods. It is convenient to divide the water into three types: Langmuir or monolayer water, capillary water, and loosely bound water. The strongly interacting water can be attracted strongly and held in a rigid and orderly state. In this state the water molecules have reduced solvent capacity and may not form ice. It is difficult to provide a rigid definition for water strongly interacting with food solids because much depends on the technique used for its measurement. There are two commonly used but not agreeable definitions for bound water as follows:

1. Bound water is the water that remains unfrozen below −20 °C (note that all water molecules can form ice but they may have strong thermodynamic interactions with solids and not show ice formation).
2. Bound water is the amount of water in a system that is unavailable as a solvent (note that all water molecules can act as solvent but their thermodynamic state may not favor solvent capacity).

The amount of unfrozen water, based on protein content, appears to vary only slightly from one food to another. About 8–10% of the total water in animal tissue may remain unfrozen (Meryman 1966). Egg white, egg yolk, meat, and fish all contain approximately 0.4 g of unfrozen water per gram of dry protein. This corresponds to 11.4% of total water in lean meat. Most fruits and vegetables contain less than 6% unfrozen water; whole grain corn, 34%. However, the present knowledge is that in most food materials an unfrozen maximally freeze-concentrated water-solids phase forms at a low but composition specific temperature. Such unfrozen phase typically contains 20% unfrozen water and 80% solids (Levine and Slade 1986; Roos and Drusch 2015).

The "free" or bulk water is sometimes determined by pressing a food sample between filter paper, by diluting with an added colored substance, or by centrifugation. None of these methods permits a distinct division between bulk and strongly hydrogen bonding water, and results obtained are not necessarily identical between methods. This is not surprising since the adsorption isotherm indicates that the division between the different forms of water is gradual rather than sharp. A promising new method is the use of nuclear magnetic resonance, which can be expected to give results based on the freedom of movement of the hydrogen nuclei.

The main reason for the increased water content at high values of water activity must be capillary condensation. A liquid with surface tension σ in a capillary with radius r is subject to a pressure loss, the capillary pressure $p_o = 2\sigma/r$, as evidenced by the rising of the liquid in the capillary. As a result, there is a reduction in vapor pressure in the capillary, which can be expressed by the Thomson equation,

$$\ln \frac{p}{p_o} = -\frac{2\sigma}{r} \cdot \frac{V}{RT}$$

where
p = vapor pressure of liquid
p_o = capillary vapor pressure
σ = surface tension
V = mole volume of liquid
R = gas constant
T = absolute temperature

This permits the calculation of water activity in capillaries of different radii, as indicated in Table 1.9. In water-rich foods, such as meat and potatoes, the water is present in part in capillaries with a radius of 1 μm or more. The pressure

Table 1.9 Capillary radius and water activity

Radius (nm)	Activity (a)
0.51	0.116
1	0.340
2	0.583
5	0.806
10	0.898
20	0.948
50	0.979
100	0.989
1000	0.999

Table 1.10 Pressure required to press water from tissue at 20 °C

Radius	Pressure in Pa
0.1 μm	140,003
1 μm	145,531
10 μm	14,553
0.1 mm	1455
1 mm	145.5

necessary to remove this water is small. Calculated values of this pressure are given in Table 1.10 for water contained in capillaries ranging from 0.1 μm to 1 mm radius. It is evident that water from capillaries of 0.1 μm or larger can easily drip out. Structural damage caused, for instance, by freezing can easily result in drip loss in these products. The fact that water serves as a solvent for many solutes such as salts and sugars is an additional factor in reducing the vapor pressure.

At low temperatures in some food systems water can remain unfrozen at ordinary pressures. Freezing point depression can be caused by: 1—equilibrium effects such as freezing point depression due to hydration or osmotic effects, 2—supercooling which is the delay or absence of formation of an ice crystal in the absence of efficient ice nucleators, 3—the high viscosity produced by high concentrations of solutes (Wolfe et al. 2002).

The freezing point of water can be lowered as a result of the osmotic effects of dissolved solutes such as salt, the hydration effects of macromolecules or ultrastructures, such as membranes. For each mole of soluble molecules (i.e. sugar) or ions (i.e. Na^+ or Cl^-) in kilogram of water, one lowers the equilibrium freezing point by (approximately) 2°. At equilibrium, freezing is a balance of minimizing energy and maximizing entropy. Molecules in ice have lower energy than liquid water, because of interaction with neighboring molecules, but they also have lower entropy because they cannot move about. Solutes are minimally soluble in ice, so the entropy of ice is unaffected by their presence. However, dissolved solutes in water increase the entropy of the water molecules which results in lowering energy and maximizing entropy at low temperatures (Wolfe et al. 2002).

The depression of freezing point by hydration occurs when water within 1 nm (0.000001 mm) of a hydrophilic surface freezes at a lower temperature than water. The closer to the surface, the lower the freezing temperature of the water. The water molecules closest to the surface, are very difficult to freeze or to remove. This is because the equilibrium hydration effect. The water near a surface is affected by that surface. The surface of biomolecules usually affects the energy and orientation of nearby water molecules. Some surfaces are charged or dipolar, and their electric field affects the orientation and energy of nearby water molecules. The molecules comprising the surface form hydrogen bonds with water disrupting the water near the surface. Most surfaces don't form hydrogen bonds with water, but they disrupt the water hydrogen bonds of the water near the surface (Wolfe et al. 2002).

This disturbance of the attracted water extends several molecular layers from a hydrophilic surface.

The increasing energy required to remove water molecules that are closer to the hydrophilic surface similarly gives rise to the greater freezing point depression. Undisturbed, bulk water freezes below its equilibrium freezing temperature (which depends on the solute concentration). The more strongly the water near a surface is held (i.e. the closer it is to the surface), the lower the temperature at which it freezes (Wolfe et al. 2002).

Freezing point depression also occurs in non-equilibrium systems such as super cooling. Small volumes of water, or large volumes of very pure water, can be cooled below 0 °C before they freeze. The water in biological systems is found in very small volumes, such as the interiors of cells and organelles, where supercooling occurs in processing such as freezing foods. Before freezing nuclei are formed and these nuclei must reach a minimum size for crystallization to occur. The size of an ice crystal decreases with sub-freezing temperature, but just below freezing it is large (Wolfe and Bryant 1999). The likelihood of a large number of molecules spontaneously forming ice crystals is small. Consequently, pure water can be cooled to well below zero (sometimes to −40 °C) before ice forms. Usually water will freeze at only a few degrees below zero. This is because a range of substances act as

ice nucleators: they act as surfaces on which an ice crystal can form an embryo for their growth. The more efficient the nucleator, and the more of them there are, the easier it is to freeze. Conversely, small volumes of very pure water can be supercooled most easily.

When some of the water in a system freezes, the solutes are concentrated in the remaining unfrozen water, producing more concentrated solutions. The increased concentration of solutes results in increased viscosity of the unfrozen water. In the more viscous solution water molecules diffuse and rotate more slowly making it less likely for ice nuclei to form and increased likelihood for supercooling to occur. At the same time water cannot quickly diffuse from the region near a hydrophilic surface to a region where ice has already started to form. Thus the amount of unfrozen water is higher than one would expect at equilibrium.

High viscosity and low temperature can produce a glass. A glass is a non-crystalline solid. Window glass is an example. Glasses are formed when the viscosity of a liquid becomes so high that it can resist shear stresses (resist changes in shape) for extremely long periods. When the water in cells forms a glass, diffusion, freezing and biochemistry are virtually stopped.

For a solute to cause significant freezing point depression it must be sufficiently soluble to achieve high concentrations in water. Supersaturation often occurs at freezing temperatures. For osmotic freezing point depression, the direct effect of small solutes is similar at low concentration. Provided that one counts dissociating solutes ($MgCl_2$ in solution is three solute ions), the freezing point depression is approximately proportional to concentration. At high concentration, the osmotic effects of salts may be less than proportional to concentration. Conversely, the osmotic effect of many solutes such as sugars increases at high concentration by more than simple proportionality (Wolfe and Bryant 2001).

The thermal behavior of water has been studied by Riedel (1959), who found that water in bread did not freeze at all when moisture content was below 18%. With this method it was possible to determine the unfrozen water. For bread, the value was 0.30 g/g dry matter, and for fish and meat, 0.40 g/g protein. The unfrozen and Langmuir water are probably not exactly the same. Wierbicki and Deatherage (1958) used a pressure method to determine free water in meat. The amount of free water in beef, pork, veal, and lamb varies from 30 to 50% of total moisture, depending on the kind of meat and the period of aging. A sharp drop in such water occurs during the first day after slaughter, and is followed by a gradual, slight increase. Hamm and Deatherage (1960b) determined the changes in hydration during the heating of meat. At the normal pH of meat there is a considerable reduction of bound water. It is important to note that "bound" water and "free" water may refer to water that can be mechanically removed from food and such definitions are not justified for quantification of water freezing. A new method to determine unfrozen water contents in multicomponent food materials was presented by Roos and Potes (2015).

Phase Diagram

A phase diagram illustrates what phases of a substance are present at any given temperature and pressure. The phases are simply the solid, liquid or vapor states of a pure substance. Figure 1.21 demonstrates a phase diagram of a pure substance

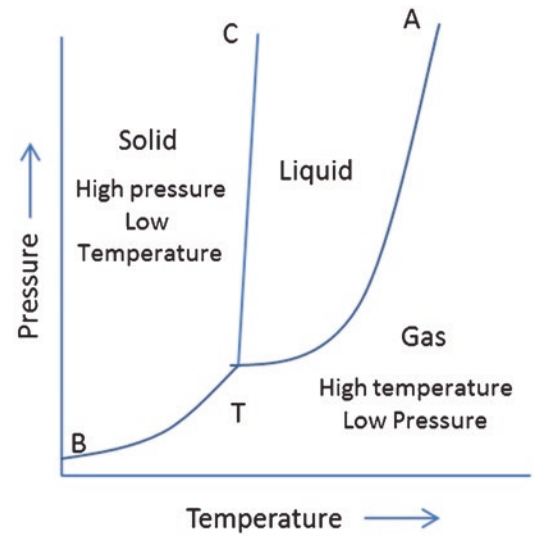

Fig. 1.21 Phase diagram of water. More detailed discussion: http://www1.lsbu.ac.uk/water/water_phase_diagram.html

such as water. The phase diagram indicates the existence of three phases: solid, liquid, and gas. The conditions under which they exist are separated by three equilibrium lines known as phase boundaries: the vapor pressure line TA, the melting pressure line TC, and the sublimation pressure line BT. The three lines meet at point T (triple point), where all three phases are in equilibrium. The diagram also illustrates that when ice is heated at pressures below 6.1 mbar, it changes directly into the vapor form. This is the basis of freeze drying.

The Glass Transition

In aqueous systems containing polymeric substances or some low molecular weight materials including sugars and other carbohydrates, lowering of the temperature may result in formation of a glass. A glass is an amorphous solid material rather than a crystalline solid. A glass is an supercooled liquid of high viscosity that exists in a metastable solid state (Levine and Slade 1999). A glass is formed when a liquid or an aqueous solution is cooled to a temperature that is considerably lower than its melting temperature. This is usually achieved at high cooling rates. The normal process of crystallization involves the conversion of a disordered liquid molecular structure to a highly ordered arrangement in a crystal. In a crystal, molecules, atoms or ions are arranged in a regular, three-dimensional array. In the formation of a glass, the disordered liquid state is immobilized into a disordered glassy solid, which has solid-like rheological properties but no ordered crystalline structure.

The relationships among melting point (T_m), glass transition temperature (T_g), and crystallization are schematically represented in Fig. 1.22. At low degree of supercooling (just below T_m), nucleation is at a minimum and crystal growth predominates. As the degree of supercooling increases, nucleation becomes the dominating effect. The maximum overall crystallization rate is at a point about halfway between T_m and T_g. At high cooling rates and a degree of supercooling that moves the temperature to below T_g, no crystals are formed and a glassy solid results. During the transition from the molten state to the glassy state, the moisture content plays an important role. This is illustrated by the phase diagram of Fig. 1.23. When the temperature is lowered at sufficiently high moisture content, the system goes through a rubbery state before becoming glassy (Chirife and Buera 1996). The glass transition temperature is characterized by very high

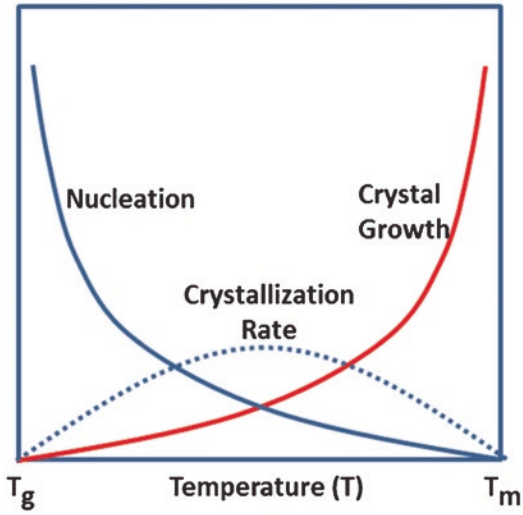

Fig. 1.22 Relationships among crystal growth, nucleation, and crystallization rate between melting temperature (T_m) and glass temperature (T_g)

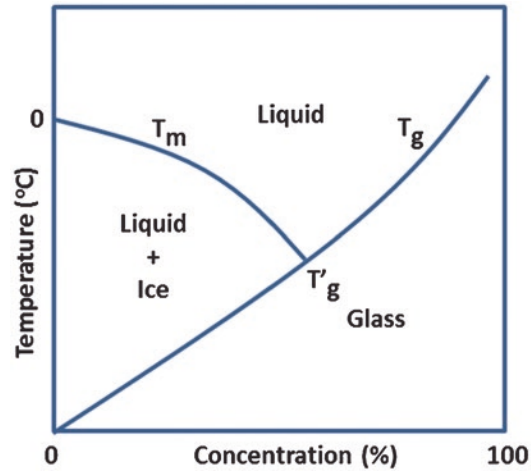

Fig. 1.23 Simplified state diagram showing the effect of moisture content on melting temperature (T_m) and glass transition temperature (T_g)

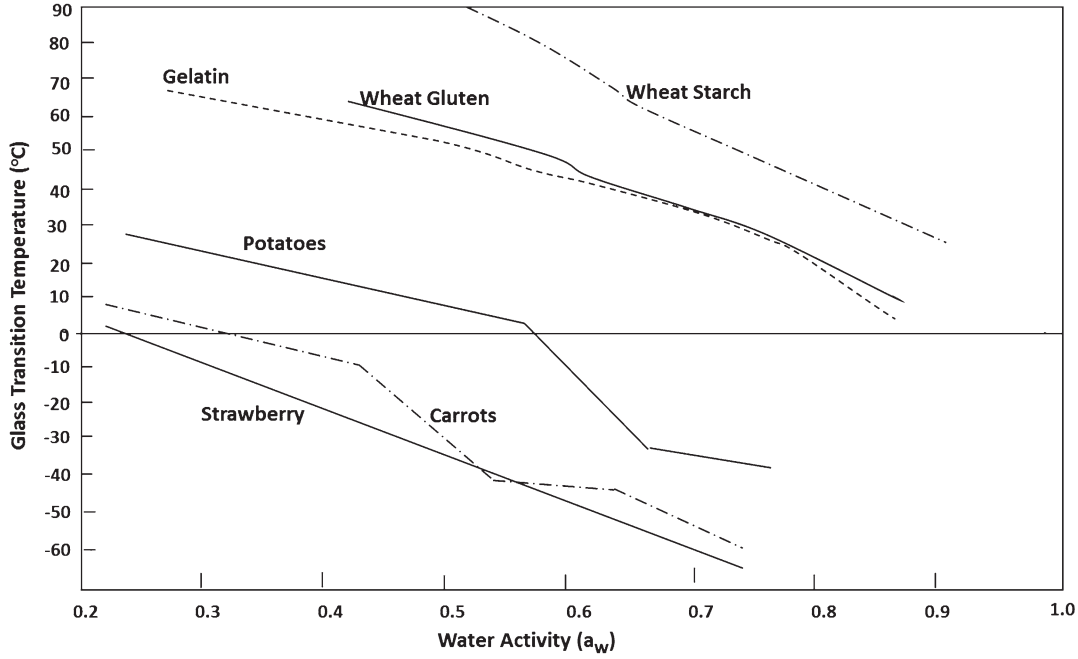

Fig. 1.24 Relationship between water activity (a_w) and glass transition temperature (T_g) of some plant materials and biopolymers. Source: Reprinted with permission from J. Cherife and M. del Pinar Buera, Water Activity, Water Glass Dynamics and the Control of Microbiological Growth in Foods, Critical Review Food Sci. Nutr., Vol. 36, No. 5, p. 490, ©1996. Copyright CRC Press, Boca Raton, Florida

apparent viscosities of more than 10^5 Pa s (Aguilera et al. 1990). The rate of diffusion limited processes is more rapid in the rubbery state than in the glassy state, and this may be important in the storage stability of certain foods. The effect of water activity on the glass transition temperature of a number of plant products (carrots, strawberries, and potatoes) as well as some biopolymers (gelatin, wheat gluten, and wheat starch) is shown in Fig. 1.24 (Chirife and Buera 1996). In the rubbery state the rates of chemical reactions appear to be higher than in the glassy state (Roos and Karel 1991d).

When water-containing foods are cooled to below the freezing point of water, ice may be formed and the remaining water is increasingly high in dissolved solids. When the glass transition temperature is reached, the freeze-concentrated solids with remaining unfrozen water is transformed into a glass. Ice formation during freezing may destabilize sensitive products by rupturing cell walls and breaking emulsions. The presence of glass-forming substances may help prevent this from occurring. Such stabilization of frozen products is known as *cryoprotection,* and the agents are known as *cryoprotectants.*

When water is removed from foods during processes such as extrusion, drying, or freezing, a glassy state may be produced (Roos and Drusch 2015). The T_g values of high molecular mass food polymers, proteins, and polysaccharides are high and cannot be determined experimentally, because of thermal decomposition. An example of measured T_g values for low molecular mass carbohydrates is given in Fig. 1.25. The value of T_g for starch is obtained by extrapolation.

Roos and Drusch (2015) used a combined sorption isotherm and state diagram to obtain critical water activity and water content values that result in depressing T_g to below ambient temperature (Fig. 1.26). This type of plot can be used to evaluate the stability of low-moisture foods under different storage conditions. When the T_g is decreased to below ambient temperature, molecules are mobilized because of plasticization and

reaction rates increase because of increased diffusion, which in turn may lead to deterioration. Roos and Himberg (1994) and Roos et al. (1996) have described how glass transition temperatures influence nonenzymatic browning in model systems. This deteriorative reaction showed an increased reaction rate as water content increased.

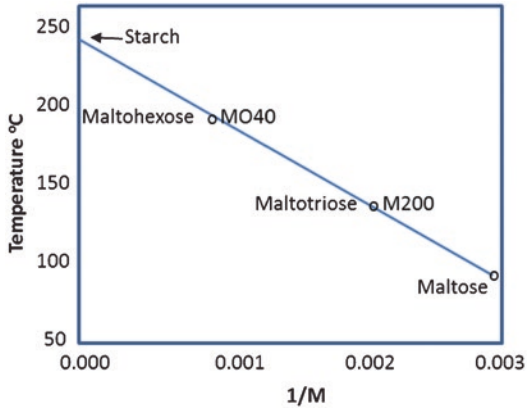

Fig. 1.25 Glass transition temperature (T_g) for maltose, maltose polymers, and extrapolated value for starch. M indicates molecular weight. Adopted from from Y.H. Roos, Glass Transition-Related Physico-Chemical Changes in Foods, Food Technology, Vol. 49, No. 10, p. 98, © 1995, Institute of Food Technologists

Carbohydrate polymers with different molecular mass have different glass transition temperatures. Figure 1.27 illustrates the glass transition temperature (T_g) of different starch hydrolysates at different moisture contents. From the figure it can be seen that the T_g decreases with decreasing molecular weight. All of the starches demonstrate the reduction of T_g with increasing water content.

The water present in foods may act as a plasticizer. Plasticizers increase plasticity and flexibility of food polymers as a result of weakening of the intermolecular forces existing between molecules. Increasing water content decreases T_g. Roos and Karel (1991a) studied the plasticizing effect of water on thermal behavior and crystallization of amorphous food models. They found that dried foods containing sugars behave like amorphous materials, and that small amounts of water decrease T_g to room temperature with the result of structural collapse and formation of stickiness. Roos and Karel (1991d) reported a linearity between water activity (a_w) and T_g over the a_w range of 0.1–0.8. This allows prediction of T_g at the a_w range typical of dehydrated and intermediate moisture foods.

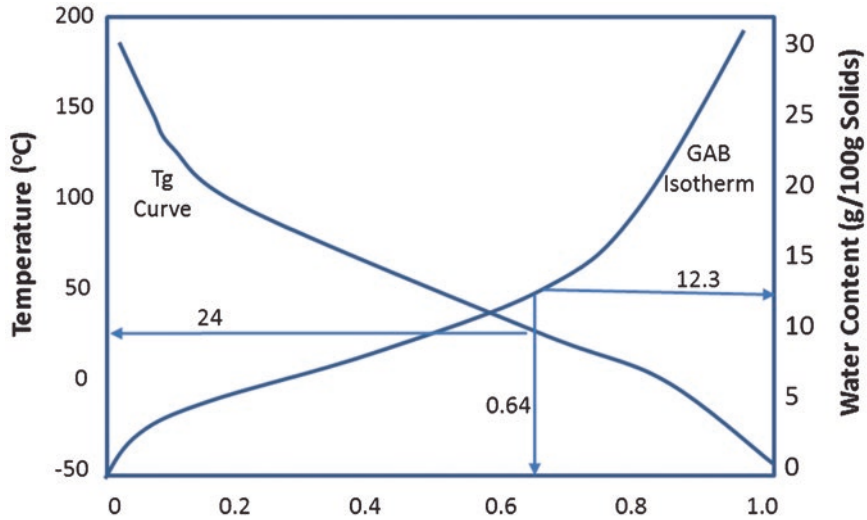

Fig. 1.26 Modified state diagram showing relationship between glass transition temperature (T_g), water activity (GAB isotherm), and water content for an extruded snack food model. Crispness is lost as water plasticization depresses T_g to below 24 °C. Plasticization is indicated with critical values for water activity and water content. Adapted from: Y.H. Roos, Glass Transition-Related Physico-Chemical Changes in Foods, Food Technology, Vol. 49, No. 10, p. 99, © 1995, Institute of Food Technologists

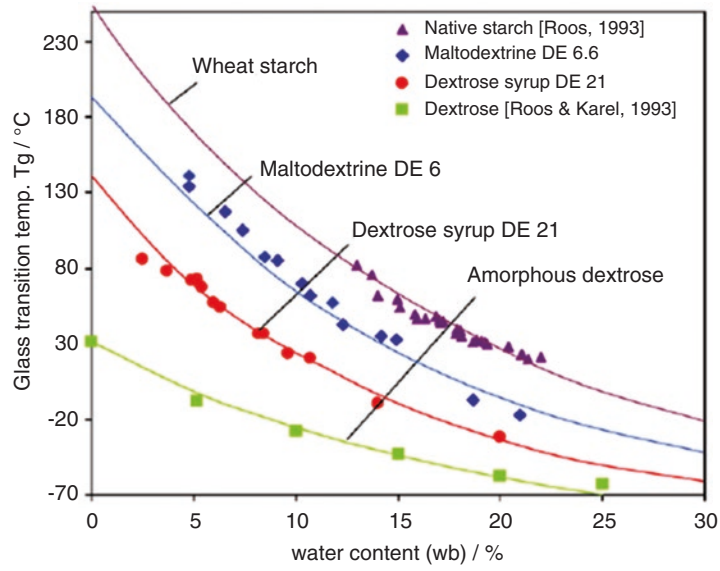

Fig. 1.27 Glass transition temperature of starch, maltodextrin, dextrose syrup and dextrose as a function of the water content. Palzer, S. (2009) Influence of material properties pm the agglomeration of water soluble particles. Powder Technology 189:318

Water Activity and Reaction Rate

Water activity has a profound effect on the rate of many chemical reactions in foods and on the rate of microbial growth (Labuza 1980). This information is summarized in Table 1.11. Enzyme activity is virtually nonexistent in the monolayer water (a_w between 0 and 0.2). Not surprisingly, growth of microorganisms at this level of a_w is also virtually zero. Molds and yeasts start to grow at a_w between 0.7 and 0.8, the upper limit of capillary water. Bacterial growth takes place when a_w reaches 0.8, the limit of loosely bound water. Enzyme activity increases gradually between a_w of 0.3 and 0.8, then increases rapidly in the loosely bound water area (a_w 0.8–1.0). Hydrolytic reactions and non-enzymic browning do not proceed in the monolayer water range of a_w (0.0–0.25). However, lipid oxidation rates are high in this area, passing from a minimum at a_w 0.3–0.4, to a maximum at a_w 0.8. The influence of a_w on chemical reactivity has been reviewed by Leung (1987). The relationship between water activity and rates of several reactions and enzyme activity is presented graphically in Fig. 1.28 (Bone 1987).

Table 1.11 Reaction rates in foods as determined by water activity

Reaction	Monolayer water	Capillary water	Loosely bound water
Enzyme activity	Zero	Low	High
Mold growth	Zero	Low[a]	High
Yeast growth	Zero	Low[a]	High
Bacterial growth	Zero	Zero	High
Hydrolysis	Zero	Rapid increase	High
Nonenzymic browning	Zero	Rapid increase	High
Lipid oxidation	High	Rapid increase	High

[a]Growth starts at a_w of 0.7–0.8

Water Activity and Food Spoilage

The influence of water activity on food quality and spoilage is well established and widely recognized as an important factor (Rockland and Nishi 1980). Moisture content and water activity affect the progress of chemical and microbiological spoilage reactions in foods. Dried or freeze-dried foods, which have great storage stability,

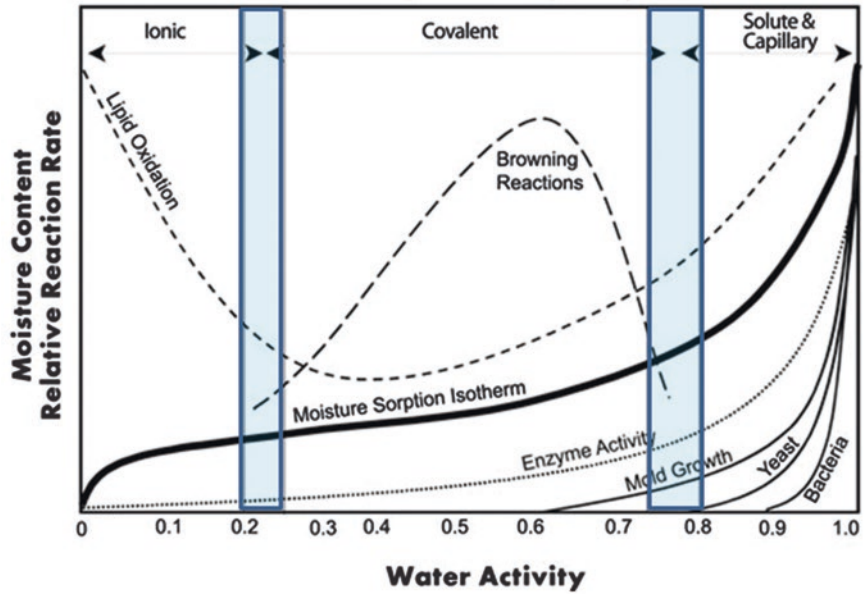

Fig. 1.28 Relationship between water activity and a number of reaction rates. http://www.aqualab.com/education/measurement-of-water-activity-for-product-quality/

usually have water contents in the range of about 5–15%. The group of intermediate-moisture foods, such as dates and cakes, may have moisture contents in the range of about 20–40%. The dried foods correspond to the lower part of the sorption isotherms. This includes water in the monolayer and multilayer category. Intermediate-moisture foods have water activities generally above 0.5, including the capillary water. Reduction of water activity can be obtained by drying or by adding water-soluble substances, such as sugar to jams or salt to pickled preserves. Bacterial growth is virtually impossible below a water activity of 0.90. Molds and yeasts are usually inhibited between 0.88 and 0.80, although some osmophile yeast strains grow at water activities down to 0.65.

Uncontrolled enzyme activity in intermediate moisture foods can have detrimental effects on quality.

Most enzymes are inactive when the water activity falls below 0.85. Such enzymes include invertases, amylases, phenoloxidases, peroxidases, lipoxygenases and lipases (see Table 1.12). All of these can adversely affect product quality. Lipase can retain activity as at a_w values as low as 0.3 or sometimes 0.1 (Loncin et al. 1968). Acker (1969) provided examples of the effect of water activity on some enzymatic reactions. Practical observations of lipase and lipoxygenase activity can be observed in soy flour, whole grain wheat flours, crackers and peanuts where rancid aroma is observed in otherwise sable products with a_w of 0.3 or lower. Table 1.12 contains the minimum a_w and temperatures for enzyme activity. The low water activity for lipases is taken advantage of to produce interesterified lipids in non-aqueous systems.

Acker found that for reactions in which lipolytic enzyme activity was measured, the manner in which components of the food system were put into contact significantly influenced the enzyme activity. Separation of substrate and enzyme could greatly retard the reaction. Also, the substrate has to be in liquid form; for example, liquid oil could be hydrolyzed at water activity as low as 0.15, but solid fat was only slightly hydrolyzed. Oxidizing enzymes were affected by water activity in about the same way as hydrolytic

Table 1.12 Minimum a_w values for enzyme activity in selected foods and model systems

Product/substrate	Enzyme	T (°C)	a_w threshold
Grains	Phytases	20	0.90
Wheat germ	Glucoside-hydrolases	20	0.20
Rye flour	Amylases Proteases	30	0.75
Macaroni	Phospholipases	25-30	0.45
Wheat flour dough	Proteases	35	0.96
Bread	Amylases Proteases	30	0.36
Starch	Amylases	37	0.40/0.75
Potato	Polyphenoloxidase	25	0.25
Galactose	Galactosidase	30	0.40/0.60
Olive oil	Lipase	5-40	0.025
Triolein, trilaurin	Phospholipases	30	0.45
Glucose	Glucose oxidase	30	0.40
Linoleic acid	Lipoxygenase	25	0.50/0.70
Casein	Trypsin	30	0.50

Adapted from Drapon (1985). Potato data from Acker (1969)

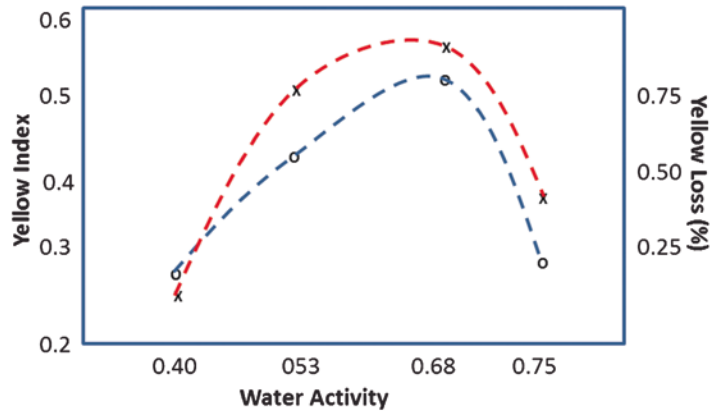

Fig. 1.29 Color change of milk powder (*blue color dashed line*) and loss of free lysine in milk powder (*red color dashed line*) kept at 40 °C for 10 days as a function of water activity. Data adopted from: From M. Loncin, J.J. Bimbenet, and J. Lenges, Influence of the Activity of Water on the Spoilage of Foodstuffs, *J. Food Technol.*, Vol. 3, pp. 131–142, 1968

enzymes, as was shown by the example of phenoloxidase from potato.

Nonenzymatic browning or Maillard reactions are one of the most important factors causing spoilage in foods. These reactions are strongly dependent on water activity and reach a maximum rate at *a* values of 0.6–0.7 (Loncin et al. 1968). This is illustrated by the browning of milk powder kept at 40 °C for 10 days as a function of water activity (Fig. 1.29). The loss in lysine resulting from the browning reaction parallels the color change, as is shown in Fig. 1.29.

Labuza et al. (1970) have shown that, even at low water activities, sucrose may be hydrolyzed to form reducing sugars that may take part in browning reactions. Browning reactions are usually slow at low humidities and increase to a maximum in the range of intermediate-moisture foods. Beyond this range the rate again decreases. This behavior can be explained by the fact that, in the intermediate range, the reactants are all dis-

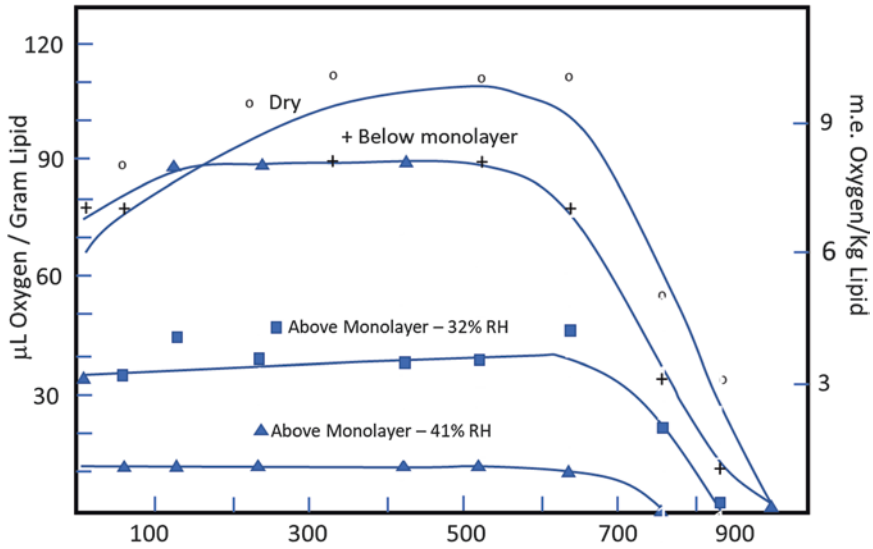

Fig. 1.30 Peroxide production in freeze-dried salmon stored at different relative humidities. *Source*: From F. Martinez and T.P. Labuza, Effect of Moisture Content on Rate of Deterioration of Freeze-Dried Salmon, *J. Food Sci*., Vol. 33, pp. 241–247, 1968

solved, and that further increase in moisture content leads to dilution of the reactants.

The effect of water activity on oxidation of fats is complex. Storage of freeze-dried and dehydrated foods at moisture levels above those giving monolayer coverage appears to give maximum protection against oxidation. This has been demonstrated by Martinez and Labuza (1968) with the oxidation of lipids in freeze-dried salmon (Fig. 1.30). Oxidation of the lipids was reduced as water content increased. Thus, conditions that are optimal for protection against oxidation may be conducive to other spoilage reactions, such as browning.

Other reactions that may be influenced by water activity are hydrolysis of protopectin, splitting and demethylation of pectin, autocatalytic hydrolysis of fats, and the transformation of chlorophyll into pheophytin (Loncin et al. 1968).

Water Activity and Packaging

Because water activity is a major factor influencing the keeping quality of a number of foods, it is obvious that packaging can do much to maintain optimal conditions for long storage life. Sorption isotherms play an important role in the selection of packaging materials. Hygroscopic products always have a steep sorption isotherm and reach the critical area of moisture content before reaching external climatic conditions. Such foods have to be packaged in glass containers with moisture-proof seals or in watertight plastic (thick Polyvinylchloride). For example, consider instant coffee, where the critical area is at about 50% RH. Under these conditions the product cakes and loses its flowability. Other products might not be hygroscopic and no unfavorable reactions occur at normal conditions of storage. Such products can be packaged in polyethylene containers.

There are some foods where the equilibrium relative humidity is above that of the external climatic conditions. The packaging material then serves the purpose of protecting the product from moisture loss. This is the case with processed cheese and baked goods.

Different problems may arise in composite foods, such as soup mixes, where several distinct ingredients are packaged together. In Fig. 1.31, for example, substance B with the steep isotherm is more sensitive to moisture, and is mixed in

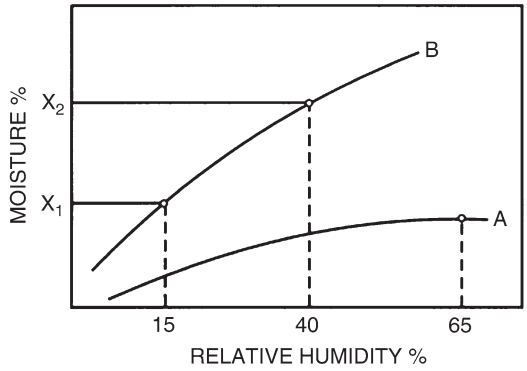

Fig. 1.31 Sorption isotherms of materials A and B

equal quantities with substance A in an impermeable package. The initial moisture content of B is X_1 and after equilibration with A, the moisture content is X_2. The substances A and B will reach a mean relative humidity of about 40%, but not a mean moisture content. If this were a dry soup mix and the sensitive component was a freeze-dried vegetable with a moisture content of 2% and the other component, a starch or flour with a moisture content of 13%, the vegetable would be moistened to up to 9%. This would result in rapid quality deterioration due to nonenzymatic browning reactions. In this case, the starch would have to be postdried.

Salwin and Slawson (1959) found that stability in dehydrated foods was impaired if several products were packaged together. A transfer of water could take place from items of higher moisture-vapor pressure to those of lower moisture-vapor pressure. These authors determined packaging compatibility by examining the respective sorption isotherms. They suggested a formula for calculation of the final equilibrium moisture content of each component from the isotherms of the mixed food and its equilibrium relative humidity:

The initial relative humidity of A is 65% and of B, 15%.

$$a_w = \frac{\left(W_1 \cdot S_1 \cdot a_{w1'}\right) + \left(W_2 \cdot S_2 \cdot a_{w2'}\right)}{\left(W_1 \cdot S_1\right) + \left(W_2 \cdot S_2\right)}$$

where

W_1 = gram solids of ingredient 1
S_1 = linear slope of ingredient 1
$a_{w1'}$ = initial a_w of ingredient 1

Water Activity and Food Processing

Water activity is one of the criteria for establishing good manufacturing practice (GMP) regulations governing processing requirements and classification of foods (Johnston and Lin 1987). Process requirements for foods are governed by a_w and pH; a_w controlled foods are those with pH greater than 4.6 and a_w less than 0.85. At pH less than 4.6 and a_w greater than 0.85, foods fall into the category of low-acid foods; when packaged in hermetically sealed containers, these foods must be processed to achieve commercially sterile conditions.

Intermediate moisture foods are in the a_w range of 0.90–0.60. They can achieve stability by a combination of a_w with other factors, such as pH, heat, preservatives, and E_h (equilibrium relative humidity).

The a_w level of 0.85 is applied as a point for determining whether a low-acid canned food or an acidified food is covered by FDA regulations. Low-acid canned foods can be preserved by controlling water activity at levels above 0.85. The minimum a_w level for the growth of *C. botulinum* is approximately 0.93. The regulations (21 CFR 113.3(e) (1) (ii)) state that "commercial sterility can be achieved by the control of water activity and the application of heat. The heat is generally necessary at a_w levels above 0.85 to destroy vegetative cells of microorganisms of public health significance (e.g., *staphylococci*) and spoilage microorganisms which can grow in a reduced a_w environment". (See the following other sections of the regulations which deal with a_w controlled products:

21 CFR 113.10—Attendance at an approved school giving instruction appropriate to the preservation technology involved.
21 CFR 113.40(i)—Equipment and procedures for thermal processing of foods where critical factors such as water activity are used.
21 CFR 113.81(f)—Additional factors to be controlled to prevent the growth of microoganisms not destroyed by the thermal process.
21 CFR 113.100(a) (6)—Record keeping requirements for a_w determinations.

Low-acid canned foods (LACF) include foods with a finished equilibrium pH greater than 4.6

and a water activity greater than 0.85. This excludes tomatoes and tomato products having a finished equilibrium pH less than 4.7.

Acidified foods (AF) are low-acid food to which acid(s) or acid food(s) have been added and with a finished equilibrium pH of 4.6 or below and a water activity (a_w) greater than 0.85.

Some examples of water activity controlled low-acid canned foods, that may have an a_w of greater than 0.85, are: canned cake, bread, bean paste, some chutney, salted vegetables, salted fish, guava paste, lupini beans, syrup, toppings, puddings, and some oriental specialty sauces. Water activity in these products can be controlled by the use of salt or sugar. Some examples of foods with a range of water activity are included in Table 1.13.

Table 1.13 Approximate a_w values of plant and animal food categories

Animal products	a_w
Fresh meat, poultry, fish	0.99–1.00
Natural cheeses	0.95–1.00
Pudding	0.97–0.99
Eggs	0.97
Cured meat	0.87–0.95
Sweetened condensed milk	0.83
Parmesan cheese	0.68–0.76
Honey	0.75
Dried whole egg	0.40
Dried whole milk	0.20
Plant products	a_w
Fresh fruits, vegetables	0.97–1.00
Bread	~0.96
Bread, white	0.94–0.97
Bread, crust	0.30
Baked cake	0.90–0.94
Maple syrup	0.85
Jam	0.75–0.80
Jellies	0.82–0.94
Uncooked rice	0.80–0.87
Fruit juice concentrates	0.79–0.84
Fruit cake	0.73–0.83
Cake icing	0.76–0.84
Flour	0.67–0.87
Dried fruit	0.55–0.80
Cereal	0.10–0.20
Sugar	0.19
Crackers	0.10

http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm094145.htm

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Lipids

2

John W. Finley and John M. deMan

It is difficult to provide a clear and comprehensive definition for the class of substances called lipids. Early definitions were mainly based on whether the substance is soluble in organic solvents like ether, benzene, or chloroform and is not soluble in water. In addition, definitions usually emphasize the presence of fatty acids. Every definition proposed so far has some limitations. For example, monoglycerides of the short-chain fatty acids are undoubtedly lipids, but they would not fit the definition on the basis of solubility because they are more soluble in water than in organic solvents. Instead of trying to find a definition that would include all lipids, it is better to provide a scheme describing the lipids and their components, as illustrated in Fig. 2.1 shows. The basic components of lipids are listed in the central column with the fatty acids occupying the prominent position. The left column lists the lipids known as phospholipids. The right column of the diagram includes the compounds most important from a quantitative standpoint in foods. These are mostly esters of fatty acids and glycerol. Up to 99% of the lipids in plant and animal material consist of such esters, known as fats and oils.

The fat content of foods can range from very low to very high in both vegetable and animal products, as indicated in Table 2.1. In nonmodified foods, such as meat, milk, cereals, and fish, the lipids are mixtures of many of the compounds listed in Fig. 2.1, with triglycerides comprising the major portion. The fats and oils used for making fabricated foods, such as margarine and shortening, are almost pure triglyceride mixtures. Fats are sometimes divided into visible and invisible fats. In the United States, about 60% of total fat and oil consumed consists of invisible fats—that is, those contained in dairy products (excluding butter), eggs, meat, poultry, fish, fruits, vegetables, and grain products. The visible fats, including lard, butter, margarine, shortening, and cooking oils, account for 40% of total fat intake. The interrelationship of most of the lipids is represented in Fig. 2.1. A number of minor components, such as hydrocarbons, fat-soluble vitamins, and pigments are not included in this scheme.

Fats and oils may differ considerably in composition, depending on their origin. Both fatty acid and glyceride composition may result in different properties. Fats and oils can be classified broadly as of animal or vegetable origin. Animal fats can be further subdivided into mammal depot fat (lard and tallow) and milk fat (ruminant) and marine oils (fish and whale oil). Vegetable oils and fats can be divided into seed oils (such as soybean, canola), fruit coat fats (palm and olive oils), and kernel oils (coconut and palm kernel).

The scientific names for esters of glycerol and fatty acids is acylglycerols. Triacylglycerols, diacylglycerols, and monoacylglycerols which have three, two, or one fatty acid ester linkages respectively. The common names for these compounds are glycerides, e.g. triglycerides, diglycerides,

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_2

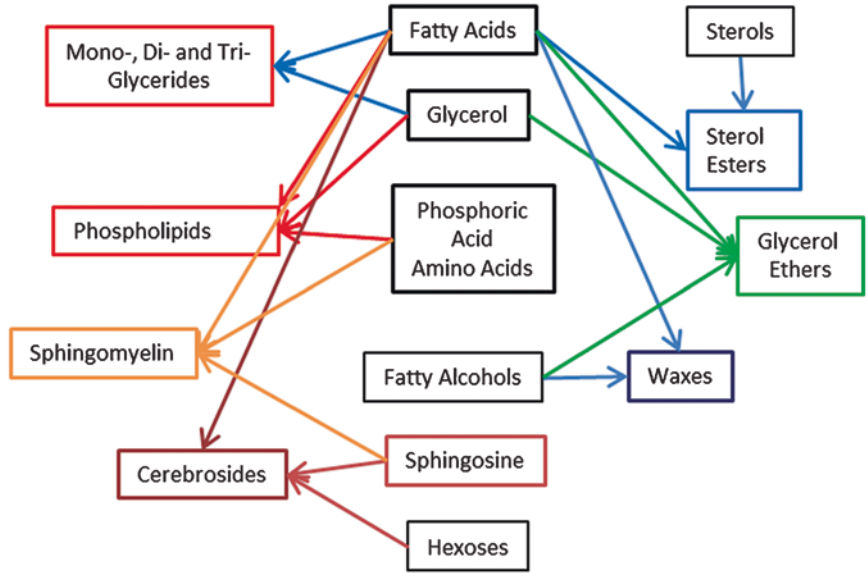


Fig. 2.1 Interrelationships of lipids

Table 2.1 Composition of Lipids in various foods expressed as % of total lipids (Belitz et al. 2009)

	Milk	Soya	Wheat	Apple
Total lipids	3.6	23	1.5	0.088
Triacyglycerols	94	88	41	5
Mono-, Diacylglycerol	1.5		1	
Sterol	<1		1	15
Steroid Ester			1	2
Phospholipids	1.5	10	20	47
Glycolipids		1.5	29	17
Sulfolipids				1
Others		0.54	7	15

and monoglycerides. The scientific and common names are used interchangeably in the literature, and this practice is followed in this book.

Lipids are organic molecules generally formed by the esterification of an fatty acid to an alcohol. The simplest definition is a lipid as any molecule that is insoluble in water and soluble in organic solvents. Most lipids are soluble to some extent in organic solvents such as hexane, ether, chloroform or benzene. They constitute a complex collection organic compounds that include fatty acids, (A,D, E and K). Lipids have many important functions in vivo and in foods. The diversity of some of the most abundant lipid structures is illustrated in Fig. 2.2.

There is no single common structure for lipids, however, the most abundant group of lipids in foods are triglycerides, which are commonly grouped as fats and oils. Glycerides have a glycerol backbone appended with from one to three fatty acids through ester linkages. Triglycerides are found in most plant, animal and microbial tissue. They serve as energy storage or energy sources and are included in membranes. In animals triglycerides are found in all tissues to varying degrees.

Table 2.2 includes the principal lipid fractions found in some common foods. The data illustrate that most foods contain both triglycerides and phospholipids. The compositions very widely, the contents of cholesterol, saturated fat and trans-fat in foods have raised some health concerns.

Shorthand Description of Fatty Acids and Glycerides

To describe the composition of fatty acids it is sometimes useful to use a shorthand designation. In this convention the composition of a fatty acid can be described by two numbers separated by a colon. The first number indicates the

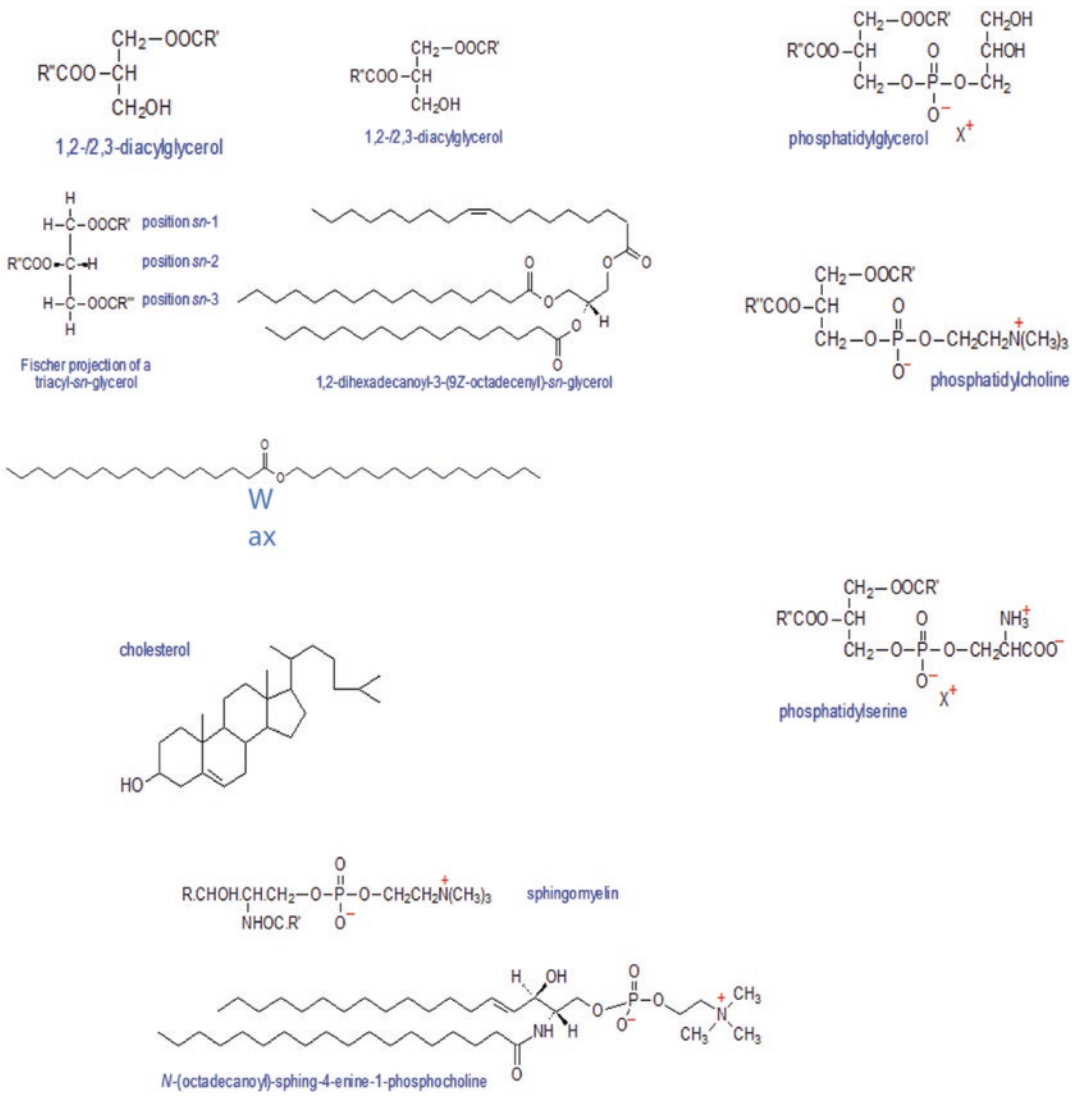


Fig. 2.2 Major lipid structures

Table 2.2 Fat contents of some foods

Food	Total fat g/100 g	Saturated Fat g/100 g	Mono- g/100 g	Poly- g/100 g	Trans g/100 g	Cholesterol mg/100 g
Cooked rice	0.370	0.074	0.074	0.091	0	0
Macaroni cooked	0.930	0.176	0.131	0.319	0	0
Instant Oat Meal	1.360	0.226	0.391	0.426	0	0
Miniwheats	2.000	0.500	0.300	1.100	0	0
Whole Wheat Bread	3.500	0.722	0.620	1.592	0	0
Cooked Asparagas	0.220	0.048	0.000	0.105	0	0
Pinto Beans	0.480	0.058	0.035	0.276	0	0

(continued)

Table 2.2 (continued)

Food	Total fat g/100 g	Saturated Fat g/100 g	Mono- g/100 g	Poly- g/100 g	Trans g/100 g	Cholesterol mg/100 g
Carrots	0.180	0.030	0.006	0.089	0	0
Sweet corn	1.500	0.197	0.374	0.603	0	0
Almonds	49.930	3.802	31.551	12.329	0	0
English Walnuts	65.210	6.126	8.933	55.194	0	0
Mayonnaise	74.850	11.703	16.843	44.690	0.187	42
Margarine (80%)	80.170	14.224	36.435	26.741	5.827	0
Butter (salted)	81.110	51.368	21.021	3.043	3.278	215
Cheddar Cheese	33.820	19.368	8.428	1.433	1.179	102
Whole Milk	3.250	1.865	0.812	0.195	0	10
Chicken	13.600	3.790	5.340	2.970	0	88
Beef Steak	11.360	4.407	5.034	0.569	0.565	85
Ground Beef	21.830	8.805	9.356	0.656	0	84

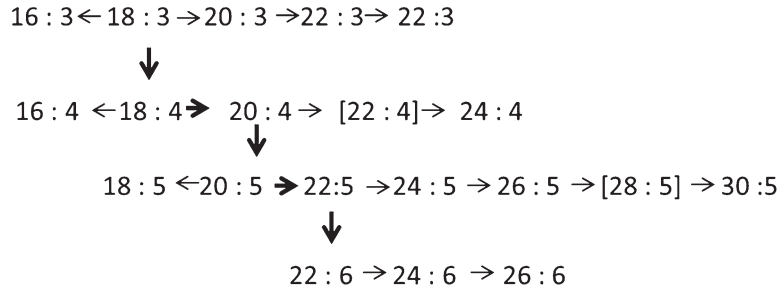


Fig. 2.3 The $n-3$ Family Polyunsaturated Fatty Acids Based on Linolenic Acid. The *heavy arrows* show the relationship between the most important $n-3$ acids through desaturation (*vertical arrows*) and chain elongation (*horizontal arrows*)

number of carbon atoms in the fatty acid chain, the second number indicates the number of double bonds. Thus, 4:0 is short for butyric acid, 16:0 for palmitic acid, 18:1 for oleic acid, etc. The two numbers provide a complete description of a saturated fatty acid. For unsaturated fatty acids, information about the location of double bonds and their stereo isomers can be given as follows: oleic acid (the *cis* isomer) is 18:1*c*9; elaidic acid (the *trans* isomer) is 18:1*t*9. The numbering of carbon atoms in fatty acids starts normally with the carboxyl carbon as number one. In some cases polyunsaturated fatty acids are numbered starting at the methyl end; for instance, linoleic acid is represented as $18:2n-6$ and linolenic acid $18:3n-3$. These symbols indicate straight-chain, 18-carbon fatty acids with two and three methylene interrupted *cis* double bonds that start at the sixth and third carbon from the methyl end, respectively. These have also been described as ω6 and ω3. The reason for this type of description is that the members of each group $n-6$ or $n-3$ are related biosynthetically through processes involving desaturation, chain elongation, and chain shortening (Gunstone 1986) (Fig. 2.3).

Triglycerides can be abbreviated by using the first letters of the common names of the component fatty acids. SSS indicates tristearin, PPP tripalmitin, and SOS a triglyceride with two palmitic acid residues in the 1 and 3 positions and oleic acid in the 2 position. In some cases, glyceride compositions are discussed in terms of saturated and unsaturated component fatty acids. In this case, S and U are used and glycerides would be indicated as SSS for trisaturated glyceride and SUS for a glyceride with an unsaturated fatty acid in the 2 position. In other cases, the total number of carbon atoms in a glyceride is important, and this can be shortened to glycerides with

carbon numbers 54, 52, and so on. A glyceride with carbon number 54 could be made up of three fatty acids with 18 carbons, most likely to happen if the glyceride originated from one of the seed oils. A glyceride with carbon number 52 could have two component fatty acids with 18 carbons and one with 16 carbons. The carbon number does not give any information about saturation and unsaturation.

Fatty Acids

Fatty acids are composed of hydrocarbon chains with a carboxylic acid on one end of the molecule. Generally in foods fatty acids exist as esters of alcohols. When esterified to a long chain fatty alcohol they are called waxes and when bound to glycerol they are called glycerides. The variation in chain length and saturation of the hydrocarbon chain of fatty acids is important in determining the physical and biological properties of lipids.

Fatty acids in biological systems are generally composed of an even number of carbon atoms, typically between 14 and 24, although the 16- and 18-carbon fatty acids are the most abundant. Fatty acids typically contain an even number of carbon atoms because of the way in which fatty acids are biosynthesized. The hydrocarbon side chains in animal fatty acids have hydrocarbon chains which are almost invariably un-branched. The side chain may be saturated or it may contain one or more double bonds. In unsaturated fatty acids, the double bonds are in the cis formation. In polyunsaturated fatty acids the double bonds are generally separated by at least one methylene group. The chain length and degree of saturation control the properties that are found within the fatty acids and lipids. Unsaturated fatty acids have lower melting points than saturated fatty acids of the same length. The cis-double bonds cause curvature in the hydrocarbon chain which prevents alignment with other side chains and limits crystallization. The fatty acids cannot compact tightly together, reducing the van der Waals interaction between the fatty acids. The melting point of fatty acids is also affected by chain length. The longer the hydrocarbon chain is, the higher the melting point. Short chain length and unsaturation enhance the fluidity of lipids.

The common feature of these lipids is that they are all esters of moderate to long chain fatty acids. Acid or base-catalyzed hydrolysis yields the component fatty acid, some examples of which are given in the following table, together with the alcohol component of the lipid. These long-chain carboxylic acids are generally referred to by their common names, which in most cases reflect their sources. Natural fatty acids may be saturated or unsaturated, and as the following data indicate, the saturated acids have higher melting points than unsaturated acids of corresponding size. The double bonds in the unsaturated compounds listed on the right are all cis (or Z).

Lipid Nomenclature

In nature even numbered straight chain fatty acids constitute the majority of the fatty acids found in triglycerides and phospholipids. Branched chain fatty acids and hydroxyl fatty acids are found in nature but not commonly. One hydroxyl fatty acid of importance is ricinoleic acid which is found in Castor plant (*Ricinus communis*L., Euphorbiaceae) seeds or in sclerotium of ergot (*Claviceps purpurea* Tul., Clavicipitaceae). In castor oil approximately 90% of the fatty acid content in the triglyceride is ricinoleic acid. Ricinoleic acid exerts analgesic and anti-inflammatory effects. It is also frequently used in lubricants including motor oil (Gunstone et al. 2007; Vieira et al. 2000).

Fatty acids are also broken in groups by chain length. Short chain fatty acids include: Formic acid, Acetic acid, Propionic acid, Isobutyric acid (2-methylpropanoic acid), Butyric acid, Isovaleric acid (3-methylbutanoic acid), Valeric acid (pentanoic acid). Formic **acid** is the simplest carboxylic acid. Its chemical formula is HCOOH or HCO_2H. It is an important intermediate in chemical synthesis and occurs naturally, most notably in ant venom. Acetic acid is widely distributed in nature and is an important food additive contributing pH control, typical acid taste and antibacterial properties. Propionic acid is an

important aroma constituent in Swiss cheese, at higher concentrations it is a pungent unpleasant aroma. Butyric acid, the only short chain fatty acid commonly found in triglycerides is a major component in butter fat. Free butyric acid exhibits a strong aroma which at low levels is an important note in dairy flavors ranging from very small amounts in butter to high levels in limburger cheese. The other short chain fatty acids exhibit strong aromas and are found at low levels in food flavors.

Medium chain fatty acids generally include saturated fatty acids with 6–12 carbons including caproic, caprylic, capric and lauric acids. The medium chain fatty acids are found in triglycerides which are frequently used in foods because of their oxidative stability and low melting pint making them liquid at room temperatures. They are also frequently used in cosmetic formulations.

The long chain fatty acids are 14 or greater straight chain fatty acids. Long chain fatty acids occur as saturated fatty acids or with varying degrees of unsaturation.

Even-numbered, straight-chain saturated and unsaturated fatty acids make up the greatest proportion of the fatty acids of natural fats. However, it is now known that many other fatty acids may be present in small amounts. Some of these include odd carbon number acids, branched-chain acids, and hydroxyl-acids. These may occur in natural fats (products that occur in nature), as well as in processed fats. The latter category may, in addition, contain a variety of isomeric fatty acids not normally found in natural fats. It is customary to divide the fatty acids into different groups, for example, into saturated and unsaturated ones. This particular division is useful in food technology because saturated fatty acids have a much higher melting point than unsaturated ones, so the ratio of saturated fatty acids to unsaturated ones significantly affects the physical properties of a fat or oil. Another common division is into short-chain, medium-chain, and long-chain fatty acids. Unfortunately, there is no generally accepted division of these groups. Generally, short-chain fatty acids have from 4 to 10 carbon atoms; medium-chain fatty acids, 12 or 14 carbon atoms; and long-chain fatty acids, 16 or more carbon atoms. Yet another division differentiates between essential and nonessential fatty acids.

Some of the more important saturated fatty acids are listed with their systematic and common names in Table 2.3, and some of the unsaturated fatty acids are listed in Table 2.3. The naturally occurring unsaturated fatty acids in fats are almost exclusively in the *cis*-form (Table 2.3), although *trans*-acids are present in ruminant milk fats and in catalytically hydrogenated fats.

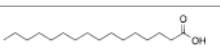
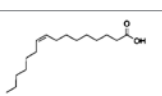
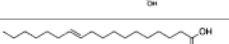
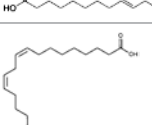
Cis and *trans* Fatty Acids

In fats and oils fatty acids containing double bonds can occur in two forms *cis* and *trans* as shown in Fig. 2.4. The fatty acids have identical chemical composition the only differences is the orientation around the double bond. As we will see late this can have a profound effect on the physical properties of the fat or oil.

Most fatty acids found in human body are *cis* fatty acids except for Retinoic acid present in the eye. Vegetable oils are typically rich in mono- and polyunsaturated fatty acids. Saturated fatty acids can pack together more effectively resulting in a more solid fat whereas unsaturated fatty acids containing *cis* double bonds do not line up effectively to form solid structures thus resisting solidification. Vegetable oils tend to be rich in mono and poly- unsaturated fatty acids. The unsaturated vegetable oils are converted from liquids to solids by the hydrogenation reaction. The hydrogenation results in hardening of the fats making them solid or semi-solids. Vegetable oils which are partially hydrogenated, are partially saturated so the melting point increases to the point where a solid is present at room temperature. The degree of hydrogenation of unsaturated oils controls the final consistency of the product.

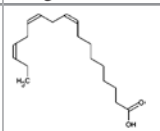
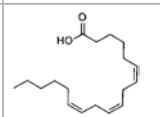
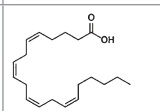
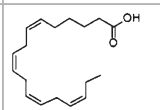
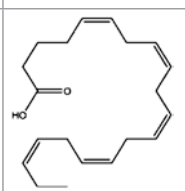
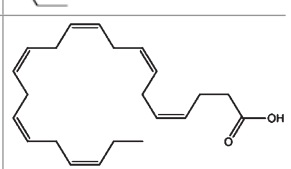
Partial hydrogenation results in the production of *trans* fats. Trans fats are the result of a side reaction with the catalyst of the hydrogenation process. Unsaturated fat which is normally found as a *cis* isomer converts to a *trans* isomer of the unsaturated fat. Isomers are molecules that have

Table 2.3 Structures and nomenclature of common fatty acids found in plants and animals

Lipid number	Name		Formula		Mass (g/mol)	Melting Point	Diagram
	Common	Systematic	Molecular	Structural			
Short and medium chain fatty acids							
C4:0	Butyric acid	Butanoic acid	$C_4H_8O_2$	$CH_3(CH_2)_2COOH$	88.11	- 5.1 °C	
C6:0	Caproic acid	Hexanoic acid	$C_6H_{12}O_2$	$CH_3(CH_2)_4COOH$	116.16	- 3.4 °C	
C8:0	Caprylic acid	Octanoic acid	$C_8H_{16}O_2$	$CH_3(CH_2)_6COOH$	144.21	16.7 °C	
C10:0	Capric acid	Decanoic acid	$C_{10}H_{20}O_2$	$CH_3(CH_2)_8COOH$	172.26	31.6 °C	
C12:0	Lauric acid	C12:0	$C_{12}H_{24}O_2$	$CH_3(CH_2)_{10}COOH$	200.32	43.8 °C	
C14:0	Myristic Acid	Tetradecanoic acid	$C_{12}H_{24}O_2$	$CH_3(CH_2)_{10}COOH$	228.37	54.4 °C	
Long chain saturated fatty acids							
C16:0	Palmitic Acid	Hexadecanoic acid	$C_{16}H_{32}O_2$	$CH_3(CH_2)_{14}CO_2H$		62.9 °C	
C18:0	stearic acid	Octadecanoic Acid	$C_{18}H_{36}O_2$	$CH_3(CH_2)_{16}CO_2H$	284.48	69.3 °C	
C20:0	arachidic acid	Eicassanoic	$C_{20}H_{40}O_2$	$CH_3(CH_2)_{18}CO_2H$	312.53	75.5 °C	
Unsaturated fatty acids							
C16:1	Palmitoleic acid	9-*cis*-Hexadecenoic acid	$C_{16}H_{30}O_2$	$CH_3(CH_2)_5CH = CH(CH_2)7COOH$	254.41	0.1 °C	
C18:1(CH	Oleic acid	*cis*-9-Octadecenoic acid	$C_{18}H_{34}O_2$	$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$	282.46	13–14 °C	
C18:1 t	Vaccenic	(E)-Octadec-11-enoic acid	$C_{18}H_{34}O_2$	$CH_3(CH_2)_5CH = CH(CH_2)_9COOH$	282.46	44–45 °C	
C18:1 t	Elacdic Acid	(*E*)-octadec-9-enoic acid	$C_{18}H_{34}O_2$	$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$	282.46	41–45 °C	
C18:2ω6	Linoleic acid	(9*Z*,12*Z*)-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	$CH_3(CH_2)_4(CH = CHCH_2)2(CH_2)_6COOH$	280.45	−5 °C	

(continued)

Table 2.3 (continued)

Lipid number	Name		Formula		Mass (g/mol)	Melting Point	Diagram
	Common	Systematic	Molecular	Structural			
C18:3 ω3	α−Linolenic acid	*cis*,*cis*,*cis*-9,12,15-Octadecatrienoic acid; (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	$C_{18}H_{30}O_2$	$CH_3(CH_2CH=CH)_3(CH_2)_7COOH$	278.43	−11 °C	
C18:3 ω6	γ−Linolenic acid	Cis-cis-*cis*-6,9,12-octadecatrienoic acid	$C_{18}H_{30}O_2$	$CH_3(CH_2)_4(CH_2CH=CH)_3(CH_2)_3COOH$	278.43	- 11 °C	
C20:4 ω6	Arachidonic acid	(5Z,8Z,11Z,14Z)-5,8,11,14-Eicosatetraenoic acid	$C_{20}H_{32}O_2$	$CH_3(CH_2)_4(CH_2CH=CH)_4(CH_2)_2COOH$	304.47	−49 °C	
C18:4 ω3	*Stearidonic* (18:4)	(6Z,9Z,12Z,15Z)-6,9,12,15-octadecatetraenoic acid		$CH_3CH_2(CH=CH\ CH_2)_4(CH_2)_3COOH$	276.40	N/A	
C20:5 ω3	*Eicosapentaenoic* EPA	(5Z,8Z,11Z,14Z,17Z)-5,8,11,14,17-icosapentaenoic acid	$C_{20}H_{30}O_2$	$CH_3CH_2(CH=CH\ CH_2)_5(CH_2)_2COOH$	302.45		
C22:6 ω3	*Docosahexaenoic* DHA	(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid	$C_{22}H_{32}O_2$	$CH_3CH_2(CH=CHCH_2)_5(CH_2)_2COOH$	328.49		

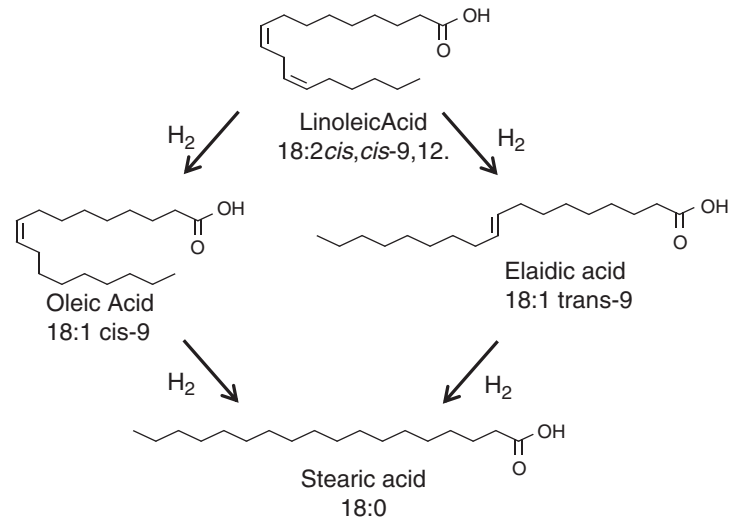


Fig. 2.4 Hydrogenation of linoleic acid yields monounsaturated fatty acids and ultimately reaches saturated stearic acid. Eladic acid is the preferred pathway for monounsaturated because *trans* forms are more thermodynamically stable

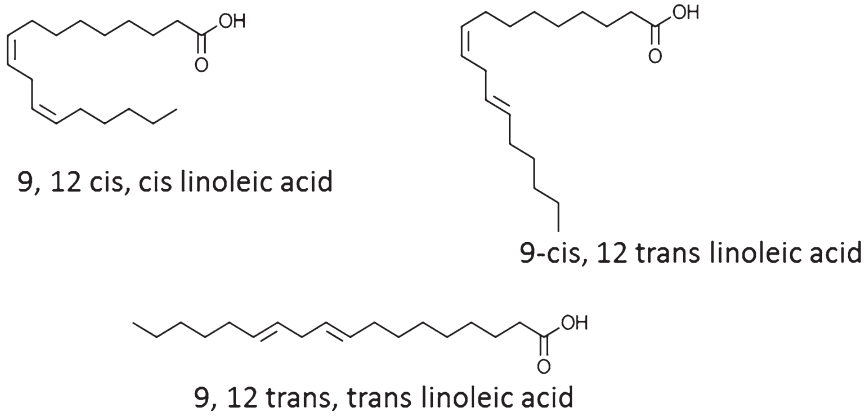


Fig. 2.5 Isomers of linoleic acid

the same molecular formula but are bonded together differently. Focusing on the Sp2 double bonded carbons, a *cis* isomer has the hydrogens on the same side. Due to the added energy from the hydrogenation process, the activation energy is reached to convert the *cis* isomers of the unsaturated fat to a *trans* isomer of the unsaturated fat. The effect is putting one of the hydrogens on the opposite side of one of the carbons. This results in a *trans* configuration of the double bonded carbons.

Unsaturated fatty acids are converted to saturated fatty acids by the relatively simple hydrogenation reaction. The addition of hydrogen to an alkene (unsaturated double bond) results in an alkane (saturated). The hydrogenation reaction is (Fig. 2.5):

The *trans* fatty acids are chemically "monounsaturated" or "polyunsaturated" but they differ from the *cis* monounsaturated or polyunsaturated fatty acids. The *trans* fatty acids (although chemically still unsaturated) produced by the partial hydrogenation process act more like saturated fat because they form similar linear structures that result in higher melting points than a *cis* fatty acid of the same length.

The methods of hydrogenation of fats were developed in early 1900s for the purpose of developing solid fats for making soaps. Later they were used to hydrogenate dietary fatty acids such as

soybean oils. Hydrogenated oils are more stable to oxidation and therefore are less prone to producing rancid or oxidized off flavors in the products. The hydrogenation process allowed processors to dial in melting ranges and physical properties of the hydrogenated fats for specific food applications such as bakery shortening or frying oil.

While there are many modifications of the process, the major process is to heat the unsaturated oils to above 200 °C, add powdered nickel (as catalyst) bubble hydrogen through it. The double bonds get saturated. However, all the double bonds do not get saturated. The first hydrogen addition is reversible. When the c-c bonding rotates to the trans form, which is more stable, subtraction of the hydrogen results in the formation of the trans-fat. The *trans* configuration is more stable than *cis*. In that process at the newer position they become *trans* double bond. *Cis* configuration has more strain in it than *trans*. As it is seen on the packet ingredient list, they are written as "partially hydrogenated".

Fatty acids can also form structures known as micelles in an aqueous solution. The structure is formed when the hydrocarbon tails form a hydrophobic center, while the polar heads form a hydrophilic shell outside the interior. The significance of micelles is that they act as emulsifiers, thus dissolving fat-soluble vitamins or other lipids that need to be absorbed.

Triglycerides

The predominant form of lipids in fats and oils are as the triesters of fatty acids with glycerol (1,2,3-trihydroxypropane). Glycerol appended with either one or two fatty acids are referred to as mono- and di- glycerides. The mono- and diglycerides are frequently used as emulsifiers in food systems. Triglycerides (or triacylglycerols) are found in both plants and animals, and compose one of the major food groups of our diet. Figure 2.6 represents a typical glyceride structures. It should be noted that the fatty acid in monoglycerides can occur at any of the three positions on the glycerol and the two fatty acids on diglycerides can be on any two of the glycerol hydroxyl positions.

Triglycerides of animal origins such as lard or beef tallow tend to be solid or semisolid at room temperature are classified as fats. Those triglycerides that are liquid are called oils and originate chiefly in plants, although triglycerides from fish are also largely oils. Some examples of the composition of triglycerides from various sources are given in Table 2.4. Animal fats exhibit large variations in fatty acid composition. Chicken and turkey tend to have higher levels of polyunsaturates than beef, sheep and pig (Table 2.5).

Natural fats can be defined as mixtures of mixed triglycerides. Simple triglycerides are virtually absent in natural fats, and the distribution of fatty acids both between and within glycerides is selective rather than random. When asymmetric substitution in a glycerol molecule occurs, enantiomorphic forms are produced (Kuksis 1972; Villeneuve and Foglia 1997). This is illustrated in Fig. 2.7. Glycerol has a plane of symmetry or mirror plane, because two of the four substituents on the central carbon atom are identical. When one of the carbon atoms is esterified with a fatty acid, a monoglyceride results and two non-superimposable structures exist. These are called enantiomers and are also referred to as

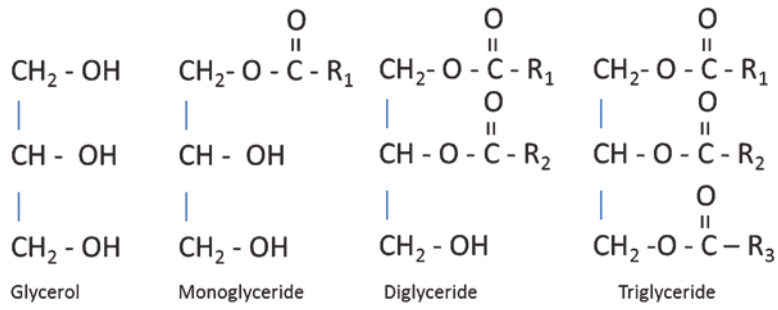


Fig. 2.6 Structures of glycerol lipids

Table 2.4 Principal fatty acid composition and melting points of food fats and oils

	% Total Fatty acids												
Oil or Fat	Saturated								Monounsaturated		Polyunsaturated		Melting point
	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	16:1	18:1	18:2	18:3	°C
Beef tallow						3	24	19	4	43	3	1	41–42
Butterfat	4	2	1	3	3	11	27	12	2	29	2	1	−10
Canola oil							4	2		62	22	10	14
Cocoa butter							26	34		34	3		24–38
Coconut		1	8	6	47	18	9	3		6	2		25
Corn							11	2		28	58	1	11
Cottonseed						1	22	3	1	19	54	1	48
Lard						2	26	14	3	44	10		36–45
Olive							13	3	1	71	10		6
Palm						1	45	4		40	10		35
Palm Kernal			3	4	48	16	8	3		15	2		3
Peanut							11	2		48	32		3
Safflower							7	2		13	78		−17
Soybean							11	4		24	54	7	−16
Sunflower							7	5		19	68	1	−17

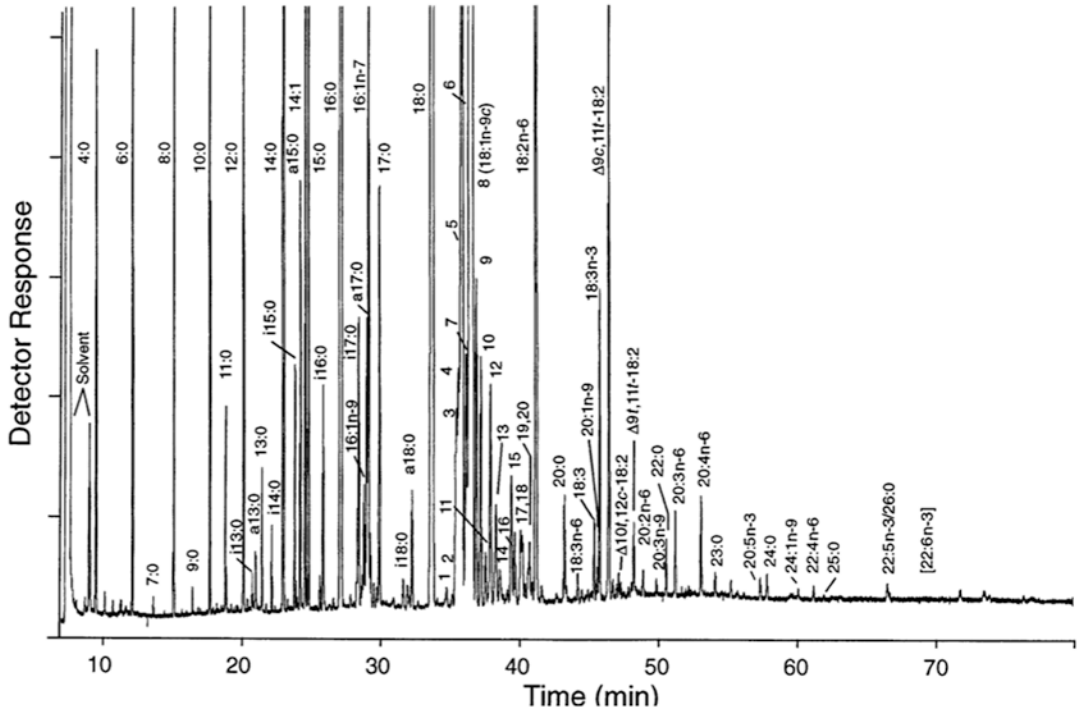


Fig. 2.7 A typical gas–liquid chromatogram of total bovine milk fatty acid methyl ester prepared by NaOCH3/methanol (10 min at 50 °C) followed by HCl/methanol (10 min at 80 °C), and separated on a 100-m fused silica capillary column (SP-2560; Supelco Inc., Bellefonte, PA). *i* iso, *a* anteiso; numbers 1–20 are an arbitrary consecutive numbering of all the peaks in the region between 18:0 and 18:2n − 6 using this column. [22:6n − 3] is not present in these milk samples but the position at which it should emerge in the chromatogram is indicated. Kramer, J.K.G., Fellner, V., Dugan, M.E.R. et al. Lipids (1997) 32: 1219. doi:10.1007/s11745-997-0156-3

Table 2.5 Component fatty acids of animal depot fats

	Fatty Acids Wt %						
Animal	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Pig	1	24	3	13	41	10	1
Beef	4	25	5	19	36	4	Trace
Sheep	3	21	2	25	34	5	3
Chicken	1	24	6	6	40	17	1
Turkey	1	20	6	6	38	24	2

chiral. A racemic mixture is a mixture of equal amounts of enantiomers. Asymmetric or chiral compounds are formed in 1-monoglycerides; all 1, 2-diglycerides; 1, 3-diglycerides containing unlike substituents; and all triglycerides in which the 1- and 3-positions carry different acyl groups.

The depot fats of higher land animals, especially mammals, have relatively simple fatty acid composition. The fats of birds are somewhat more complex. The fatty acid compositions of some major food fats of this group are listed in Table 2.4. The kind of feed consumed by the animals may greatly influence the composition of the depot fats. Animal depot fats are characterized by the presence of 20–30% palmitic acid, a property shared by human depot fat. Many of the seed oils, in contrast, are very low in palmitic acid. The influence of food consumption applies equally for the depot fat of chicken and turkey (Marion et al. 1970; Jen et al. 1971). The animal depot fats are generally low in polyunsaturated fatty acids. The iodine value of beef fat is about 50 and of lard about 60. Iodine value is generally used in the food industry as a measure of total unsaturation in a fat.

Iodine value is a measure of the degree of unsaturation of an oil or wax. The oil is reacted with is reacted with iodine, which adds across the double bond. The iodine unreacted iodine is then measured and the differences is the iodine number. A saturate fat would have a value of zero.

Cocoa butter typically has iodine values of 30–40 and highly unsaturated oil like soy has a value of 130–136. These values are used by the industry as an index of unsaturation and vulnerability to oxidation. The iodine addition reaction is shown as:

$$R - CH = CH - R' + I_2 \longrightarrow R - CHI - CHI - R'$$

Ruminant milk fat is extremely complex in fatty acid composition. By using gas chromatography in combination with fractional distillation of the methyl esters and adsorption chromatography, Magidman et al. (1962) and Herb et al. (1962) identified at least 60 fatty acids in cow's milk fat. Several additional minor fatty acid components have been found in other recent studies. About 12 fatty acids occur in amounts greater than 1% (Jensen and Newburg 1995). Among these, the short-chain fatty acids from butyric to capric are characteristic of ruminant milk fat. Data provided by Hilditch and Williams (1964) and Markiewicz-Kęszycka et al. 2013, on the component fatty acids of some milk fats are listed in Table 2.6. Fatty acid compositions are usually reported in percentage by weight, but in the case of fats containing short-chain fatty acids (or very long-chain fatty acids) this method may not give a good impression of the molecular proportions of fatty acids present. Therefore, in many instances, the fatty acid composition is reported in mole percent, as is the case with the data in Table 2.7.

Table 2.6 The component fatty acids of some milk fats in g/100 g

Fatty acids in g/100 g fat	Goat	Sheep	Cow
C4:0 Butyric	2.03	2.57	2.87
C6:0 Caproic	2.78	1.87	2.01
C8:0 Caprylic	2.92	1.87	1.39
C10:0 Capric	9.59	6.33	3.03
C12:0 Lauric	4.52	3.99	3.64
C14:0 Myristic	9.83	10.17	10.92
C16:0 Palmitic	24.64	25.10	28.70
C18:0 Stearic	8.87	8.85	11.23
C18:1 *cis-9* Oleic	18.65	20.18	22.39
C18:2 *cis-9, cis-12* Linoleic	2.25	2.32	2.57
C18:2 *cis-9, trans-11* CLA	0.45	0.76	0.57
C18:3 *cis-9, cis-12, cis-15* Linolenic	0.77	0.92	0.50

Adapted from Markiewicz-Kęszycka et al. (2013)

It is impossible to determine all of the constituents of milk fatty acids by a normal chromatographic technique, because many of the minor component fatty acids are either not resolved or are covered by peaks of other major fatty acids. A milk fat chromatogram of fatty acid composition is shown in Fig. 2.6. Such fatty acid compositions as reported are therefore only to be considered as approximations of the major component fatty acids; these are listed in Table 2.6. This table reports results of the major component fatty acids in bovine milk fat as well as their distribution among the sn-1, sn-2, and sn-3 positions in the triacylglycerols (Jensen and Newburg 1995). Milk fatty acids can vary considerably. Seasonal variations because of changes in feed are one of the major factors (Jensen 2000; Månsson 2008). The composition of bovine milk lipids over a 5 year period was reviewed by Jensen (2002).

In most natural fats the double bonds of unsaturated fatty acids occur in the *cis* configuration. In milk fat a considerable proportion is in the *trans* configuration. These *trans* bonds result from microbial action in the rumen where polyunsaturated fatty acids of the feed are partially hydrogenated. Catalytic hydrogenation of oils in the fat industry also results in *trans* isomer

Table 2.7 Major fatty acids of bovine milk fat and their distribution in the triacylglycerols

	Bovine milk fat			
Fatty acids (mol%)	TG	sn-1	sn-2	sn-3
4:0	11.8	–	–	35.4
6:0	4.6	–	0.9	12.9
8:0	1.9	1.4	0.7	3.6
10:0	37	1.9	3.0	6.2
12:0	3.9	4.9	6.2	0.6
14:0	11.2	9.7	17.5	6.4
15:0	2.1	2.0	2.9	1.4
16:0	23.9	34.0	32.3	5.4
16:1	2.6	2.8	3.6	1.4
17:0	0.8	1.3	1.0	0.1
18:0	7.0	10.3	9.5	1.2
18:1	24.0	30.0	18.9	23.1
18:2	2.5	1.7	3.5	2.3
18:3	Trace	–	–	–

Adapted from R.G. Jensen and D.S. Newburg, Milk Lipids, in *Handbook of Milk Composition*, R.G. Jensen, ed., p. 546, © 1995, Academic Press

formation. The level of *trans* isomers in milk fat has been reported as 2–4% (deMan and deMan 1983). Since the total content of unsaturated fatty acids in milk fat is about 34%, *trans* isomers may constitute about 10% of total unsaturation (Akoh 1997). The complexity of the mixture of different isomers is demonstrated by the distribution of positional and geometric isomers in the monoenoic fatty acids of milk fat (Table 2.8) and in the unconjugated 18:2 fatty acids (Table 2.9). The iodine value of milk fat is in the range of 30–35, much lower than that of lard, shortening, or margarine, which have similar consistencies.

The higher melting points of the saturated fatty acids are because the uniform rod-like shape of their molecules which allows the fatty acids to align in crystal like forms. Unsaturated fatty acids there contain one or more double bonds causing curvature in the chain and results in lower the melting point of the fatty acid. In most fats and oils the natural form of the fatty acid \double bond is in the *cis* form. The *cis*-double bond(s) in the unsaturated fatty acids introduces a kink in chain, which makes it more difficult to pack their molecules together in a stable repeating array or crystalline lattice. The *trans*-double bond isomer of oleic acid, known as elaidic acid, has a linear shape and a melting point of 45 °C (32 °C higher than its cis isomer). The shapes of stearic and oleic acids are displayed in the models below.

Marine oils have also been found to contain a large number of component fatty acids. Ackman (1972) has reported as many as 50 or 60 components. Only about 14 of these are of importance in terms of weight percent of the total. These consist of relatively few saturated fatty acids (14:0, 16:0, and 18:0) and a larger number of unsaturated fatty acids with 16–22 carbon atoms and up to 6 double bonds. This provides the possibility for many positional isomers.

The complexity of the fatty acid composition of marine oils is evident from the chromatogram shown in Fig. 2.8. The end structure of the polyunsaturated fatty acids is of nutritional importance, especially eicosapentaenoic acid (EPA), 20:5ω3 or 20:5 n-3, and docosahexaenoic acid (DHA), 22:6ω3 or 22:6 n-3. The double bonds in marine oils occur exclusively in the *cis* configuration. EPA and DHA can be produced slowly from linolenic acid by herbivore animals, but not by humans. EPA and DHA occur in major amounts in fish from cold, deep waters, such as cod, mackerel, tuna, swordfish, sardines, and

Table 2.8 Positional and geometric isomers of bovine milk lipid monoenoic fatty acids (Wt%)

	cis Isomers				trans Isomers	
Position of double bond	14:1	16:1	17:1	18:1	16:1	18:1
5	1.0	Tr			2.2	
6	0.8	1.3	3.4		7.8	1.0
7	0.9	5.6	2.1		6.7	0.8
8	0.6	Tr	20.1	1.7	5.0	3.2
9	96.6	88.7	71.3	95.8	32.8	10.2
10	–	Tr	Tr	Tr	1.7	10.5
11	–	2.6	2.9	2.5	10.6	35.7
12	–	Tr	Tr	–	12.9	4.1
13	–	–	–	–	10.6	10.5
14	–	–	–	–	–	9.0
15	–	–	–	–	–	6.8
16	–	–	–	–	–	7.5

Source: From R.G. Jensen, Composition of Bovine Milk Lipids, *J. Am. Oil Chem. Soc*., Vol. 50, pp. 186–192, 1973

Table 2.9 Location of double bonds in unconjugated 18:2 isomers of milk lipids

cis, cis	cis, trans *or* trans, cis	trans, trans
11, 15	11, 16 and/or 11, 15	12, 16
10,15	10, 16 and/or 10, 15	11, 16 and/or 11, 15
9, 15	9, 15 and/or 9, 16	10,16 and/or 10, 15
8, 15 and/or 8, 126	8, 16 and/or 8, 15 and/or 8, 12	9, 16 and/or 9, 15 and/or 9, 13
7, 15 and/or 7, 12		
15 and/or 6, 12		

Adopted from R.G. Jensen, Composition of Bovine Milk Lipids, *J. Am. Oil Chem. Soc*., Vol. 50, pp. 186–192, 1973; Morrison, W.R., in "Topics in Lipid Chemistry," Vol. 1, Ed. by F.D. Gunstone, Logos Press, London, 1970, p. 51.; Van der Wel, H., and K. De Jong, Fette Seifen Anstrichm. 67:279 (1967)

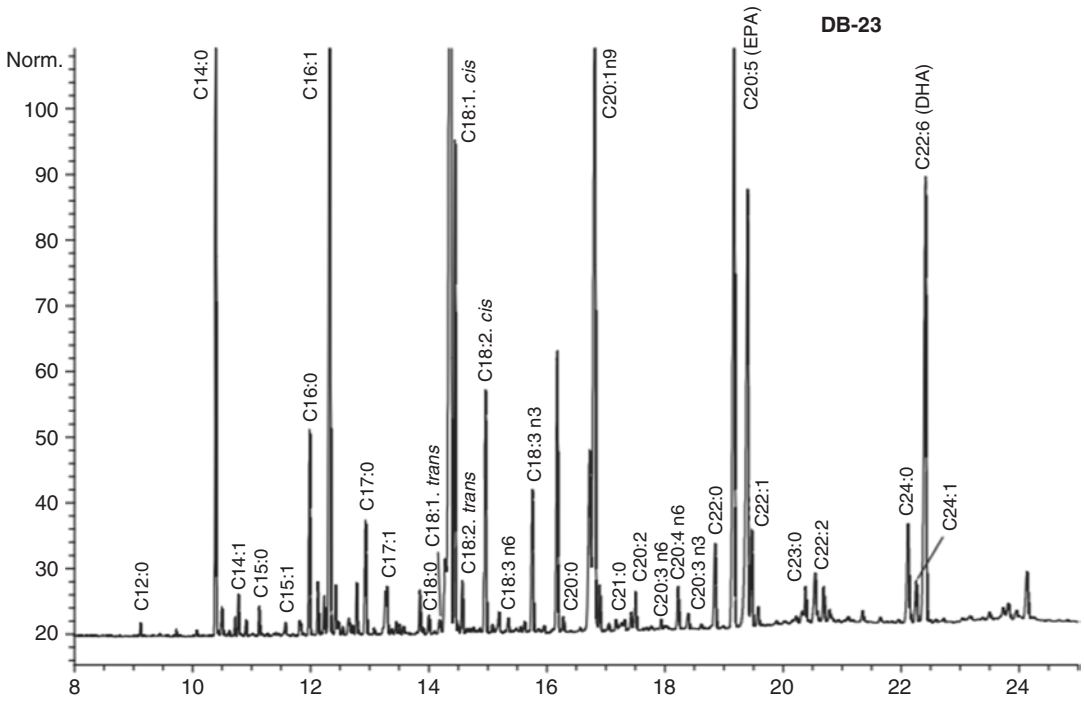


Fig. 2.8 Analysis fatty acid methyl esters of a typical marine oil sample. www.agilent.com/cs/library/applications/5989-3760EN.pdf

herring (Ackman 1988a; Simopoulos 1988). Arachidonic acid is the precursor in the human system of prostanoids and leukotrienes.

Ackman (1988b) has drawn attention to the view that the fatty acid compositions of marine oils are all much the same and vary only in the proportions of fatty acids. The previously held view was that marine oils were species-specific. The major fatty acids of commonly consumed seafood are found in Table 2.10.

The fatty acid composition of egg yolk is given in Table 2.11. The main fatty acids are palmitic, oleic, and linoleic. The yolk constitutes about one-third of the weight of the edible egg portion. The relative amounts of egg yolk and white vary with the size of the egg. Small eggs have relatively higher amounts of yolk. The egg white is virtually devoid of fat.

Diet can exert a significant impact on the lipid profile of foods. Milinsk et al. fed chickens diets enriched with various lipids and observed that the fatty acid profiles in egg yolk are altered by the diet. The control diet was based on soy and the flax, soy and canola diets were all enriched with 3% flax, soy or canola oil. The flax oil which is rich in omega-3 fatty acids resulted in higher levels of omega-3 fatty acids in the egg yolk. All three experimental diets resulted in higher levels of mono and poly unsaturated fatty acids (Table 2.12). Commonly used food oils have a wide range of fatty acid composition which impacts functionality in food systems, nutritional profile and oxidative stability. Table 2.13 summarizes some of the variations seen between vegetable oils.

Component Glycerides

Natural fats can be defined as mixtures of mixed triglycerides. Simple triglycerides are virtually absent in natural fats, and the distribution of fatty acids both between and within glycerides is selective rather than random. When asymmetric substitution in a glycerol molecule occurs, enantiomorphic forms are produced (Kuksis 1972; Villeneuve and Foglia 1997). This is illustrated in Fig. 2.9. Glycerol has a plane of symmetry or mirror plane, because two of the four substituents

Table 2.10 Total fat content, fatty acid content of raw seafood

	Anchovy	Atlantic Salmon	Cod Raw	Shrimp raw	Tuna Raw, Bluefin	Bluefish Raw
Nutrient	g/100 g Edible portion					
Water	73.37	68.5	81.22	78.45	68.09	70.86
Total lipid (fat)	4.84	6.34	0.67	0.51	4.9	4.24
Lipids						
Fatty acids, total saturated	1.282	0.981	0.131	0.101	1.257	0.915
C14:0	0.302	0.137	0.009	0.001	0.139	0.179
C16:0	0.715	0.632	0.091	0.052	0.81	0.576
C18:0	0.252	0.21	0.03	0.037	0.307	0.16
Fatty acids, total monounsaturated	1.182	2.103	0.094	0.086	1.6	1.793
C16:1	0.4	0.251	0.016	0.003	0.162	0.277
C18:1	0.624	1.351	0.061	0.038	0.924	0.684
C20:1	0	0.223	0.015	0.002	0.277	0.34
C22:1	0.115	0.279	0.003	0	0.237	0.492
Fatty acids, total polyunsaturated	1.637	2.539	0.231	0.152	1.433	1.06
C18:2	0.097	0.172	0.005	0.032	0.053	0.06
C18:3	0	0.295	0.001	0.002	0	0
C18:4	0.055	0.083	0.001	0	0.039	0.167
C20:4	0.007	0.267	0.022	0.012	0.043	0
20:5 n-3 (EPA)	0.538	0.321	0.064	0.03	0.283	0.252
22:5 n-3 (DPA)	0.029	0.287	0.01	0.002	0.125	0.062
22:6 n-3 (DHA)	0.911	1.115	0.12	0.031	0.89	0.519
Cholesterol (mg)	60	55	43	161	38	59

National Nutrient Database for Standard Reference Release 28 slightly revised May, 2016 https://ndb.nal.usda.gov/ndb/foods

Table 2.11 Fatty acid composition of egg yolk

Fatty Acid	%
Total saturated	36.2
14:0	0.3
16:0	26.6
18:0	9.3
Total monounsaturated	48.2
16:1	4.0
18:1	44.1
Total polyunsaturated	14.7
18:2	13.4
18:3	0.3
20:4	1.0

on the central carbon atom are identical. When one of the carbon atoms is esterified with a fatty acid, a monoglyceride results and two nonsuperimposable structures exist. These are called enantiomers and are also referred to as chiral. A racemic mixture is a mixture of equal amounts of enantiomers. Asymmetric or chiral compounds are formed in 1-monoglycerides; all 1, 2-diglycerides; 1, 3-diglycerides containing unlike substituents; and all triglycerides in which the 1- and 3-positions carry different acyl groups.

The glyceride molecule can be represented in the wedge and slash form (Fig. 2.10). In this spatial representation, the wedge indicates a substituent coming out of the plane toward the observer, and the slash indicates a substituent going away from the observer. The three carbon atoms of the glycerol are then described by the stereospecific numbering (*sn*) with the three carbon atoms designated *sn*-1 from the top to *sn*-3 at the bottom.

When a fat or oil is characterized by determination of its component fatty acids, there still remains the question as to how these acids are distributed among and within the glycerides.

Table 2.12 Influence of lipid supplementation on fatty acid distribution in egg yolk fatty acids

	Control	Flax	Soy	Canola
C14:0	1.27	0.29	0.29	0.3
C16:0	25	22.5	23.2	22.9
C:16:1n9	0.7	0.94	0.83	0.94
C16:1n7	2.14	2.47	1.91	1.91
C17:0	0.17	0.16	0.18	0.18
C17:1n10	0.12	0.16	0.13	0.14
C18:0	12.4	8.87	9.46	8.83
C18:1n9	39.6	43.3	41.5	44
C18:2n6	14.7	13.7	17.8	15.8
C18:3n6	0.06	0.08	0.13	0.13
C18:3n3	0.22	3.4	0.63	0.55
C18:4n3	Nd	0.06	0.05	0.08
C20:1n9	0.27	0.17	0.24	0.34
C20:2n6	0.22	0.21	0.24	0.26
C20:3n6	0.2	0.18	0.23	0.3
C20:4n6	2.63	1.17	1.74	1.94
C20:5n3	Nd	0.18	Nd	Nd
C22:4n6	0.52	0.09	0.2	0.18
C22:5n6	Nd	0.08	0.38	0.4
C22:5n3	Nd	0.29	0.1	0.1
C22:6n3	0.64	1.55	0.65	0.65
Total Fat	4.12	4.44	5.22	5.70
0Saturated FA	38.84	31.82	33.13	32.21
Mono Unsaturated	42.83	47.04	44.61	47.33
Total PUFA	19.19	20.99	22.15	20.39
n-3 FA	0.86	5.48	1.43	1.38

Adapted from Milinsk et al. (2003)

Originally theories of glyceride distribution were attempts by means of mathematical schemes to explain the occurrence of particular kinds and amounts of glycerides in natural fats. Subsequent theories have been refinements attempting to relate to the biochemical mechanisms of glyceride synthesis. Hilditch proposed the concept of even distribution (Gunstone 1967). In the rule of even (or widest) distribution, each fatty acid in a fat is distributed as widely as possible among glyceride molecules. This means that when a given fatty acid A constitutes about 35 mole percent or more of the total fatty acids (A + X), it will occur at least once in all triglyceride molecules, as represented by GAX_2. If A occurs at levels of 35–70 mole percent, it will occur twice in an increasing number of triglycerides GA_2X. At levels over 70%, simple triglycerides GA_3 are formed. In strictly random distribution the amount of GA_3 in a fat would be proportional to the cube of the percentage of A present. For example, at 30% A there would be 2.7% of GA_3, which under rules of even distribution would occur only at levels of A over 70% (Fig. 2.11).

The theory of restricted random distribution was proposed by Kartha (1953). In this theory the fatty acids are distributed at random, but the content of fully saturated glycerides is limited to the amount that can remain fluid *in vivo*. This theory is followed by the 1,3 random, 2 random distribution hypothesis of Vander Wal (1964). According to this theory, all acyl groups at the 2-positions of the glycerol moieties of a fat are distributed therein at random. Equally, all acyl groups at the 1- and 3-positions are distributed at random and these positions are identical. Application of this theory to the results obtained with a number of fats gave good agreement (Vander Wal 1964), as Table 2.14 shows.

In vegetable fats and oils, the saturated fatty acyl groups have a tendency to occupy the 1- and 3- positions in the glycerides and the unsaturated acyl groups occupy the 2-position (Fig. 2.12). Since these fats contain a limited number of fatty acids, it is customary to show the glyceride composition in terms of saturated (S) and unsaturated (U) acids. The predominant glyceride types in these fats and oils are S-U-S and S-U-U. Lard is an exception—saturated acyl groups predominate in the 2-position. The glyceride distribution of cocoa butter results in a fat with a sharp melting point of about 30–34 °C. It is hard and brittle below the melting point, which makes the fat useful for chocolate and confectionery manufacture. Other fats with similar fatty acid composition, such as sheep depot fat (see Table 2.4), have a greater variety of glycerides, giving the fat a higher melting point (about 45 °C) and a wider melting range, and a greasy and soft appearance.

Brockerhoff et al. (1966) studied the fatty acid distribution in the 1-, 2-, and 3-positions of the triglycerides of animal depot fats by stereospecific analysis. The distribution among the three positions was nonrandom. The distribution of fatty acids seems to be governed by chain length and unsaturation. In most fats a short chain and unsatu-

Table 2.13 Typical component fatty acids of some vegetable oils (Wt %)

Oil or Fat	Unsat/ sat ratio	Saturated					Mono unsaturated	Poly unsaturated	
		Capric acid	Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid (ω−6)	Alpha linolenic acid (ω−3)
		C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
Canola Oil	15.7	–	–	–	4	2	62	22	10
Cocoa Butter	0.6	–	–	–	25	38	32	3	–
Cocoanut Oil	0.1	6	47	18	9	3	6	2	–
Corn Oil	6.7	–	–	–	11	2	28	58	1
Cottonseed Oil	2.8	–	–	–	22	3	19	54	1
Flaxseed Oil	9	–	–	–	3	7	21	16	53
Olive Oil	4.6	–	–	–	13	3	71	10	1
Palm Oil	1	–	–	1	45	4	40	10	–
Palm Olein	1.3	–	–	1	37	4	46	11	–
Palm Kernal Oil	0.2	4	48	16	8	3	15	2	–
Peanut Oil	4	–	–	–	11	2	48	32	–
Safflower	10.1	–	–	–	7	2	13	78	–
Sesame Oil	6.6	–	–	–	9	4	41	45	–
Soybean Oil	5.7	–	–	–	11	4	24	54	7

http://www.scientificpsychic.com/fitness/fattyacids1.html

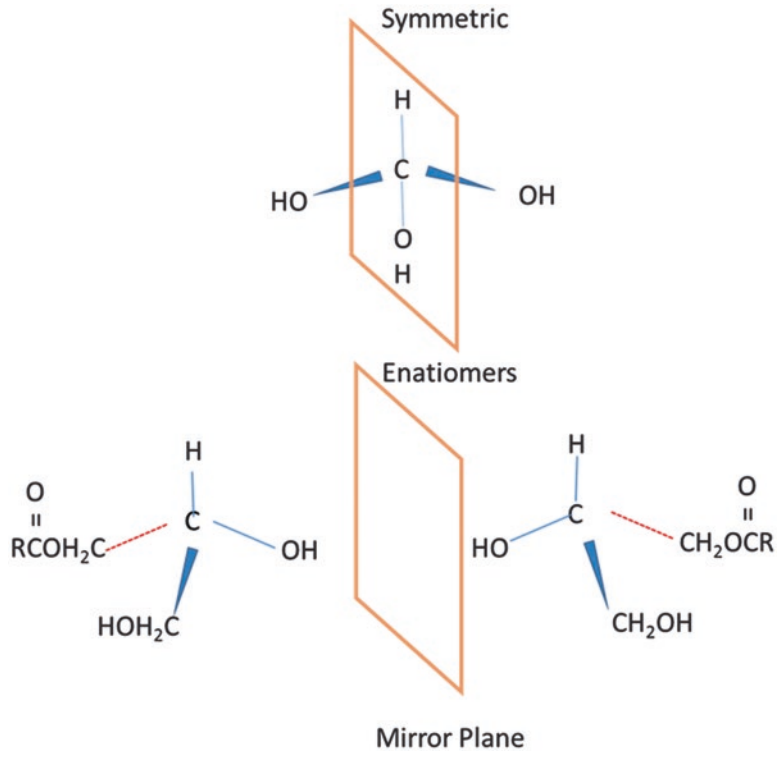


Fig. 2.9 Plane of symmetry of a glycerol molecule (*top*) and mirror image of two enantiomers of a mono-acylglycerol (*bottom*)

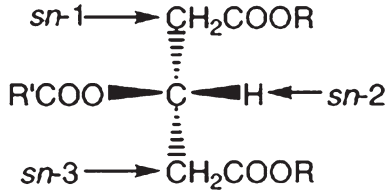


Fig. 2.10 Stereospecific numbering of the carbons in a triacylglycerol

ration direct a fatty acid toward position 2. The depot fat of pigs is an exception, palmitic acid being predominant in position 2. In the fats of marine animals, chain length is the directing factor, with polyunsaturated and short-chain fatty acids accumulated in the 2-position and long chains in the 1- and 3- positions. In the fats of birds, unsaturation seems to be the only directing factor and these acids accumulate in the 2-position.

The positional distribution of fatty acids in pig fat (lard) and cocoa butter is shown in Table 2.15. Most of the unsaturation in lard is located in the 1- and 3-positions, whereas in cocoa butter the major portion of the unsaturation is located in the 2-position. This difference accounts for the difference in physical properties of the two fats (deMan et al. 1987).

Milk fat, with its great variety of fatty acids, also has a very large number of glycerides. It is possible, by, for example, fractional crystallization, to separate milk fat in a number of fractions with different melting points (Chen and deMan 1966). Milk fat is peculiar in some respects. Its short-chain fatty acids are classified chemically as saturated compounds but behave physically like unsaturated fatty acids. One of the unsaturated fatty acids, the so-called oleic acid, is partly *trans* and has a much higher melting point than the *cis* isomers. In the highest melting fraction from milk fat, there is very little short-chain fatty acid and little unsaturation, mostly in the *trans* configuration (Woodrow and deMan 1968). The low melting fractions are high in short-chain fatty acids and unsaturation (*cis*). The general

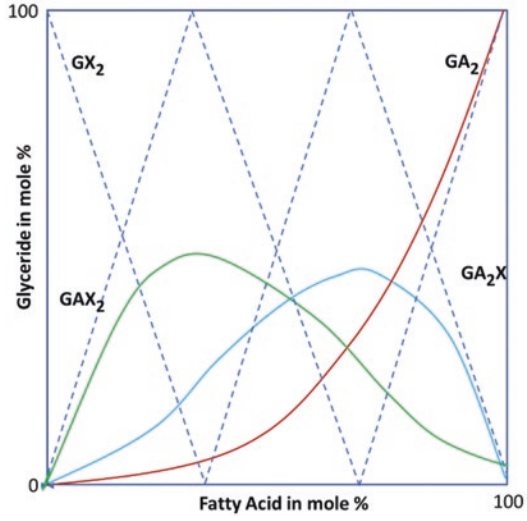
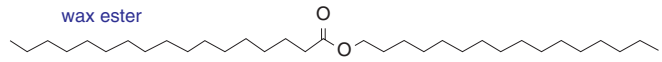


Fig. 2.11 Calculated values for glyceride types in random distribution (*solid lines*) and even distribution (*dotted lines*). *Adapted from:* F.D. Gunstone, *An Introduction to the Chemistry of Fats and Fatty Acids,* 1967, Chapman and Hall

distribution of major fatty acids in whole milk fat is as follows (Morrison 1970): 4:0 and 6:0 are located largely sn1 and sn-3 positions; 18:0 and 18:1 are preferentially in primary positions; 10:0, 12:0, and 16:0 are distributed randomly or with a slight preference for the secondary position; and 14:0 is predominantly in the secondary position. Prosser et al. (Table 2.16) have shown that C14 and C16 are predominantly in the sn-2 position of the triglyceride in both human and cow milk.

Waxes

Waxes are esters of fatty acids with long chain monohydric alcohols (one hydroxyl group). Natural waxes are often mixtures of such esters, and may also contain hydrocarbons. The formulas for three well known waxes are given below, with the carboxylic acid moiety colored red and the alcohol colored blue.



Spermaceti	Beeswax	Carnuba wax
$CH_3(CH_2)_{14}CO_2$-$(CH_2)_{15}CH_3$	$CH_3(CH_2)_{24}CO_2$-$(CH_2)_{29}CH_3$	$CH_3(CH_2)_{30}CO_2$-$(CH_2)_{33}CH_3$

Spermaceti	Beeswax	Carnuba wax
$CH_3(CH_2)_{14}CO_2$—$(CH_2)_{15}CH_3$	$CH_3(CH_2)_{24}CO_2$—$(CH_2)_{29}CH_3$	$CH_3(CH_2)_{30}CO_2$—$(CH_2)_{33}CH_3$

Table 2.14 Comparison of the glyceride composition of some natural fats as determined experimentally and as calculated by 1,3 random, 2 random hypothesis

		Molecular species					
Fat	Method	SSS (Mole %)	SUS (Mole %)	SSU (Mole %)	USU (Mole %)	UUS (Mole %)	UUU (Mole %)
Lard	Experiment	8	0	29	36	15	12
Lard	Calculated	6	2	29	36	12	15
Chicken fat	Experiment	3	10	9	12	38	28
Chicken fat	Calculated	3	10	10	9	36	32
Cocoa butter	Experiment	5	66	7	3	20	1
Cocoa butter	Calculated	5	69	2	0	22	2

Adapted from *R*.J. Vander Wal, Triglyceride Structure, *Adv. Lipid Res.,* Vol. 2, pp. 1–16, 1964

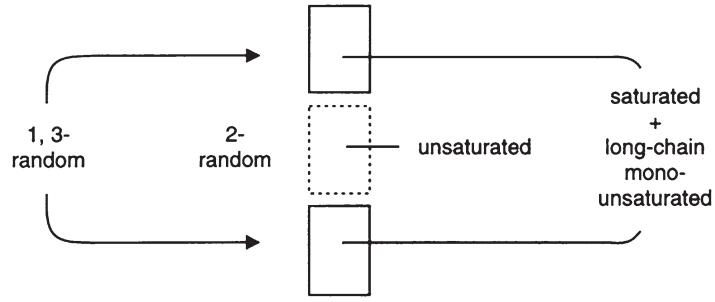


Fig. 2.12 Fatty acid distribution in the triacylglycerols of vegetable oils

Table 2.15 Positional distribution fatty acids in pig fat and cocoa butter

	Fatty acid (Mole %)						
Fat	Position	14:0	16:0	16:1	18:0	18:1	18:2
Pig fat	1	0.9	9.5	2.4	29.5	51.3	6.4
	2	4.1	72.3	4.8	2.1	13.4	3.3
	3	0	0.4	1.5	7.4	72.7	18.2
Cocoa butter	1	–	34.0	0.6	50.4	12.3	1.3
	2	–	1.7	0.2	2.1	87.4	8.6
	3	–	36.5	0.3	52.8	8.6	0.4

Source: From W.C. Breckenridge, Stereospecific Analysis of Triacylglycerols, in *Fatty Acids and Glycerides,* A. Kuksis, ed., 1978, Plenum Press

Waxes are widely distributed in nature on leaves and fruits of many plants. The waxy coatings may protect the leaves and fruit from dehydration and small predators. Bird feathers and the fur of some animals have similar coatings which serve as a water repellent.

Waxes occur primarily in plants and are generally composed of long chain alcohols esterified to fatty acids. The chain-length and degree of unsaturation and branching of the aliphatic constituents will vary with the origin of the wax. Some waxes of marine origin or from some higher animals, the

Table 2.16 Fatty acid contents of cow milk and breast milk and the fatty acid distribution in the *sn-2* position of the triglycerides Adapted from Prosser et al. 2010

	Cow Milk		Breast Milk	
	Total Fatty acids	In *sn-2* position	Total Fatty acids	In *sn-2* position
	mol/100 mol	mole/100 mol	mol/100 mol	mole/100 mol
C4:0	8.4	ND		
C6:0	4.5	0.9		
C8:0	2.2	1.7	0.1	ND
C10:0	4.3	4.2	1.2	0.3
C12:0	4.3	5.9	5.5	4.2
C14:0	13.0	21.7	6.0	9.6
C15:0	1.4	2.0	0.2	0.5
C16:0	31.0	40.8	21.0	57.0
C17:0	0.8	0.7	0.4	0.4
C18:0	9.6	4.9	7.4	1.4
C18:1n9	14.0	10.2	42.0	16.0
C18:2n6	0.6	1.2	18.0	13.0
C18:3n3	0.7	1.6	0.8	0.7
C20:0	0.1	ND	0.2	0.1

aliphatic moieties tend to be saturated or monoenoic. A number of waxes are produced commercially in large amounts for use in food coatings, cosmetics, lubricants, polishes, inks and many other applications. Bees secrete a wax, which they use to construct the honeycomb. The wax is recovered as a by-product when the honey is harvested and refined. It contains a high proportion of wax esters (35–80%). The hydrocarbon content is highly variable. The wax esters consist of C40 to C46 molecular species, based on 16:0 and 18:0 fatty acids some with hydroxyl groups in the ω-2 and ω-3 positions. In addition, some diesters with up to 64 carbons may be present, together with triesters, hydroxy-polyesters and free acids (which are different in composition and nature from the esterified acids). The jojoba plant (Simmondsia chinensis), which grows in the semi-arid regions of Mexico and the U.S.A., produces wax esters rather than triacylglycerols in its seeds. The wax consists mainly of 18:1 (6%), 20:1 (35%) and 22:1 (7%) fatty acids linked to 20:1 (22%), 22:1 (21%) and 24:1 (4%) fatty alcohols. Therefore, it contains C38 to C44 esters with one double bond in each alkyl moiety. As methylene-interrupted double bonds are absent, the wax is relatively resistant to oxidation.

The leaves of the carnauba palm, *Copernicia cerifera* which grows in Brazil, have a thick coating of wax, which is harvested from the dried leaves. It contains mainly wax esters (85%), accompanied by small amounts of free acids and alcohols, hydrocarbons and resins. The wax esters constitute C:16 to C:20 fatty acids linked to C30 to C34 alcohols, resulting in C:46 to C:54 molecular species.

Waxes are widely distributed in nature on leaves and fruits of many plants. The waxy coatings, may protect the leaves and fruit from dehydration and small predators. Bird feathers and the fur of some animals have similar coatings which serve as a water repellent.

Phospholipids

After triglycerides, phospholipids are the second most abundant class of lipids found in nature. Phospholipids are found in animal, plant and microbial cell membranes. Like triglycerides phospholipids contain a glycerol backbone appended with two fatty acids, plus phosphoric acid and a low-molecular-weight alcohol on one of the hydroxyl groups of the glycerol backbone. Common phospholipids include lecithins and cephalins.

Phospholipids are the primary constituents of cell membranes. They resemble the triglycerides in being ester derivatives of glycerol appended with fatty acids and phosphoric acid. The phosphate moiety of the resulting phosphatidic acid is further esterified with ethanolamine, choline or serine. Figure 2.13 illustrates some of the structural components of fatty acids and Fig. 2.14 illustrates the structures of the most abundant phospholipids. Note that the fatty acid components (R & R') may be saturated or unsaturated.

Phospholipids are an important class of biomolecules. Phospholipids are the fundamental building blocks of cellular membranes. These molecules have a glycerol backbone, a polar or charged head group and a pair of nonpolar fatty

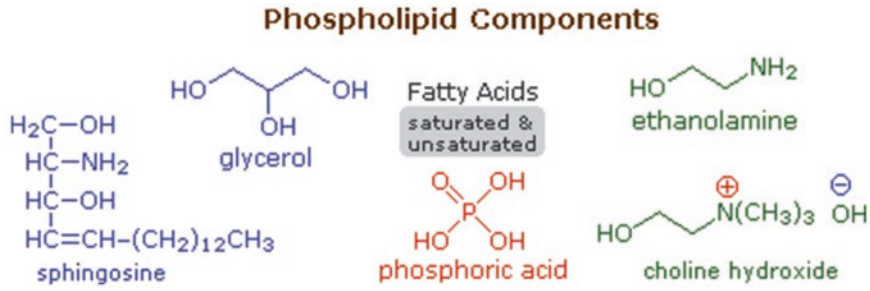


Fig. 2.13 Components of phospholipids

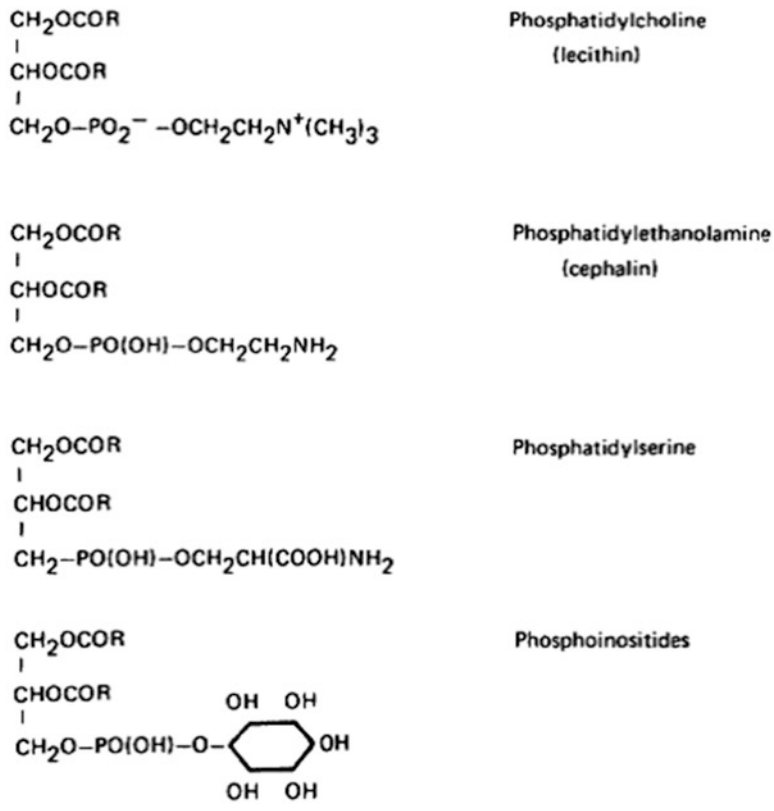


Fig. 2.14 Structure of the major phospholipids

acid tails esterified to the glycerol backbone. The combination of polar and nonpolar segments is termed amphiphilic, and the word describes the tendency of these molecules to assemble at interfaces between polar and nonpolar phases.

Two fatty acid chains, each typically having an even number of carbon atoms between 14 and 20, attach via esterification to the first and second carbons of the glycerol molecule, denoted as the sn1 and sn2 positions, respectively. The third hydroxyl group of glycerol, at position sn3, is appended with phosphoric acid to form phosphatidate bond. Phospholipids are widely distributed in nature, generally one of the groups are bound to the phosphatidic acid moiety such as serine, ethanolamine, choline, glyercol, or inositol. The resulting phospholipids may be charged, for example, phosphatidyl serine (PS), phosphatidyl inositol (PI), and phosphatidyl glyercol (PG); or dipolar (having separate positively and negatively charged regions), for example, phosphatidyl choline (PC), and phosphatidyl ethanolamine (PE). Phosphatidyl choline commercially called lecithin is important in foods as an emulsifier. A typical phospholipid contains a saturated fatty acid, such as palmitic or stearic acid, at the sn1 position, and an unsaturated or polyunsaturated fatty acid, such as oleic or arachodonic acid.

Phosphatidylcholine (lecithin) is usually the most abundant phospholipid in plants, often amounting to almost 50% of the total phospholipid in the system. Phosphotidyl choline is a key building block of membrane bilayers. It makes up a very high proportion of the outer leaflet of the plasma membrane. Phosphatidylcholine is also the principal phospholipid circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL.

In food systems where it is more commonly referred to as lecithin it is a valuable emulsifier. Phospholipids are amphoteric (mixed ionic charges), thus, they can aggregate or self-assemble when mixed with water. This aggregation differs from the surface activity of soaps and

detergents. Soaps and detergents tend to form micelles. The two pendant alkyl chains present in phospholipids and the unusual mixed charges in their head groups, micelle formation is unfavorable relative to a bilayer structure. As shown in Fig. 2.15, the polar head groups on the faces of the bilayer contact water, and the hydrophobic alkyl chains form a nonpolar interior. The phospholipid molecules can move about in their half the bilayer, but there is a significant energy barrier preventing migration to the other side of the bilayer. The bilayer membrane structure is also found in aggregate structures called liposomes. Liposomes are microscopic vesicles consisting of an aqueous core enclosed in one or more phospholipid layers. They are formed when phospholipids are vigorously mixed with water. Unlike micelles, liposomes have both aqueous interiors and exteriors. Liposomes therefore are extremely valuable for encapsulation of aqueous food, flavor components or lipid soluble components. The liposome protects the guest material from oxidation or enzymatic attack.

Soy beans are a rich source of phospholipids which are extracted and refined for use as emulsifiers n foods. Table 2.17 illustrates the range and

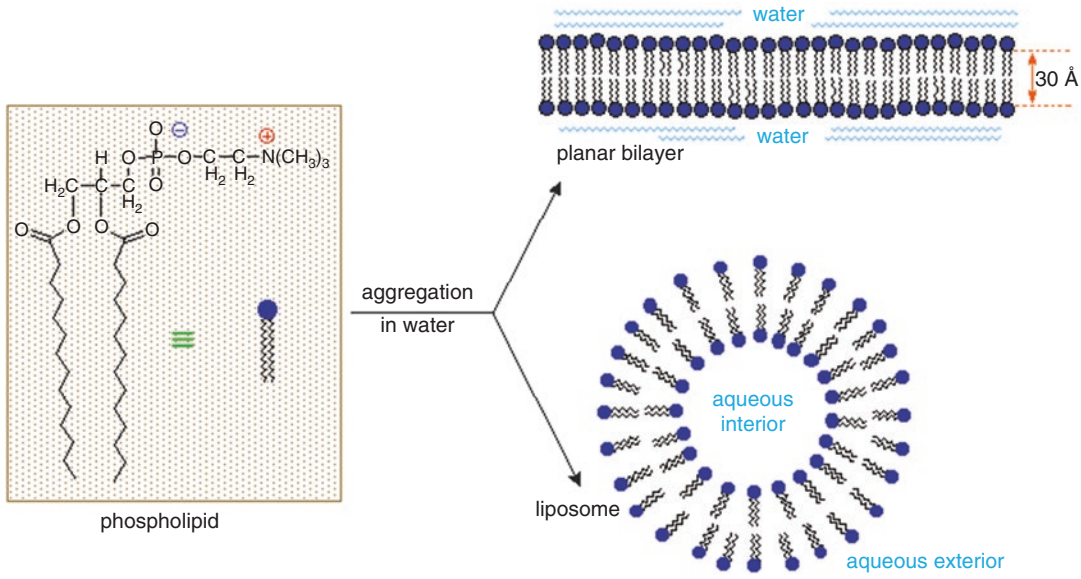


Fig. 2.15 Phospholipids form lipid bilayers and lipid micelles

Table 2.17 Range of phospholipids in soy

Component	Shorthand abbreviation	Low	Intermediate	High
Phosphatidylcholine	PC	12.0–21.0	29.0–39.0	41.0–46.0
Phosphatoidylethanolamine	PE	8.5–9.5	20.0–26.3	31.0–34.0
Phosphatidylinositol	PI	1.7–7.0	13.0–17.5	19.0–21.0
Phosphatoidic Acid	PA	0.2–1.5	5.0–9.0	14
Phosphatidyl Serine	PS	0.2	5.9–6.6	–
Lysophosphatidylcholine	LPC	1.5	8.5	–
Lysophosphatidylinositol	PLI	0.4–1.8	–	–
Lysophosphatidylserine	LPS	1	–	–
Lysophosphatidic acid	LPA	1	–	–
Phytoglycolipids	–	–	14.3–15.4	29.6

Szuhaj (1989)

Table 2.18 Total and principal phospholipids of different foods

Food	Total lipid	Total phospholipid	PC	PE	PS	PI
	g/100 g product	mg/100 g product				
Beef (L. dorsi) Fattened	12.4	690	340[a]	124	96[b]	32
Beef (L. dorsi) Lean	1.7	597	260[a]	106	48[b]	44
Pork (L. dorsi)	2.58	596	304	167	57[c]	
Chicken breast	1.12	782	391	187	100	
Chicken thigh	3.26	1386	662	352	186	tr
Turkey breast	0.73	418	231	92	33[b]	
Turkey thigh	2.48	418	231	92	34[b]	
Cod	0.59	520	359	99	26	
Tuna (Dorsal)	3.79	617	166	132	93	
Cow milk	3.66	34	12	10	1	2
Egg Yolk	31.8	10,306	6771	1917		64
Peanut	48.5	620	270	50		150
Soybean	20.8	2038	917	536		287
Corn	3.7	213	139	15		26
Hard wheat (whole grain)	2.5	1060	164	56		69

[a]PC + LPC
[b]PS + PA + CL
[c]PS + PI
PC phosphatidyl choline, *PE* phosphatidyl ethanolamine, *PS* phosphatidyl Serine, *PI* phosphatidyl Inositol, *LPC* lysophosphatidyl choline
Adapted from Weihrauch and Son (1983)

distribution of soy phospholipids. Table 2.18 contains distribution of phospholipids found in a variety of foods. Frequently the phosphatidyl choline is refined and sold as lecithin which can be used as an emulsifier in foods and cosmetics.

Unsaponifiables

The unsaponifiable fraction of fats contains sterols, terpenic alcohols, aliphatic alcohols, squalene, and hydrocarbons. The distribution of the various components of the unsaponifiable fraction in some fats and oils is given in Table 2.19. In most fats the major components of the unsaponifiable fraction are sterols.

Terpenes

Compounds classified as terpenes constitute a large and diverse group of natural compounds. A majority of these compounds are found only in plants, but some of the larger and more complex terpenes (e.g. squalene & lanosterol) occur in animals. Are classified by the number and structural organization. Terpenes are primarily made of isoprene (more accurately isopentane) units, an empirical feature known as the **isoprene rule**. Because of this, terpenes usually have 5n carbon atoms (n is an integer), and are subdivided as follows (Table 2.20):

Isoprene itself, a C5H8 gaseous hydrocarbon, is emitted by the leaves of various plants as a natural byproduct of plant metabolism. Next to methane it is the most common volatile organic compound found in the atmosphere. Examples of C10 and higher terpenes, representing the four most common classes are shown in Fig. 2.16.

Steroids

The class of lipids called steroids is recognized by their tetracyclic skeleton, consisting of three fused six-membered and one five-membered ring.

Table 2.19 Composition of the unsaponifiable fraction of some fats and oils

Oils	Hydrocarbons	Squalene	Aliphatic alcohols	Terpenic alcohols	Sterols
Olive	2.8–3.5	32–50	0.5	20–26	20–30
Linseed	3.7–14.0	1.0–3.9	2.5–5.9	29–30	34.5–52
Tea seed	3.4	2.6	–	–	22.7
Soybean	3.8	2.5	4.9	23.2	58.4
Rapeseed	8.7	4.3	7.2	9.2	63.6
Corn	1.4	2.2	5.0	6.7	81.3
Lard	23.8	4.6	2.1	7.1	47.0
Tallow	11.8	1.2	2.4	5.5	64.0

Source: From G. Jacini, E. Fedeli, and A. Lanzani, Research in the Nonglyceride Substances of Vegetable Oils, *J. Assoc. Off. Anal. Chem.*, Vol. 50, pp. 84–90, 1967

Table 2.20 Terpene

Classification	Isoprene units	Carbon atoms
monoterpenes	2	C10
sesquiterpenes	3	C15
diterpenes	4	C20
sesterterpenes	5	C25
triterpenes	6	C30

Steroids structures are not based a glycerol backbone or ester linkages. The four rings in sterols structures are designated A, B, C & D shown in Fig. 2.17. The numbers in red represent the numbering of the ring carbon atoms in the sterol structure. The substituents designated by R are often alkyl groups. The R group at the A:B ring fusion is most commonly methyl or hydrogen, the R group at the C:D fusion is generally a methyl group. The R- substituent at C-17 varies considerably, and is usually larger than methyl if it is not a functional group. The most common locations of functional groups are C-3, C-4, C-7, C-11, C-12 & C-17.

Animal fats contain cholesterol and, in some cases, minor amounts of other sterols such as lanosterol. Plant fats and oils contain phytosterols, usually at least three, and sometimes four (Fedeli and Jacini 1971). Plants can contain trace amounts or cholesterol. The predominant phytosterol found in plants is β-sitosterol; others include campesterol and stigmasterol. In rapeseed oil, brassicasterol takes the place of stigmasterol (Table 2.21). Table 2.22 contains the typical phytosterol content found in servings of plant foods. Plant sterols are beneficial in helping control cholesterol re-absorption thus possibly educing serum cholesterol. Wheat germ and rice bran oil are excellent sources of phytosterols. Sterols contain the perhydrocyclopenteno-phenanthrene nucleus, which is shared in common with many other natural compounds, including bile acids, hormones, and vitamin D. The nucleus and the description of the four rings, as well as the system of numbering of the carbon atoms, are shown in Fig. 2.18a. The sterols generally have high melting points and therefore are solid at room or body temperatures. Stereochemically they are relatively flat molecules, usually with all *trans* linkages, as shown in Fig. 2.18. The ring junction between rings A and B is *trans* in some steroids, *cis* in others. The junctions between B and C and C and D are normally *trans.* Substituents that lie above the plane, as drawn in Fig. 2.18c, are named β, those below the plane, α. The 3-OH group in cholesterol (Fig. 2.18c) is the β-configuration, and it is this group that may form ester linkages. The structuresof the major plant sterols is given in Fig. 2.15. Part of the sterols in natural fats are present as esters of fatty acids; for example, in milk fat, about 10% of the cholesterol occurs in the form of cholesterol esters.

Cholesterol has 256 stereoisomers, although only two of them are of biochemical significance (nat-cholesterol and ent-cholesterol,[l]and only one of them occurs naturally (nat-cholesterol). In animal tissues, **cholesterol** (cholest-5-en-3β-ol) is by far the most abundant member of a family of polycyclic compounds known as sterols.

In animals cholesterol has an essential structural role in membranes and in lipid metabolism. Cholesterol is the biosynthetic precursor of bile acids, vitamin D and steroid hormones (glucocor-

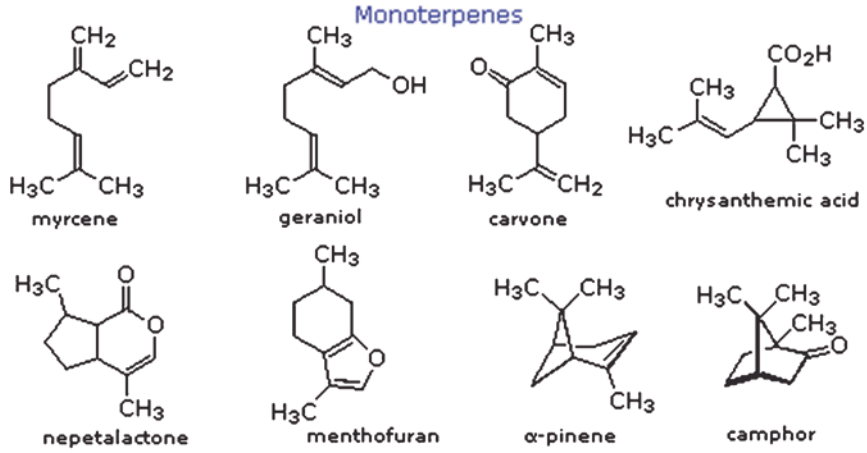


Fig. 2.16 Structures of monoterpenes

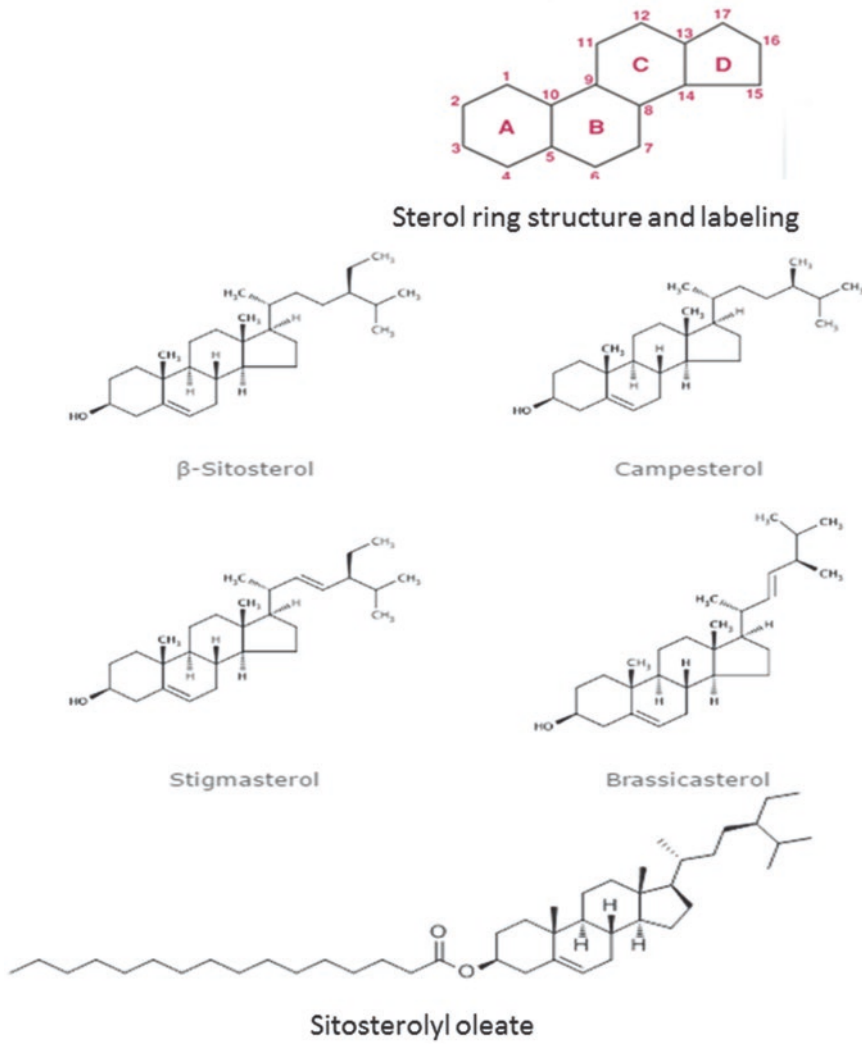


Fig. 2.17 Sterol structure and major plant sterols

Table 2.21 Sterol content of fats and oils

Fat	Sterol (%)
Lard	0.12
Beef tallow	0.08
Milk fat	0.3
Herring	0.2–0.6
Cottonseed	1.4
Soybean	0.7
Corn	1.0
Rapeseed	0.4
Coconut	0.08
Cocoa butter	0.2

Table 2.22 Phytosterol content of selected food oils

Total Phytosterol per serving of various food oils		
Food	Serving	Phytosterols (mg)
Wheat germ	½ cup (57 g)	197
Rice bran oil	1 tablespoon (14 g)	162
Sesame oil	1 tablespoon (14 g)	118
Corn oil	1 tablespoon (14 g)	102
Canola oil	1 tablespoon (14 g)	92
Peanuts	1 ounce (28 g)	62
Wheat bran	½ cup (29 g)	58
Almonds	1 ounce (28 g)	39
Brussels sprouts	½ cup (78 g)	34
Rye bread	2 slices (64 g)	33
Macadamia nuts	1 ounce (28 g)	33
Olive oil	1 tablespoon (14 g)	22
Benecol® spread	1 tablespoon (14 g)	850 mg plant stanol esters (500 mg free stanols)

http://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/phytosterols; USDA Nutrient Database for Standard Reference, Release 20. 2007. Available at: http://ndb.nal.usda.gov//. Accessed 7/24/08.; (Norman et al. 2002; Normen et al. 1999; Phillips et al. 2002)

ticoids, estrogens, progesterone's, androgens and aldosterone), the central nervous system, and it has major functions in signal transduction and males,m it is required for sperm development. Plasma cholesterol levels are also risk factors for heart disease because they can be a major contributory factor to atherogenesis. Cholesterol is ubiquitous in in the membranes all animal tissues (and of some fungi, although it is not evenly distributed. The highest levels of unesterified cholesterol are found in plasma membrane (roughly 30–50% of the lipid in the membrane or 60–80%

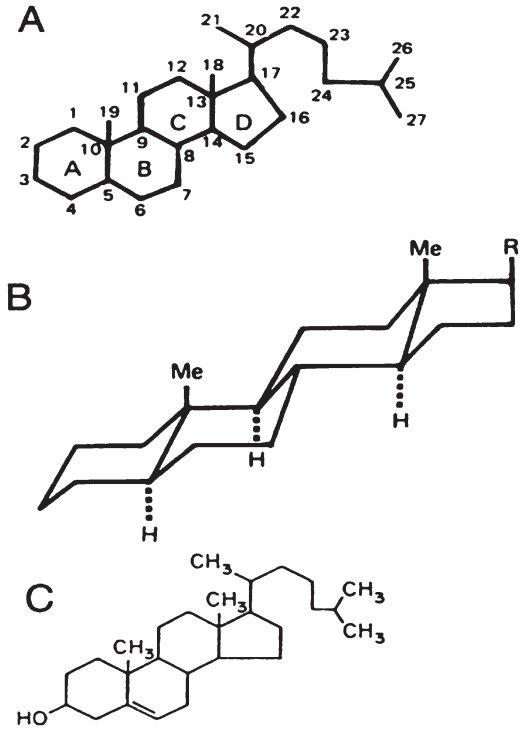


Fig. 2.18 Sterols. (**a**) Structure of the steroid nucleus, (**b**) stereochemical representation, and (**c**) Cholesterol

of the cholesterol in the cell). Cholesterol levels in mitochondria and the endoplasmic reticulum are very low and the Golgi contains intermediate levels. The brain contains more cholesterol than any other organ, where it comprises roughly a quarter of the total free cholesterol in the human body. Cholesterol can occur in the free form, esterified to long-chain fatty acids (cholesterol esters), and in other covalent and non-covalent linkages in animal tissues, including the plasma lipoproteins. In plants, it tends to be a minor component only of a complex mixture of structurally related phytosterols, although there are exceptions, but it is nevertheless importance as a precursor of some plant hormones. http://lipidlibrary.aocs.org/Lipids/simple.html.

Phytosterols

The sterols provide a method of distinguishing between animal and vegetable fats by means of their acetates. Cholesterol acetate has a melting

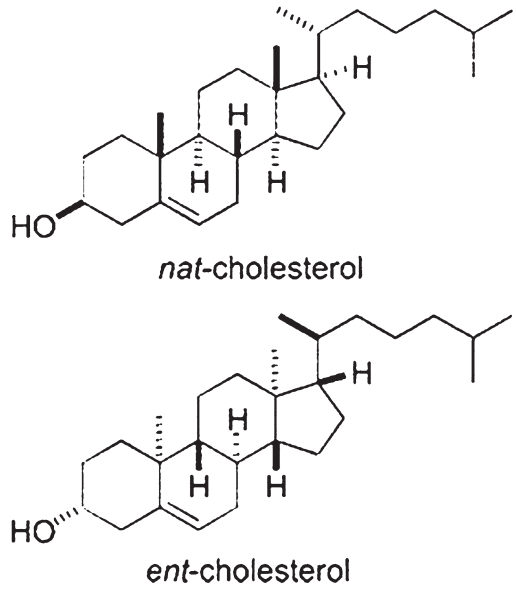


Fig. 2.19 Isomeric forms of cholesterol

point of 114 °C, whereas phytosterol acetates melt in the range of 126–137 °C. This provides a way to detect adulteration of animal fats with vegetable fats.

The most common phytosterols and phytostanols (examples of structures are shown in Fig. 2.19 are sitosterol (3β-stigmast-5-en-3ol; CAS number 83-46-5), sitostanol (3β,5α-stigmastan-3-ol; CAS Number 83-45-4), campesterol (3β-Ergost-5-en-3-ol; CAS Number 474-62-4), campestanol (3β,5α-ergostan-3-ol; CAS Number 474-60-2), stigmasterol (3β-stigmasta-5,22-dien-3-ol; CAS Number 83-48-7) and brassicasterol (3β-ergosta-5,22-dien-3-ol; CAS Number 474-67-9). Each commercial source has its typical phytosterols composition. Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil or corn oil, or from so-called "tall oil", a by-product of the manufacture of wood pulp. Phytosterols can be hydrogenated to obtain phytostanols. Phytosterols and phytostanols are high melting powders. Phytostanol and phytosterol esters are chemically stable materials, having comparable chemical and physical properties to edible fats and oils. The substances are insoluble in water, but soluble in non-polar solvents, such as hexane, iso-octane and 2-propanol. The esters are also soluble in vegetable fats and oils. Phytostanol and phytosterol esters are added to margarine and promoted for reduction of serum cholesterol.

Edible vegetable oils, extracted from oil seeds, are typically refined to remove minor oil components such as phosphatides, free fatty acids, oxidized fatty acids, pigments and odors, while minimizing damage to the glycerides. The most common processes are referred to as either physical or chemical. Chemical or alkaline refining an alkali is used to neutralize the free fatty acids which are removed as soapstock. The chemical refining process consists of water degumming which where the gums a dried and used to produce lecithin, aalkal neutralization resulting in sproduction of soap stock, dewaxing, bleaching, and deodorization yielding edible oils. In physical refining acid degumming yields gums, dewaxing bleaching, are followed by deacidification yielding fatty acids and deodorization procedure comprises degumming, neutralization, bleaching and deodorization. In physical refining the neutralization step is omitted and the residual free fatty acids are removed in the final deodorization step. Deodorization is the last step in the edible oil refining process in which volatiles are removed that can adversely affect the stability, flavor, and odor of the oil.

This process relies on the large volatility differences between the oil itself (triglycerides) and the volatile compounds to be removed and is carried out under reduced pressure, an elevated temperature in the presence of a stripping gas. The volatiles are recovered in a vapor condenser. This distillate mainly contains free fatty acids, but also significant levels of tocopherols (5–15%) and phytosterols (8–20%).

In a transesterification (methanolysis) step, the glycerides are converted into fatty acid methyl esters and glycerol and the phytosterol-esters into free phytosterols and fatty acid methyl esters. After removal of the methanol/glycerol phase, the methyl esters are removed and the free phytosterols and tocopherols removed by distillation. The phytosterols are separated from the tocoph-

Table 2.23 Hydrocarbon composition of some vegetable oils

Oils	n-Paraffins	iso-and/or ante-iso Paraffins	Unidentified	Total Hydrocarbons
Corn	$C_{11–31}$	$C_{11–21}$	8	40
Peanut	$C_{11–30}$	$C_{11–23}$	7	40
Rapeseed	$C_{11–31}$	$C_{11–17}$, $C_{19–21}$	6	36
Linseed	$C_{11–35}$	$C_{11–21}$	7	43–45
Olive	C_{11}, $C_{13–30}$	–	6	29

Source: From G. Jacini, E. Fedeli, and A. Lanzani, Research in the Nonglyceride Substances of Vegetable Oils, *J. Assoc. Off. Anal. Chem*., Vol. 50, pp. 84–90, 1967

erols by solvent crystallization and filtration using food grade solvent. The phytosterols are further purified by re-crystallisation, mainly to remove wax-esters (Table 2.23).

Plants also produce low levels of paraffin hydrocarbons. Traces of hydrocarbons remain in vegetable oils, however most are removed during refining of the oil. Table 2.23 the contents of hydrocarbons in some common vegetable oils.

Lipid Reactions

Lipids contain two main functions which become involved in chemical reactions. Lipids that are unsaturated can undergo oxidation reactions both in food and in vivo. Lipids with ester bonds such as triglycerides, phospholipids and waxes can undergo hydrolysis or interesterification. The interesterification reactions are commercially important for formation of modified triglycerides and for analytical purposes forming esters of fatty acids for analysis. Interesterifcation reactions are important for forming new triglycerides with desired functionalities.

The acidity of the carboxylic acids function on fatty acids results in react with bases to form ionic salts, as shown below (Fig. 2.20). In the case of alkali metal hydroxides and simple amines (or ammonia) the resulting salts have pronounced ionic character and are usually soluble in water.

Fatty Acid Salts

RCO_2H	+	$NaHCO_3$	→	$RCO_2(-)\ Na(+) + CO_2 + H_2O$
RCO_2H	+	$(CH_3)_3N$:	→	$RCO_2(-)\ (CH3)_3NH(+)$

Hydrolysis

Interesterification

Interesterification can be defined as a redistribution of the fatty acid moieties present in triglycerides. In the presence of certain catalysts, the fatty acid radicals can be made to move between hydroxyl positions so that an essentially random fatty acid distribution results, according to the following reaction pattem (Formo 1954):

$$RCOOR^2 + R^1COOR^3 \longrightarrow RCOOR^3 + R^1COOR^2$$

Interesterification is used in industry to modify the crystallization behavior and the physical properties of fats. It can also be used to produce solid fats for margarine and shortening that are low in *trans* fatty acids. An additional advantage is that polyunsaturated fatty acids, which are destroyed during hydrogenation, are not affected. Several types of interesterification are possible. A fat can be randomized by carrying out the reaction at temperatures above its melting point, several raw materials may be interesterified together so that a new product with desired physical properties results. Fat can be interesterified at a temperature below its melting point so that only the liquid fraction reacts (this is known as directed interesterification). The effect of randomization can be demonstrated with the case of a mixture of equal amounts of two simple glycerides, such as triolein and tristearin. Interesterification of two equal glycerides (Fig. 2.21 OOO SSS) such as triolein and tristearin results in the formation of six possible triglycerides. When the blend of the two glycerides is other than in equal quantities the results can be

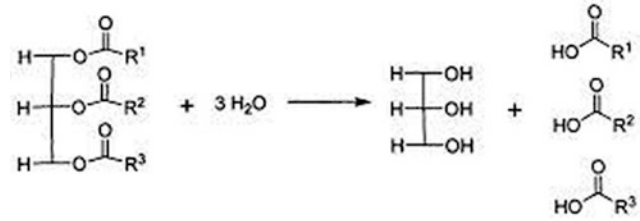


Fig. 2.20 Salt formation of fatty acids and hydrolysis of triglycrides

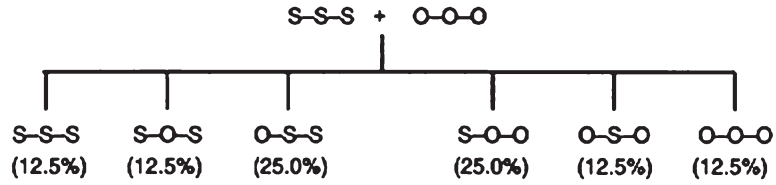


Fig. 2.21 The interesterification of equamolar quantities of triolein and tristearin

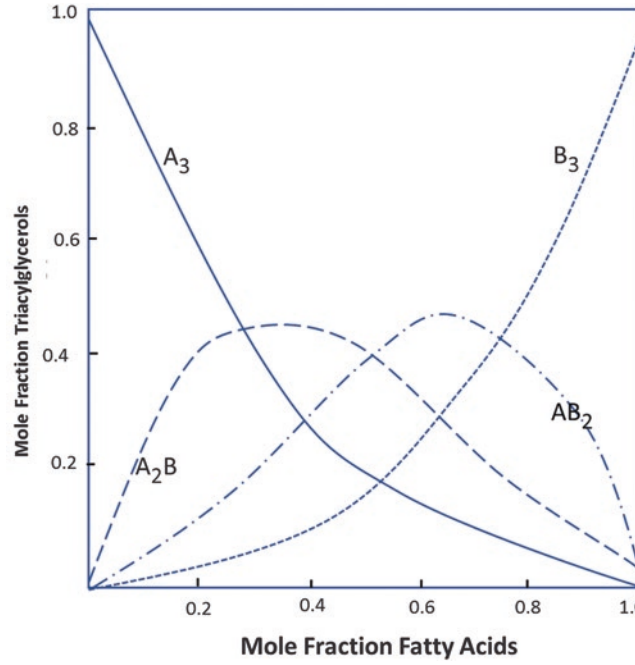


Fig. 2.22 Triacylglycerol distribution in a randomized binary mixture. *Adapted* from: A. Rozenaal, Interesterification of Oils and Fats, *Inform,* 3, pp. 1233–1235, © 1992, AOCS Press

derived from a graph such as the one in Fig. 2.22. The graph indicates that the maximum levels of the intermediate glycerides A_2B and AB_2 are formed at molar fractions of one-third A or one-third B.

The theoretical number of glycerides formed by interesterification of mixtures containing different fatty acids has been described by Rozenaal (1992) and is shown in Table 2.24. The table also gives the formula for calculating the total number

Table 2.24 Theoretical Triacylglycerol Composition after Interesterification for ***n*** Fatty Acids (A, B, C, D) with Molar Fractions a, b, c, d

Type	Number	Amount
Mono acid (AAA, BBB)	n	a^3, b^3, c^3
Diacid (AAB, AAC)	$n(n–1)$	$3a^2b$, $3ab^2$, $3a^2c$
Triacid (ABC, BCD)	$1/6\ n(n–1)\ (n–2)$	6abc, 6acd
Total	$n^3/6 + n^2/2 + n/3$	

of glycerides formed. For example, for $n = 4$ the number of glycerides formed is 20 and for $n = 6$ the number is 56. Thus, interesterification results in increased complexity of the oil.

This is results in a randomized distribution of the fatty acids on the glycerol moiety when the reaction is carried out in a single, liquid phase.

In directed interesterification, one of the reaction components is removed from the reaction mixture. This can be achieved by selecting a reaction temperature at which the trisaturated glycerides become insoluble and precipitate. The equilibrium is then disturbed and more trisaturates are formed, which can then be precipitated. Because of the low temperature employed, the reaction is up to 10–20 times slower than the random process. Another procedure of directed interesterification involves the continuous distilling of low molecular weight fatty acids, such as those present in coconut oil with high free fatty acid content (Hustedt 1976).

The reaction mechanism of interesterification using sodium methoxide as a catalyst is a two-step process (Sreenivasan 1978; Rozenaal 1992). First, the catalyst combines with the glyceride at one of the carbonyl locations (Fig. 2.23). Then the anion of the catalyst and the alkoxy group of the ester are exchanged. The catalyst has changed but remains active. At the end of the reaction there remains an amount of fatty acid methyl ester equivalent to the amount of sodium methoxide catalyst used. The randomization reaction continues until equilibrium has been reached. The reaction is terminated by destroying the catalyst through addition of water or organic acid, which converts the fatty acid methyl ester into free fatty acid. The reaction is intramolecular as well as intermolecular. An active catalyst for interesterification can be developed with sodium hydroxide and glycerol which form an active catalyst as illustrated in Fig. 2.24. Freeman (1968) has reported that the intramolecular rearrangement occurs at a faster rate than the general randomization.

Rozenaal determined the reaction rate for the randomization of palm oil (Fig. 2.25). The reaction rate, which was measured by determination of the solid fat content, increased with temperature. There is evidence of an induction period at lower temperatures.

Random interesterification can result in either an increase or a decrease in melting point and solid fat content, depending on the composition of the original fat or fat blend. When cocoa butter is interesterified, the unique melting properties are completely changed (Fig. 2.26). Cocoa butter is a relatively expensive fat, used in confectionery, because of its sharp melting point between room temperature and body temperature; chocolate literally melts in the mouth. This is due to the fairly small variation in the structure of the constituent triglycerides; 80% have palmitic acid or stearic acid in the 1 and 3 positions with oleic acid in the central 2 position. Cocoa butter substitutes have been produced from palm oil, the acid hydrolysis is accomplished by dissolving stearic acid in hexane containing enough water to activate the lipase. Olive oil may be similarly improved by exchanging its 1,3-oleic acid residues for palmityl groups. The products may be recovered by recrystallization from aqueous acetone.

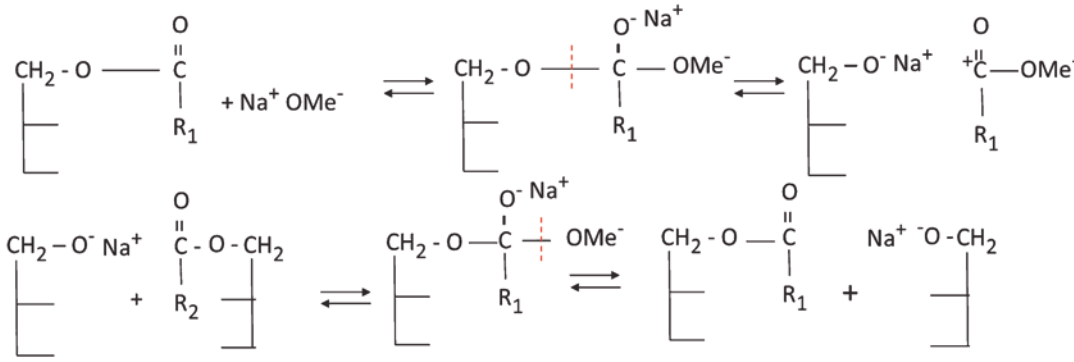


Fig. 2.23 Reaction mechanism of the interesterification process

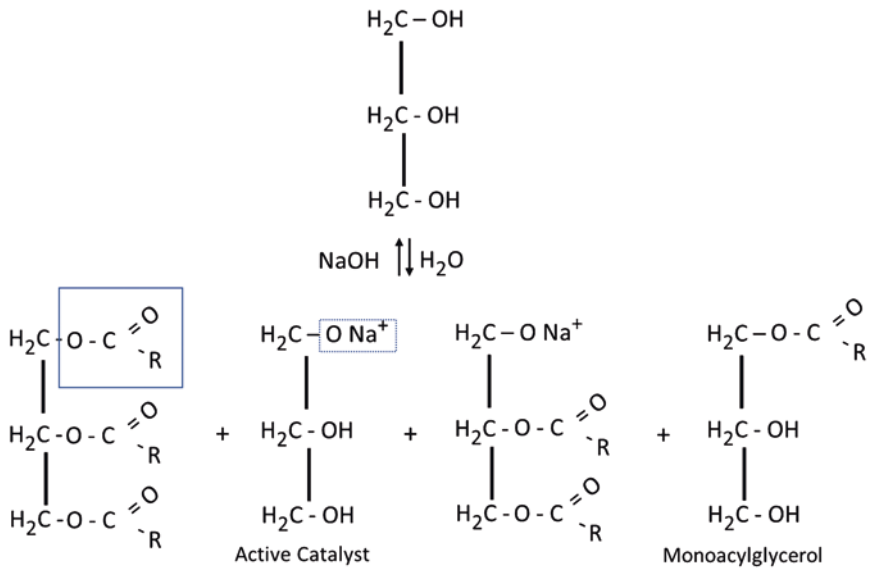


Fig. 2.24 Formation of interesterification catalyst from sodium hydroxide and glycerol in a two-step process. *Adapted from:* A. Rozenaal, Interesterification of Oils and Fats, *Inform,* 3, pp. 1233–1235, © 1992, AOCS Press

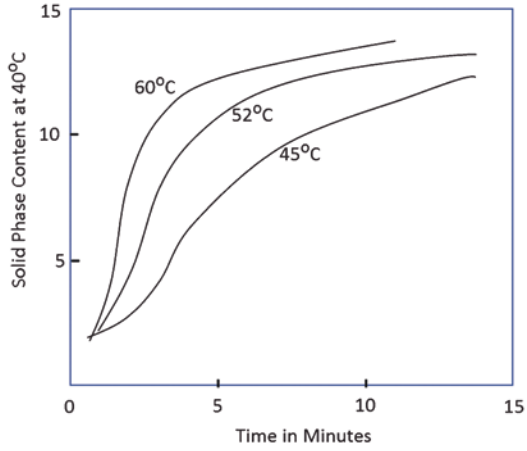


Fig. 2.25 Randomization of palm oil at different temperatures. *Adapted from:* A. Rozenaal, Interesterification of Oils and Fats, *Inform,* 3, pp. 1233–1235, © 1992, AOCS Press

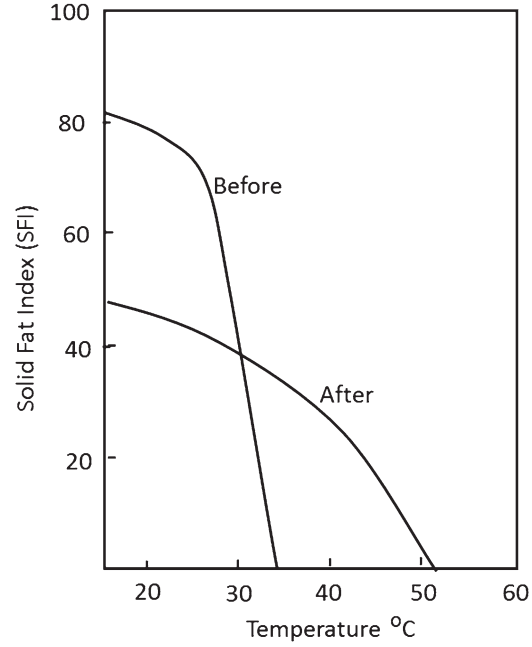


Fig. 2.26 Solid fat index (SFI) of cocoa butter before and after interesterification

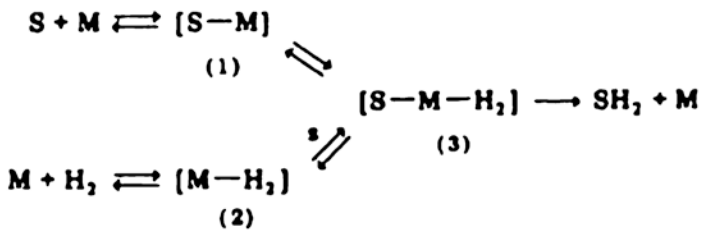
Interesterification of lard has been used extensively. Lard produces coarse crystals because it tends to crystallize in the β form. Palmitic acid is mostly located in the sn-2 position of the disaturated glycerides (S_2U). When lard is randomized, the level of palmitic acid in the sn-2 position drops from 64 to 24%. The result is a smooth-textured fat that crystallizes in the β′ form. Randomized lard has an improved plastic range and makes a better shortening. Palm oil shows the phenomenon of post-hardening or post-crystallization. This is a disadvantage in a number of applications. Interesterification eliminates this problem.

In the formulation of margarines and shortenings, a hardstock is often combined with unmodified liquid oil. A useful hardstock for the formulation of soft margarines is an interesterified blend of palm stearin and palm kernel oil or fully hydrogenated palm kernel oil. Interesterification is used to produce *trans* free fats for making margarines and shortenings. The traditional method for transforming oils into fats involves hydrogenation and this results in high *trans* levels. The physical and chemical properties of *trans* free fats made by interesterfication have been described by Petrauskaite et al. (1998).

Ester interchange of fats with a large excess of glycerol, at high temperature, under vacuum, and in the presence of a catalyst, results in an equilibrium mixture of mono-, di-, and triglycerides. After removal of excess glycerol, the mixture is called technical monoglyceride and contains about 40% of 1-monoglyceride. Technical monoglycerides are used as emulsifying agents in foods. Molecular distillation yields products with well over 90% 1-monoglycerides; the distilled monoglycerides are also widely used in foods. Interesterification can also be carried out by using lipase enzymes as a catalyst. This type of application is described in Chap. 10.

Hydrogenation

Hydrogenation of fats is a chemical reaction consisting of addition of hydrogen at double bonds of unsaturated acyl groups. This reaction is of great importance to industry, because it permits the conversion of liquid oils into plastic fats for the production of margarine and shortening. For some oils, the process also results in a decreased susceptibility to oxidative deterioration. In the hydrogenation reaction, gaseous hydrogen, liquid oil, and solid catalyst participate under agitation in a closed vessel. Although most industrial processes use solid nickel catalysts, interest in organometallic compounds that serve as homogeneous catalysts has increased greatly. Frankel and Dutton (1970) have represented catalytic hydrogenation by the following scheme, in which the reacting species are the olefinic substrate (S), the metal catalyst (M), and H_2:



The intermediates 1, 2, and 3 are organometallic species. If the reaction involves heterogeneous catalysis, the olefins and hydrogen are bound to the metal by chemisorption. If homogeneous catalysis takes place, the intermediates are organometallic complexes. The intermediates are labile and short-lived and cannot usually be isolated. In heterogeneous catalysis, the surface of the metal performs the function of catalyst and the preparation of the catalyst is of major importance. When hydrogen is added to double bonds in a natural fat consisting of many component glycerides and different component unsaturated fatty acids, the result depends on many factors, if the reaction is not carried to completion. Generally, hydrogenation of fats is not carried to completion and fats are hydrogenated only partially. Under these conditions, hydrogenation may be selective or nonselective. Selectivity means that hydrogen is added first to the most unsaturated fatty acids. Selectivity is increased by increasing hydrogenation temperature and

decreased by increasing pressure and agitation. Table 2.25 shows the effect of selectivity on the properties of soybean oil. The selectively hydrogenated oil is more resistant to oxidation because of the preferential hydrogenation of the linolenic acid. The influence of selectivity conditions on the fatty acids of hydrogenated cottonseed and peanut oil is demonstrated by the data presented in Table 2.26 The higher the selectivity, the lower the level of polyunsaturated fatty acids will be and the higher the level of monounsaturates.

It is now commonly accepted that the nickel-catalyzed hydrogenation of unsaturated fatty acids follows the Horiuti-Polanyi mechanism. According to this mechanism, molecular hydrogen is adsorbed onto the nickel surface (reaction 1 in Fig. 2.27 where adsorbed species are indicated by an asterisk) and dissociated into two hydrogen atoms (reaction 2). Fatty acids are also adsorbed onto this nickel surface by their double bond or bonds and in a first step, a hydrogen atom is added to this bond to form a half-hydrogenated intermediate. If a second hydrogen atom is then added to this intermediate, the original double bond has been saturated but because the first addition is reversible, the intermediate can also dissociate. http://lipidlibrary.aocs.org/processing/hydrog-mech/index.htm.

Figure 2.27 shows how dienes (D for short) are hydrogenated to from monoenes (M) and finally stearic acid (D). So linoleic acid (9*c*,12*c*–octadecadienoic acid, *c,c*-D) is reversibly adsorbed in reaction 3 and a hydrogen atom H* is reversibly added to the adsorbed linoleic acid (*c,c*-D*) to form a half-hydrogenated intermediate (*c*-DH*). This is still adsorbed as shown by the asterisk (*) but has only a single double bond left that has retained its *cis*-configuration. This half-hydrogenated intermediate can do one of several things. It can react irreversibly with a further hydrogen atom (H*) in reaction 10 or it can dissociate.

The hydrogen atom leaving on this dissociation can be the same as the one that has been added as shown in reaction −4 (where the minus sign indicates the reverse reaction), it can be a different atom on the same carbon atom or it can be a hydrogen atom leaving from a different carbon atom. Accordingly, the fatty acid resulting from this dissociation can have undergone geometrical isomerisation so that the original *cis*-configuration of the double bond has been changed into a *trans*-configuration as shown in reaction 5. It can also have undergone positional isomerisation meaning that the double bond has shifted one position along the fatty acid chain; this type of isomerisation is not shown in the figure. In methylene-interrupted polyunsatu-

Table 2.25 Differences in selective and nonselective hydrogenation of soybean oil

Characteristic	Selective	Non-selective
Induction period AOM (h)	240	31
Micropenetration	70 (more plastic)	30
Capillary mp (°C)	39	55
Condition		
Temp (°C)	177	121
Pressure (psi)	5	50
Ni catalyst (%)	0.05	0.05

Source: From W.O. Lundberg, *Autoxidation and Antioxidants*, 1961, John Wiley & Sons

Table 2.26 Fatty acid composition of cottonseed and peanut oil hydrogenated under different conditions of selectivity to iodine value 65

Oil	Hydrogenation conditions	Fatty acids		
		Saturated (%)	Oleic (%)	Linoleic (%)
Cottonseed	Moderately selective	31.5	64.5	4.0
Peanut	Moderately selective	27.5	72.5	–
Cottonseed	Nonselective	36.0	56.0	8.0
Peanut	Nonselective	30.0	67.0	3.0
Cottonseed	Very nonselective	39.5	48.5	12.0
Peanut	Very nonselective	33.0	61.0	6.0

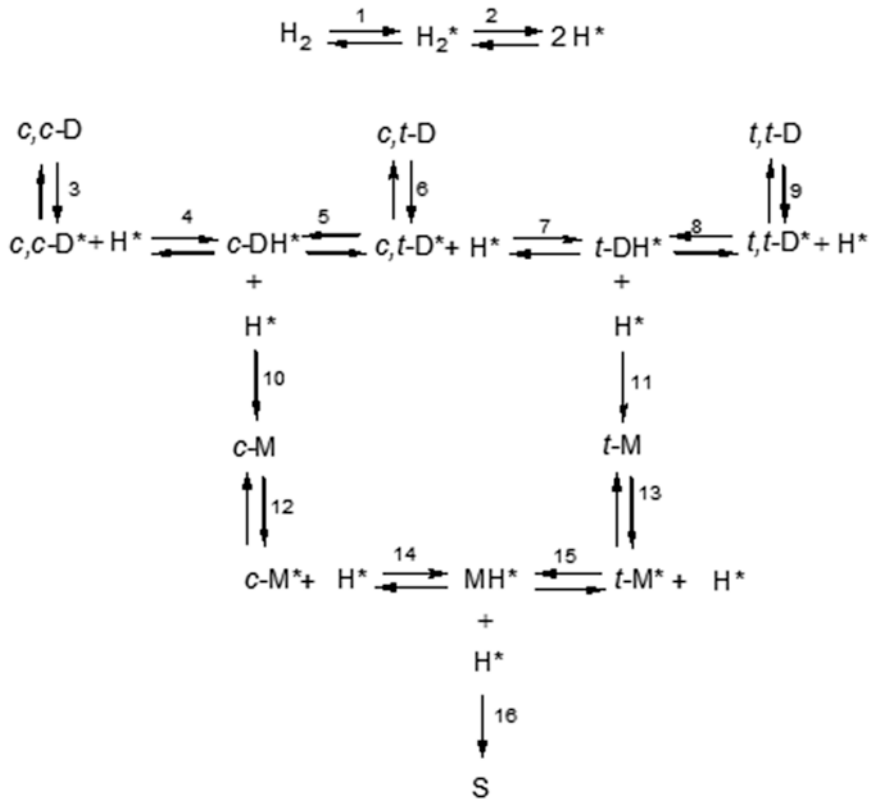


Fig. 2.27 Horiuti-Polanyi mechanism for hydrogenation with nickel catalyst. http://lipidlibrary.aocs.org/processing/hydrog-mech/index.htm

rated fatty acids this can lead to the formation of conjugated double bonds. Because the double bond is no longer present in the half-hydrogenated intermediate, this intermediate can rotate around the original double bond and this can result in an isomerisation that is both geometrical and positional. http://lipidlibrary.aocs.org/processing/hydrog-mech/index.htm.

When a half-hydrogenated intermediate is saturated by reacting with another hydrogen atom, heat is liberated since the hydrogenation process is strongly exothermic. Consequently, the reaction product is so 'hot' that it immediately leaves the catalyst surface. By sharing its kinetic energy with its surroundings, it cools down so that it can be re-adsorbed at its remaining double bond (*c*-M*). This monoene can then react with an adsorbed hydrogen to form the half-hydrogenated monoene (MH*) that just as above can react in several ways one of which (reaction 16) leads to stearic acid. (http://lipidlibrary.aocs.org/processing/hydrog-mech/index.htm).

Another important factor in hydrogenation is the formation of positional and geometrical isomers. Formation of *trans* isomers is rapid and extensive. The isomerization can be understood by the reversible character of chemisorption. When the olefinic bond reacts, two carbon-metal bonds are formed as an intermediate stage (represented by an asterisk in Fig. 2.28). The intermediate may react with an atom of adsorbed hydrogen to yield the "half-hydrogenated" compound, which remains attached by only one bond. Additional reaction with hydrogen results in formation of the saturated compound. There is also the possibility that the half-hydrogenated olefin may again attach itself to the catalyst surface at a carbon on either side of the existing bond, with simultaneous loss of hydrogen. Upon desorption of this species, a positional or geometrical isomer

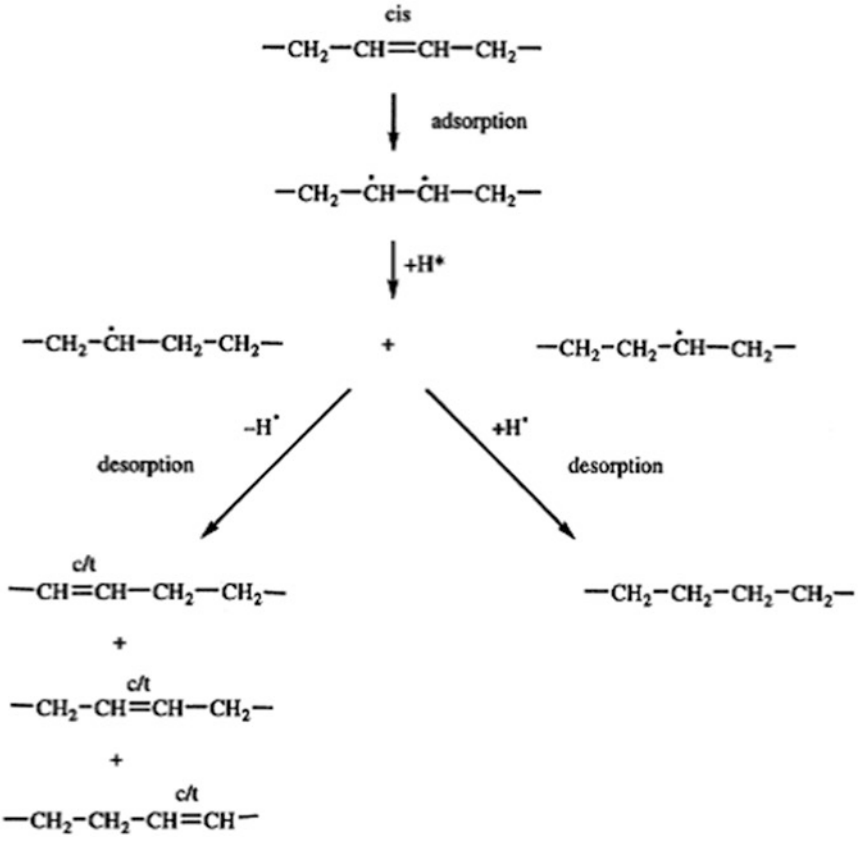


Fig. 2.28 Hydrogenation of an olefinic compound

may result. The proportion of *trans* acids is high because this is the more stable configuration.

Double bond migration occurs in both directions and more extensively in the direction away from the ester group. This is true not only for the *trans* isomers that are formed but also for the *cis* isomers. The composition of the positional isomers in a partially hydrogenated margarine fat is shown in Figs. 2.29 and 2.30 (Craig-Schmidt 1992). In a partially hydrogenated fat, the analysis of component fatty acids by gas-liquid chromatography is difficult because of the presence of many isomeric fatty acids. This is shown in the chromatogram in Fig. 2.31 (Ratnayake 1994).

Nickel catalysts are poisoned by sulfur and phosphorous compounds, free fatty acids, and residual soaps. Oils are refined and sometimes bleached before hydrogenation. Sulfur compounds are not easily removed from the oil. Oils that contain sulfur compounds are rapeseed oil, canola oil, and fish oils (Wijesundera et al. 1988). High-erucic rapeseed oil is very difficult to hydrogenate unless it is deodorized (deMan et al. 1995). Canola oils of the double-zero variety that are low in erucic acid and glucosinolates still contain traces of sulfur in the form of isothiocyanates (Abraham and deMan 1985).

When catalysts are poisoned by sulfur the hydrogenation reaction is slowed down and the formation of *trans* isomers is increased. U.S. stick margarines are reported to contain 24% of *trans* fatty acids, and soft margarines contain 14–18%. Shortenings contain 22.5%, and fats in snack foods contain up to 46% of *trans* fatty acids (Craig-Schmidt 1992).

It is difficult to eliminate oxidation-sensitive polyunsaturated fatty acids by partial hydrogenation of fish oils. This has been demonstrated by Ackman (1973) in the progressive hydrogenation of anchovetta oil. The original

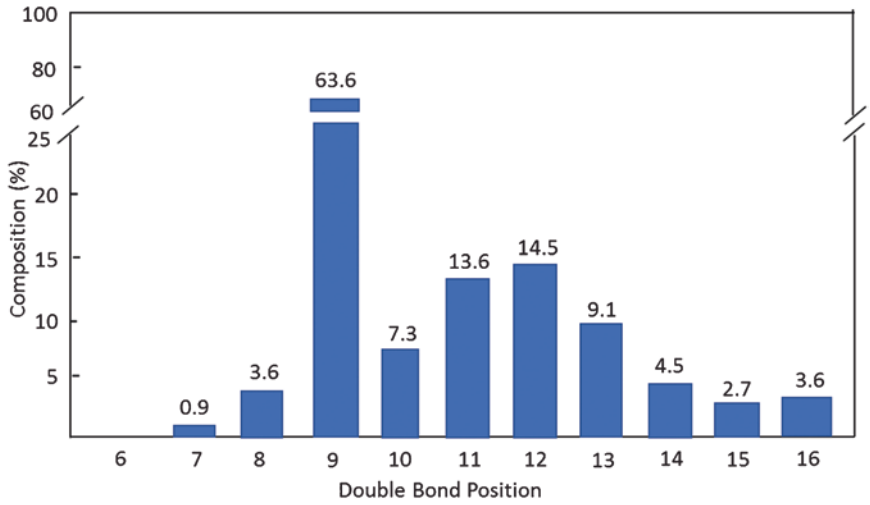


Fig. 2.29 Positional isomers of 18:1 *cis* formed in the partial hydrogenation of a margarine fat. *Adapted* from M.C. Craig-Schmidt, Fatty Acid Isomers in Foods, in *Fatty Acids in Foods and Their Health Implications*, C.K. Chow, ed., p. 369, 1992, by courtesy of Marcel Dekker, Inc.

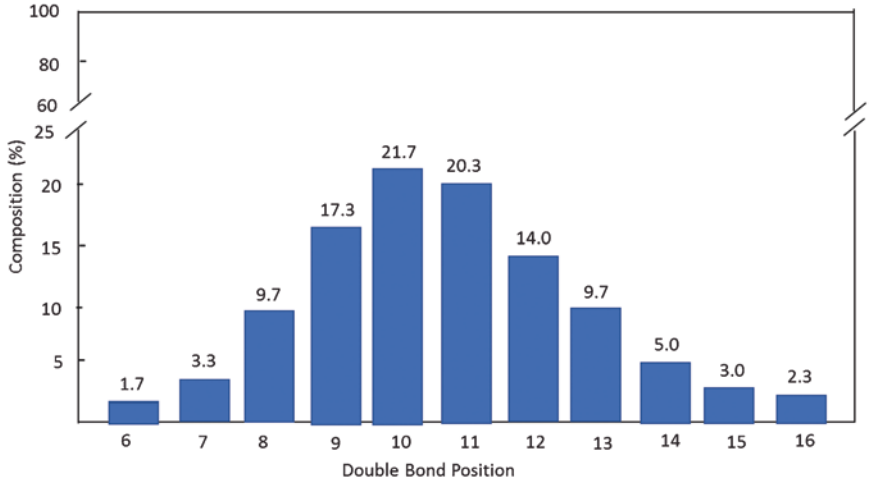


Fig. 2.30 Positional isomers of 18:1 *trans* formed in the partial hydrogenation of a margarine fat. *Adapted* from M.C. Craig-Schmidt, Fatty Acid Isomers in Foods, in *Fatty Acids in Foods and Their Health Implications*, C.K. Chow, ed., p. 369, 1992, by courtesy of Marcel Dekker, Inc.

eicosapentaenoic acid (20:5 ω 3) is not completely removed until an iodine value of 107.5 is reached. Even at this point there are other polyunsaturated fatty acids present that may be susceptible to flavor reversion.

In the nonselective hydrogenation of typical seed oils, polyunsaturated fatty acids are rapidly reduced and *trans*-isomer levels increase to high values. Figure 2.32 shows the hydrogenation of canola oil (de El-Shattory et al. 1982).

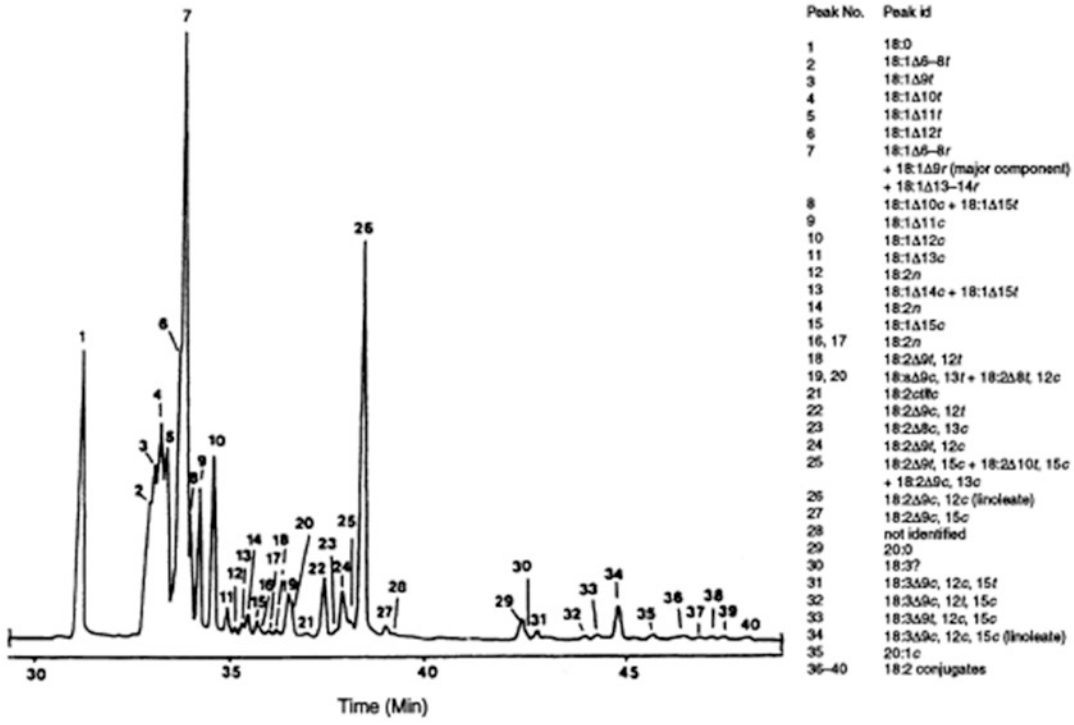
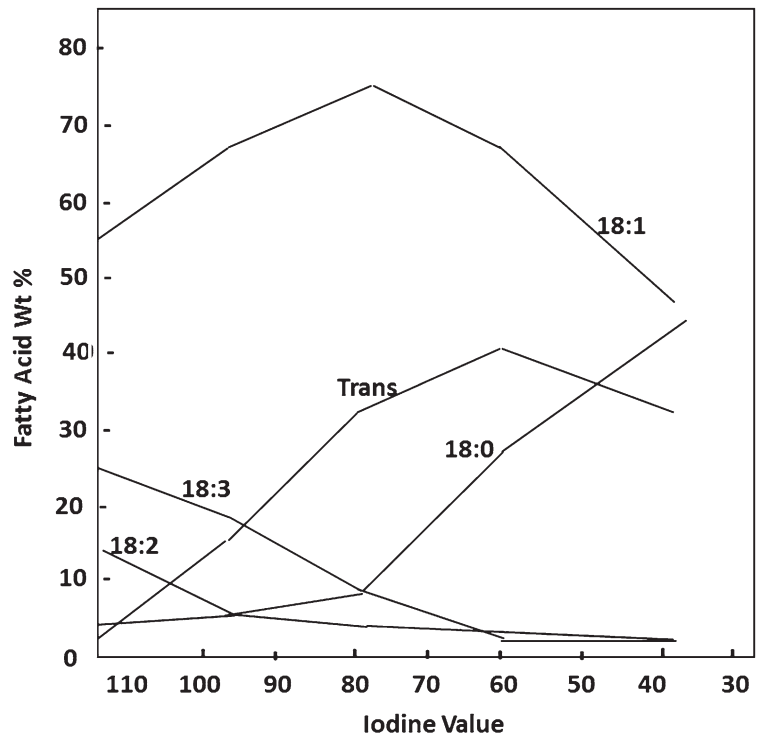


Fig. 2.31 Gas chromatogram of the fatty acid methyl esters from partially hydrogenated soybean oil, using a 100-m fused silica capillary column coated with SP2560. *Source:* Reprinted with permission from W.M.N. Ratnayake, Determination of Trans Unsaturation by Infrared Spectrophotometry and Deterinination of Fatty Acid Composition of Partially Hydrogenated Vegetable Oils and Animal Fats by Gas Chromatography/ Infrared Spectrophotometry: Collaborative Study, *J.A.O.A.C. Intern.,* Vol. 78, pp. 783–802, © 1994

Fig. 2.32 Change in fatty acid composition during hydrogenation of canola oil



Lipid Oxidation

The unsaturated bonds present in fats and oils represent active centers that, among other things, may react with oxygen. This reaction leads to the formation of primary, secondary, and tertiary oxidation products that may make the fat or fat-containing foods unsuitable for consumption.

The process of autoxidation and the resulting deterioration in flavor of fats and fatty foods are often described by the term *rancidity.* Usually rancidity refers to oxidative deterioration but, in the field of dairy science, rancidity refers usually to hydrolytic changes resulting from enzyme activity. Lundberg (1961) distinguishes several types of rancidity. In fats such as lard, common oxidative rancidity results from exposure to oxygen; this is characterized by a sweet but undesirable odor and flavor that become progressively more intense and unpleasant as oxidation progresses. Flavor reversion is the term used for the objectionable flavors that develop in oils containing linolenic acid. This type of oxidation is produced with considerably less oxygen than with common oxidation. A type of oxidation similar to reversion may take place in dairy products, where a very small amount of oxygen may result in intense oxidation off-flavors. It is interesting to note that the linolenic acid content of milk fat is quite low.

Among the many factors that affect the rate of oxidation are the following:

- amount of oxygen present
- degree of unsaturation of the lipids
- presence of antioxidants
- presence of prooxidants, especially copper, and some organic compounds such as heme-containing molecules and lipoxidase
- nature of packaging material
- light exposure
- temperature of storage

The lipid oxidation process during lipid peroxidation consists of three partially overlapping phases of radical reactions which can be distinguished: initiation, propagation, and termination (Fig. 2.33). In the initiation phase reactions prevail that form and expand the pool of radicals.

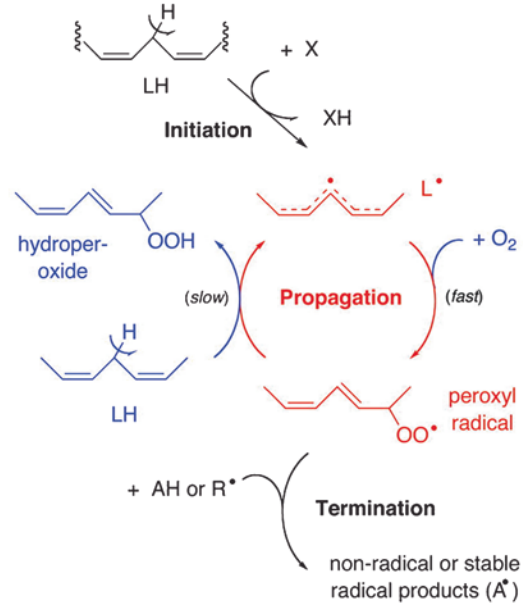


Fig. 2.33 Mechanism of lipid oxidation. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2868362/

During the propagation phase the chain reaction between fatty acid radicals and molecular oxygen leads to the formation and accumulation of the primary hydroperoxide products. Reactions between radicals leading to non-radical products dominate during the termination phase.

In the initiation part, hydrogen is abstracted from an olefinic compound to yield a free radical. The removal of hydrogen takes place at the carbon atom next to the double bond and can be initiated by light or metal ions such as iron or copper. The dissociation energy of hydrogen in various olefinic compounds has been listed by Ohloff (1973) and is shown in Table 2.27. Once a free radical has been formed, it will combine with oxygen to form a peroxy-free radical, which can in turn abstract hydrogen from another unsaturated molecule to yield a peroxide and a new free radical, thus starting the propagation reaction. This reaction may be repeated up to several thousand times and has the nature of a chain reaction.

The hydroperoxides formed in the propagation part of the reaction are the primary oxidation products. The hydroperoxide mechanism of autoxidation was first proposed by Farmer (1946). These oxidation products are generally unstable and decompose into the secondary oxidation products,

Table 2.27 Dissociation energy for the abstraction of hydrogen from olefinic compounds and peroxides

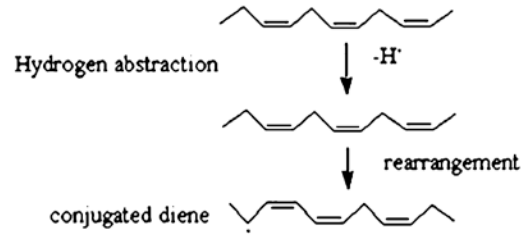
Compound	ΔE (kcal/mole)
H—CH═CH_2	103
H—CH_2—CH_2—CH_3	100
H—CH_2—CH═CH_2	85
H — CH — CH = CH — CH_2 — | CH_3	77
—CH = CH — CH — CH = CH — | H	65
H—OO—R	90

Source: From G. Ohloff, Fats as Precursors, in *Functional Properties of Fats in Foods*, J. Solms, ed., 1973, Forster Publishing

which include a variety of compounds, including carbonyls, which are the most important. The peroxides have no importance to flavor deterioration, which is wholly caused by the secondary oxidation products. The nature of the process can be represented by the curves of Fig. 2.34 (Pokorny 1971). In the initial stages of the reaction, the amount of hydroperoxides increases slowly; this stage is termed the induction period. At the end of the induction period, there is a sudden increase in peroxide content. Because peroxides are easily determined in fats, the peroxide value is frequently used to measure the progress of oxidation. Organoleptic changes are more closely related to the secondary oxidation products, which can be measured by various procedures, including the benzidine value, which is related to aldehyde decomposition products. As the aldehydes are themselves oxidized, fatty acids are formed; these free fatty acids may be considered tertiary oxidation products. The length of the induction period, therefore, depends on the method used to determine oxidation products.

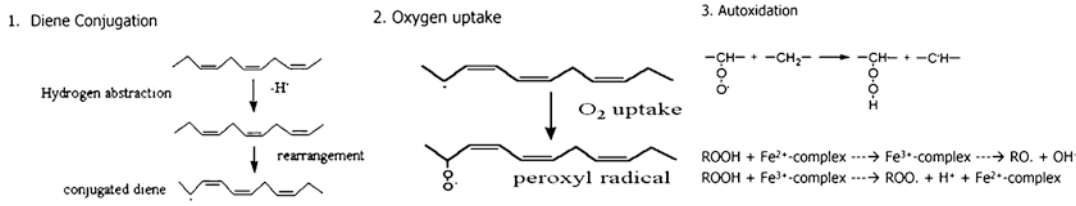
Initiation

The attack of a ROS able to abstract a hydrogen atom from a methylene group (─CH2─), generating free radicals from polyunsaturated fatty acids. OH is the most efficient ROS to do that attack.



Energy requirement for radical production by rupture of a CH bond is about 80 kcal.

Propagation



Termination

Formation of hydroperoxides: by reaction of a peroxyl radical with a-tocopherol: chain breaking

Radical-Radical reactions
R· + R· ------> RR
R· + ROO· ------> ROOR
ROO· + ROO· ------> ROOR + O2

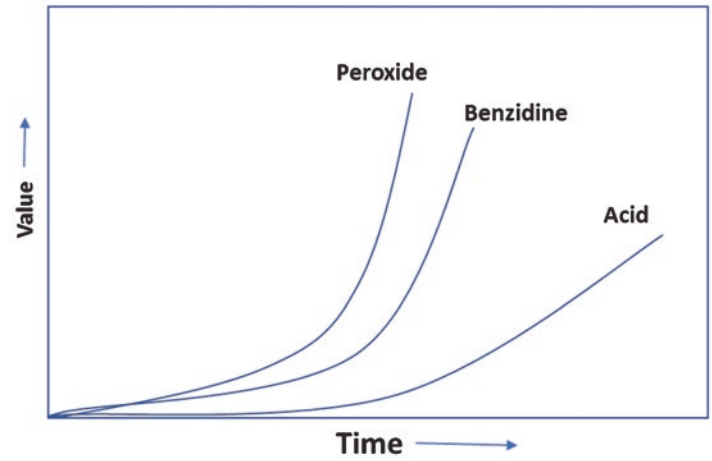


Fig. 2.34 Autoxidation of Lard comparing (**a**) peroxide value, (**b**) benzidine value, (**c**) acid value. *Adapted from* J. Pokorny, Stabilization of Fats by Phenolic Antioxidants, *Can. Inst. Food Sci. Technol. J.*, Vol. 4, pp. 68–74, 1971

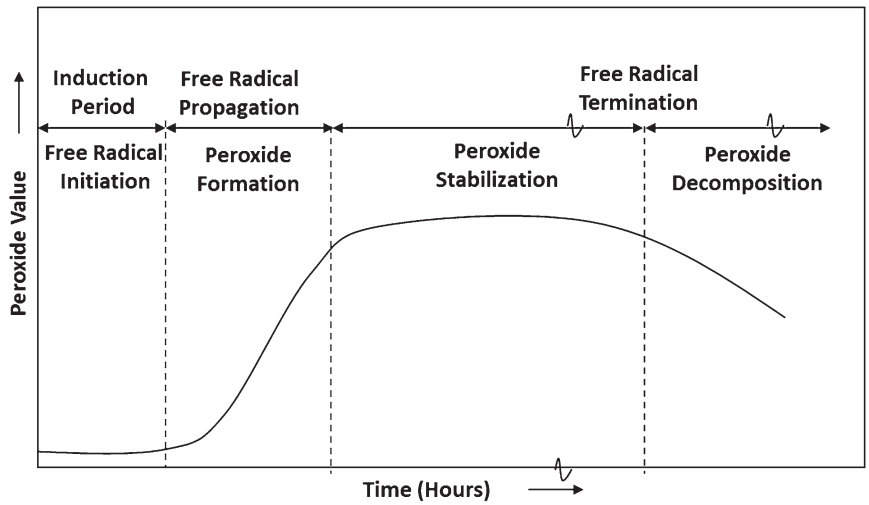


Fig. 2.35 Peroxide formation and decomposition as a function of time

The mechanism of the autoxidation of polyunsaturated fatty acids as a radical chain reaction was established in the middle of the twentieth century is illustrated in Fig. 2.34. Soon after followed the elucidation of the role of antioxidants as agents that break the radical chain (Inglold 1961), and the identification of secondary transformation products of the primary hydroperoxides through enzymatic and non-enzymatic transformations, the latter an active area of research that continues to unravel novel enzymes and products (Gardner 1989; Gerwick 1996; Grechkin 1998; Tijet and Brash 2002; Schneider et al. 2007).

The propagation can be followed by termination if the free radicals react with themselves to yield non-active products, as shown here:

$$R^{\bullet} + R^{\bullet} \rightarrow R - R$$
$$R^{\bullet} + RO_2^{\bullet} \rightarrow RO_2R$$
$$nRO_2^{\bullet} \rightarrow \left(RO_2\right)_n$$

The rate and course of autoxidation depend primarily on the composition of the fat—its degree of unsaturation and the types of unsaturated fatty acids present. The absence, or at least a low value, of peroxides does not necessarily indicate that an oil is not oxidized. As Fig. 2.35 indicates, peroxides are labile and may be transformed into sec-

ondary oxidation products. A combined index of primary and secondary oxidation products gives a better evaluation of the state of oxidation of an oil. This is expressed as Totox value: Totox value = 2 × peroxide value + anisidine value. (Anisidine value is a measure of secondary oxidation products.) Removal of oxygen from foods will prevent oxidation, but, in practice, this is not easy to accomplish in many cases. At high temperatures (100–140 °C) such as those used in the accelerated tests for oil stability (active oxygen method), formic acid is produced, which can be used to indicate the end of the induction period. The formation of formic acid results from aldehyde decomposition. Peroxidation of aldehydes establishes a resonance equilibrium between two limiting forms.

The hydroperoxides formed in the propagation part of the reaction are the primary oxidation products. The hydroperoxide mechanism of autoxidation was first proposed by Farmer (1946). These oxidation products are generally unstable and decompose into the secondary oxidation products, which include a variety of compounds, including carbonyls, which are the most important. The peroxides have no importance to flavor deterioration, which is wholly caused by the secondary oxidation products. The nature of the process can be represented by the curves of Fig. 2.36 (Labuza 1971). In the initial stages of the reaction, the amount of hydroperoxides increases slowly; this stage is termed the induction period. At the end of the induction period, there is a sudden increase in peroxide content. Because peroxides are easily determined in fats, the peroxide value is frequently used to measure the progress of oxidation. Organoleptic changes are more closely related to the secondary oxidation products, which can be measured by various procedures, including the benzidine value, which is related to aldehyde decomposition products. As the aldehydes are themselves oxidized, fatty acids are formed; these free fatty acids may be considered tertiary oxidation products.

Although even saturated fatty acids may be oxidized, the rate of oxidation greatly depends on the degree of unsaturation. In the series of 18-carbon-atom fatty acids 18:0, 18:1, 18:2, 18:3, the relative rate of oxidation has been reported to be in the ratio of 1:100:1200:2500. The reaction of unsaturated compounds proceeds by the abstraction of hydrogen from the α carbon, and the resulting free radical is stabilized by resonance as follows:

$$-\overset{1}{\dot{C}}H-\overset{2}{C}H=\overset{3}{C}H- \rightleftharpoons -\overset{1}{C}H=\overset{2}{C}H-\overset{3}{\dot{C}}H-$$

If oleic acid is taken as example of a monoethenoid compound (*cis*-9-octadecenoic acid), the reaction will proceed by abstraction of hydrogen from carbons 8 or 11, resulting in two pairs of resonance hybrids.

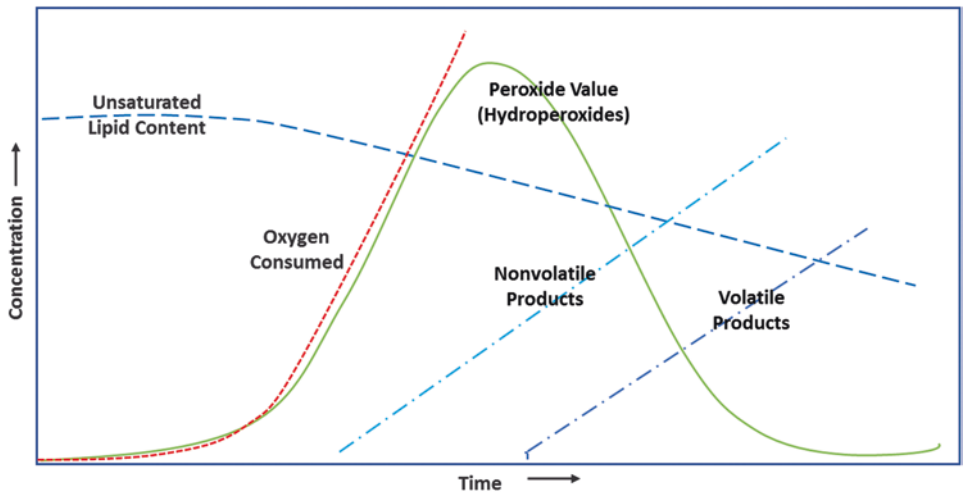
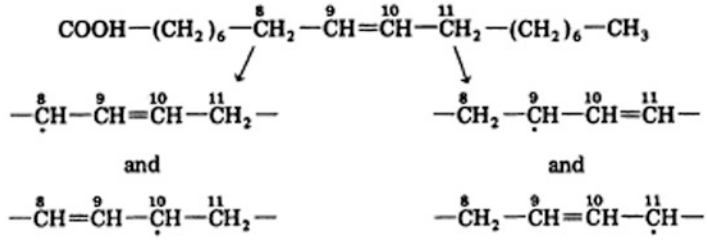
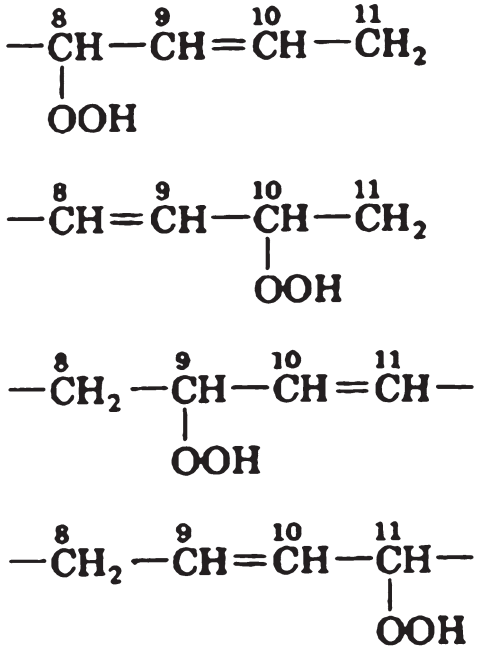


Fig. 2.36 The volatiles and non-volatiles contain the secondary oxidative by-products. Adapted from: Labuza, T. P. 1971. Kinetics of Lipid Oxidation in Foods, 2: 355–405. CRC Crit. Rev. Food Technol

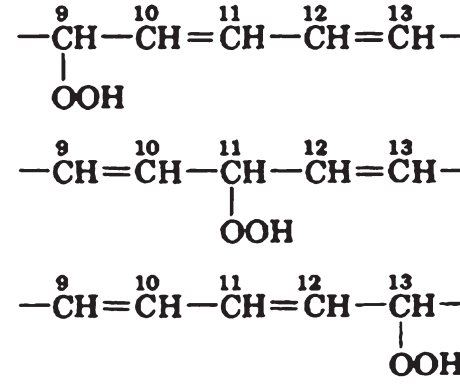


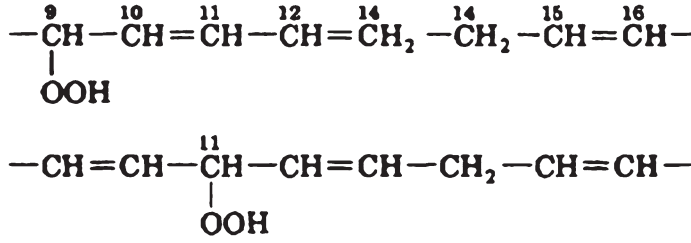
This leads to the formation of the following four isomeric hydroperoxides:

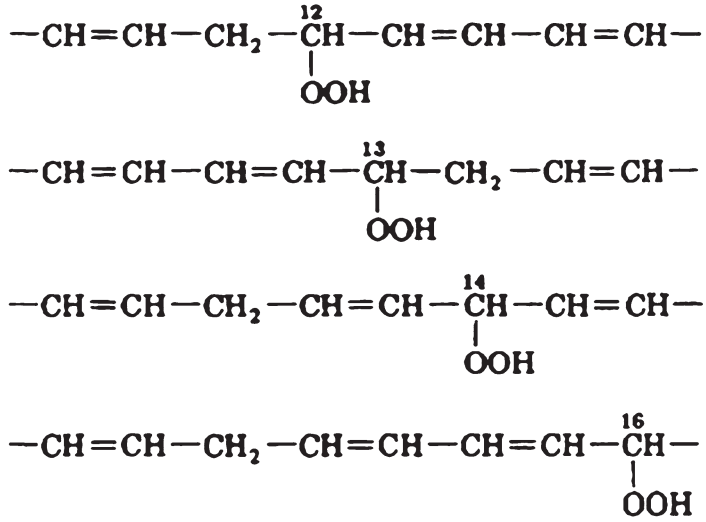


In addition to the changes in double bond position, there is isomerization from *cis* to *trans*, and 90% of the peroxides formed may be in the *trans* configuration (Lundberg 1961).

From linoleic acid (*cis-cis*-9,12-octadecadienoic acid), three isomeric hydroperoxides can be formed as shown in the next formula. In this mixture of 9, 11, and 13 hydroperoxides, the conjugated ones occur in greatest quantity because they are the more stable forms. The hydroperoxides occur in the *cis-trans* and *trans-trans* configurations, the content of the latter being greater with higher temperature and greater extent of oxidation. From the oxidation of linolenic acid (*cis, cis, cis*-9,12,15-octadecatrienoic acid), six isometric hydroperoxides can be expected according to theory, as shown:

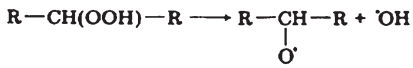




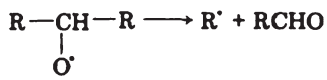


Hydroperoxides of linolenate decompose more readily than those of oleate and linoleate because active methylene groups are present. The active methylene groups are the ones located between a single double bond and a conjugated diene group. The hydrogen at this methylene group could readily be abstracted to form dihydroperoxides. The possibilities here for decomposition products are obviously more abundant than with oleate oxidation.

The decomposition of hydroperoxides has been outlined by Keeney (1962). The first step involves decomposition to the alkoxy and hydroxy free radicals.

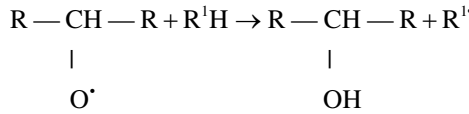


The alkoxy radical can react to form aldehydes.

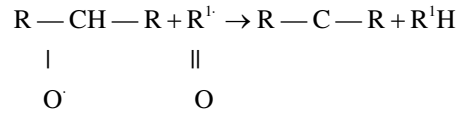


This reaction involves fission of the chain and can occur on either side of the free radical. The aldehyde that is formed can be a short-chain volatile compound, or it can be attached to the glyceride part of the molecule; in this case, the compound is nonvolatile. The volatile aldehydes are in great part responsible for the oxidized flavor of fats.

The alkoxy radical may also abstract a hydrogen atom from another molecule to yield an alcohol and a new free radical, as shown:



The new free radicals formed may participate in propagation of the chain reaction. Some of the free radicals may interact with themselves to terminate the chain, and this could lead to the formation of ketones as follows:



As indicated, a variety of aldehydes have been demonstrated in oxidized fats. Alcohols have also been identified, but the presence of ketones is not as certain. Keeney (1962) has listed the aldehydes that may be formed from breakdown of hydroperoxides of oxidized oleic, linoleic, linolenic, and arachidonic acids (Table 2.28). The aldehydes are powerful flavor compounds and have very low flavor thresholds; for example,

Table 2.28 Hydroperoxides and aldehydes (with single oxygen function) that may be formed in autoxidation of some unsaturated fatty acids

Fatty acid	Methylene Group Involved[a]	Isomeric hydroperoxides formed from the structures contributing to the intermediate free radical resonance hybrid	Aldehydes formed by decomposition of the hydroperoxides
Oleic	11	11-hydroperoxy-9-ene	octanal
		9-hydroperoxy-10-ene	2-decenal
	8	8-hydroperoxy-9-ene	2-undecenal
		10-hydroperoxy-8-ene	nonanal
Linoleic	11	13-hydroperoxy-9,11-diene	hexanal
		11-hydroperoxy-9,12-diene	2-octenal
		9-hydroperoxy-10,12-diene	2,4-decadienal
Linolenic	14	16-hydroperoxy-9,12,14-triene	propanal
		14-hydroperoxy-9,12,15-triene	2-pentenal
		12-hydroperoxy-9,13,15-triene	2,4-heptadienal
	11	13-hydroperoxy-9,11,15-triene	3-hexenal
		11-hydroperoxy-9,12,15-triene	2,5-octadienal
		9-hydroperoxy-10,12,15-triene	2,4,7-decatrienal
Arachidonic	13	15-hydroperoxy-5,8,11,13-tetraene	hexanal
		13-hydroperoxy-5,8,11,14-tetraene	2-octenal
		11-hydroperoxy-5,8,12,14-tetraene	2,4-decadienal
	10	12-hydroperoxy-5,8,10,14-tetraene	3-nonenal
		10-hydroperoxy-5,8,11,14-tetraene	2,5-undecadienal
		8-hydroperoxy-5,9,11,14-tetraene	2,4,7-tridecatrienal
	7	9-hydroperoxy-5,7,11,14-tetraene	3,6-dodecadienal
		7-hydroperoxy-5,8,11,14-tetraene	2,5,8-tetradecatrienal
		5-hydroperoxy-6,8,11,14-tetraene	2,4,7,10-hexadecatetraenal

Source: From M. Keeney, Secondary Degradation Products, in *Lipids and Their Oxidation,* H.W. Schultz et al., eds., 1962, AVI Publishing Co.

[a]Only the most active methylene groups in each acid are considered

2,4-decadienal has a flavor threshold of less than one part per billion. The presence of a double bond in an aldehyde generally lowers the flavor threshold considerably. The aldehydes can be further oxidized to carboxylic acids or other tertiary oxidation products.

When chain fission of the alkoxy radical occurs on the other side of the free radical group, the reaction will not yield volatile aldehydes but will instead form nonvolatile aldehydoglycerides. Volatile oxidation products can be removed in the refining process during deodorization, but the nonvolatile products remain; this can result in a lower oxidative stability of oils that have already oxidized before refining.

$$R - CH_2 - CO \rightleftharpoons R - CH - CHO$$

The rate and course of autoxidation depend primarily on the composition of the fat—its degree of unsaturation and the types of unsaturated fatty acids present. The absence, or at least a low value, of peroxides does not necessarily indicate that an oil is not oxidized. As Fig. 2.16 indicates, peroxides are labile and may be transformed into secondary oxidation products. A combined index of primary and secondary oxidation products gives a better evaluation of the state of oxidation of an oil. This is expressed as Totox value: Totox value = 2 × peroxide value + anisidine value. (Anisidine value is a measure of secondary oxidation products.) Removal of oxygen from foods will prevent oxidation, but, in practice, this is not easy to accomplish in many cases. At high temperatures (100–140 °C) such as those used in the accelerated tests for oil stability

(active oxygen method), formic acid is produced, which can be used to indicate the end of the induction period. The formation of formic acid results from aldehyde decomposition. Peroxidation of aldehydes establishes a resonance equilibrium between two limiting forms.

The second hybrid ties up oxygen at the α carbon to yield the α-hydroperoxy aldehyde as follows:

$$R-\dot{C}H-CHO+O_2 \rightarrow R-\begin{array}{c}O^{\cdot}\\|\\O\\|\end{array}-CH-CHO$$

Breakdown of oxygen and carbon bonds yields formic acid and a new aldehyde.

$$R-\begin{array}{c}O^{\cdot}\\|\\O\\|\end{array}-CH+CHO \rightarrow HCOOH+RCHO$$

deMan et al. (1987) investigated this reaction with a variety of oils and found that although formic acid was the main reaction product, other short-chain acids from acetic to caproic were also formed. Trace metals, especially copper, and to a lesser extent iron, will catalyze fat oxidation; metal deactivators such as citric acid can be used to reduce the effect. Lipoxygenase (lipoxidase) and heme compounds act as catalysts of lipid oxidation. Antioxidants can be very effective in slowing down oxidation and increasing the induction period. Many foods contain natural antioxidants; the tocopherols are the most important of these. They are present in greater amounts in vegetable oils than in animal fats, which may explain the former's greater stability.

Antioxidants such as tocopherols may be naturally present; they may be induced by processes such as smoking or roasting, or added as synthetic antioxidants. Antioxidants act by reacting with free radicals, thus terminating the chain. The antioxidant AH may react with the fatty acid free radical or with the peroxy free radical,

$$AH+R^{\cdot} \rightarrow RH+A^{\cdot}$$
$$AH+RO_2^{\cdot} \rightarrow RO_2H+A^{\cdot}$$

The antioxidant free radical deactivated by further oxidation to quinones, thus terminating the chain. Only phenolic compounds that can easily produce quinones are active as antioxidants (Pokorny 1971). At high concentrations antioxidants may have a prooxidant effect and one of the reactions may be as follows:

$$A^{\cdot}+RH \rightarrow AH+R$$

Tocopherols in natural fats are usually present at optimum levels. Addition of antioxidant beyond optimum amounts may result in increasing the extent of prooxidant action. Lard is an example of a fat with very low natural antioxidant activity and antioxidant must be added to it, to provide protection. The effect of antioxidants can be expressed in terms of protection factor, as shown in Fig. 2.37 (Pokorny 1971). The highly active antioxidants that are used in the food industry are active at about 10–50 parts per million (ppm). Chemical structure of the antioxidants is the most important factor affecting their activity. The number of synthetic antioxidants permitted in foods is limited, and the structure of the most widely used compounds is shown in Fig. 2.38. Propyl gallate is more soluble in water than in fats. The octyl and dodecyl esters are more fat soluble. They are heat resistant and nonvolatile with steam, making them useful for frying oils and in baked products. These are considered to have *carry-through properties.* Butylated hydroxyanisole (BHA) has carry-through properties but butylated hydroxy toluene (BHT) does not, because it is volatile with steam. The compound *tert*-butyl hydroquinine (TBHQ) is used for its effectiveness in increasing oxidative stability of polyunsaturated oils and fats. It also provides carry-through protection for fried foods. Antioxidants are frequently used in combination or together with synergists. The latter are frequently metal deactivators that have the ability to chelate metal ions. An example of the combined effect of antioxidants is shown in Fig. 2.39. It has been pointed out (Zambiazi and Przybylski 1998) that fatty acid composition can explain only about half of the oxidative stability

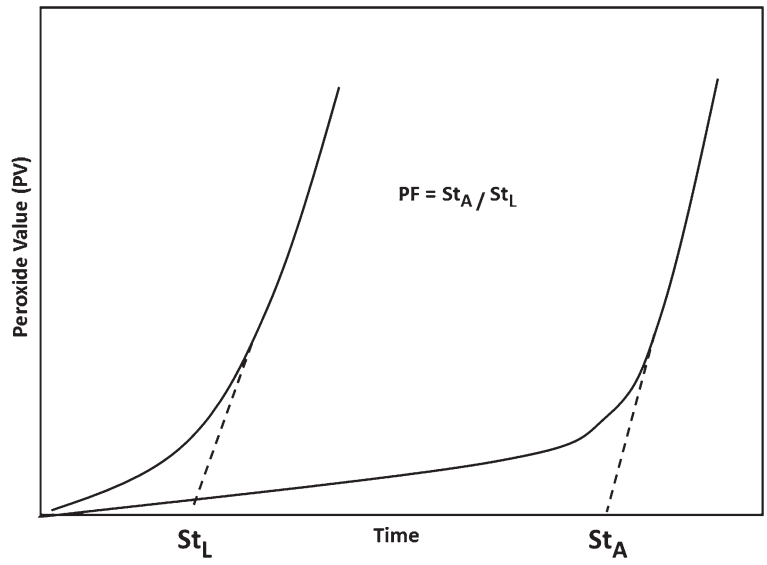


Fig. 2.37 Determination of protection factor. (**a**) Lard, (**b**) lard + antioxidant. *Adapted from* J. Pokomy, Stabilization of Fats by Phenolic Antioxidants, *Can. Inst. Food Sci. Technol. J*., Vol. 4, pp. 68–74, 1971

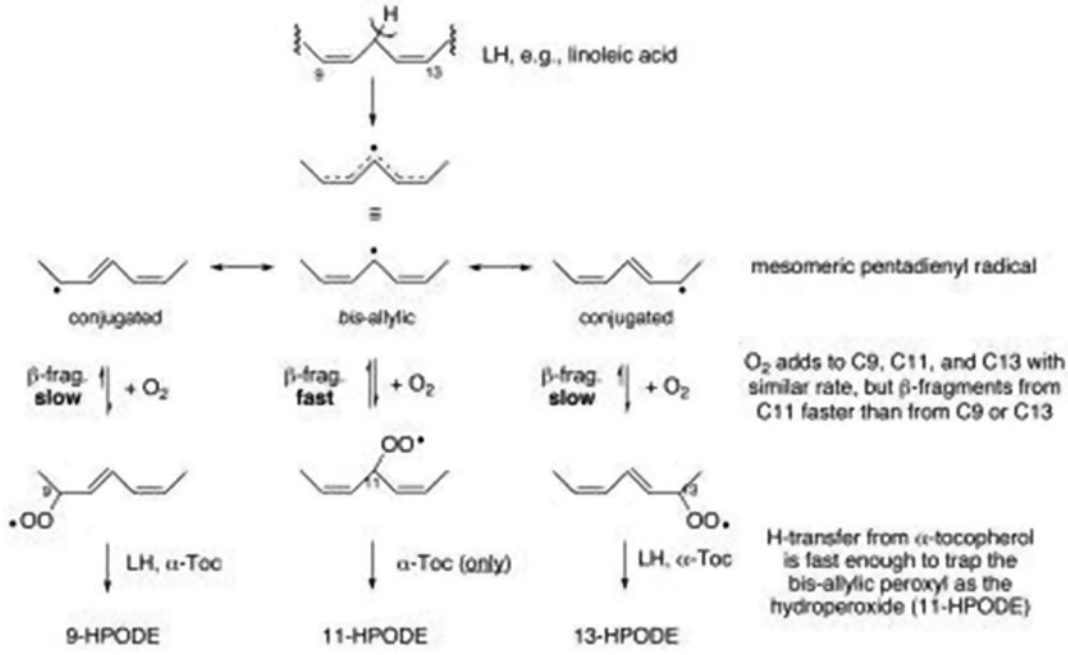


Fig. 2.38 Mechanism of oxidation, propagation and antioxidant protection

of a vegetable oil. The other half can be contributed to minor components including tocopherols, metals, pigments, free fatty acids, phenols, phospholipids, and sterols.

The formation of 9-, 11-, and 13-hydroperoxides is expected based on the three mesomeric structures for the pentadienyl radical of linoleic acid. That implicate localization (and therefore

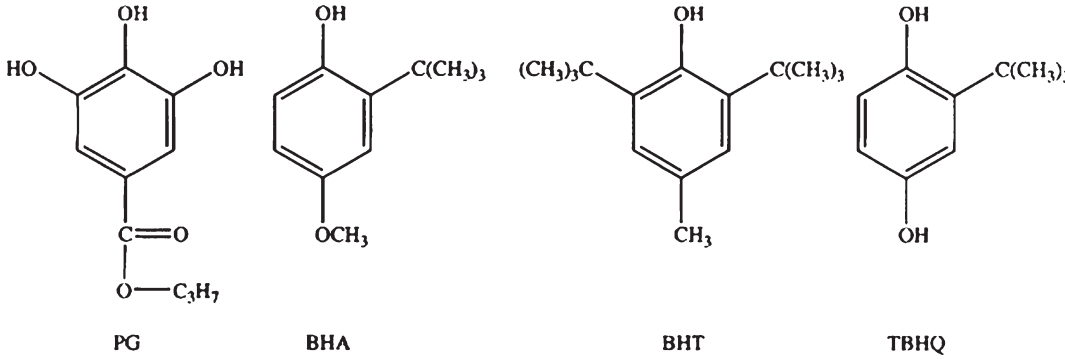


Fig. 2.39 Structure of propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), and tert-butyl hydroquinone (TBHQ)

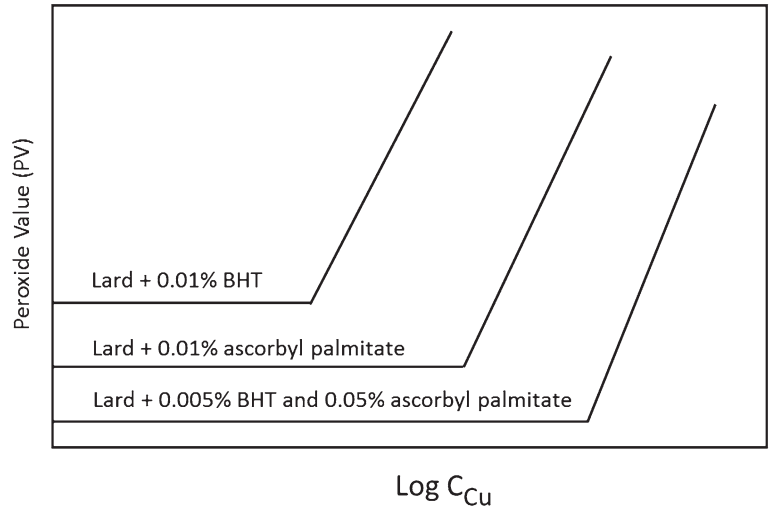


Fig. 2.40 Effect of copper concentration on protective effect of antioxidants in lard. (*Adapted from* J. Pokorny, Stabilization of Fats by Phenolic Antioxidants, *Can. Inst. Food Sci. Technol. J.*, Vol. 4, pp. 68–74, 1971

reactivity with O_2) of the radical at carbons 9, 11, and 13 (Fig. 2.38). The (9- and 13-)hydroperoxides are easily identified in autoxidation reactions, the 11-hydroperoxide has proven elusive for decades (Haslbeck et al. 1983). The instability of the intermediate *bis*-allylic peroxyl radical of this hydroperoxide has made it difficult to identify and isolate. In order to explain the mechanistic basis for formation of the *bis*-allylic hydroperoxide, the concept of radical reactions as reversible and competing reactions help explain the chemistry. This will help explain how the rate constants of different and competing reactions are a determining factors explaining products formed during lipid autoxidation (Fig. 2.40). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2868362/.

There are a number of antioxidants that can be applied to obtain certain effects in fats and oils.

Antioxidants used to control oxidation fat or oil

Butylated hydroxyanisole (BHA)	Improves oxidative stability, antioxidants
Butylated hydroxytoluene (BHT)	Improves oxidative stability, antioxidants
Carotene (Pro-Vitamin A)	Enhances color of finished foods; color additive
Citric acid	Inhibit metal-catalized oxidation and production of dark colors; metal chelating agents.
Methyl silicone	Inhibits oxidation; antifoam agent
Phosphoric acid	Inhibit metal-catalyzed oxidation and production of dark colors; metal chelating agent
tertiary butylhydroquinone (TBHQ)	Improves oxidative stability, antioxidants
Tocopherols	Natural antioxidant, improves oxidative stability

Photooxidation

Oxidation of lipids, in addition to the free radical process, can be brought about by at least two other mechanisms—photooxidation and enzymic oxidation by lipoxygenase. The latter is dealt with in Chap. 10. Light-induced oxidation or photooxidation results from the reactivity of an excited state of oxygen, known as singlet oxygen ($^1O_2$). Ground-state or normal oxygen is triplet oxygen ($^3O_2$). The activation energy for the reaction of normal oxygen with an unsaturated fatty acid is very high, of the order of 146–273 kJ/mole. When oxygen is converted from the ground state to the singlet state, energy is taken up amounting to 92 kJ/mole, and in this state the oxygen is much more reactive. Singlet-state oxygen production requires the presence of a sensitizer. The sensitizer is activated by light, and can then either react directly with the substrate (type I sensitizer) or activate oxygen to the singlet state (type II sensitizer). In both cases unsaturated fatty acid residues are converted into hydroperoxides. The light can be from the visible or ultraviolet region of the spectrum.

Singlet oxygen is short-lived and reverts back to the ground state with the emission of light. This light is fluorescent, which means that the wavelength of the emitted light is higher than that of the light that was absorbed for the excitation. The reactivity of singlet oxygen is 1500 greater than that of ground-state oxygen. Compounds that can act as sensitizers are widely occurring food components, including chlorophyll, myoglobin, riboflavin, and heavy metals. Most of these compounds promote type II oxidation reactions. In these reactions the sensitizer is transformed into the activated state by light. The activated sensitizer then reacts with oxygen to produce singlet oxygen.

$$\text{sen} \xrightarrow{hv} \text{sen}^*$$

$$\text{sen}^* + O_2 \rightarrow \text{sen} + {}^1O_2$$

The singlet oxygen can react directly with unsaturated fatty acids.

$$^1O_2 + RH \rightarrow ROOH$$

The singlet oxygen reacts directly with the double bond by addition, and shifts the double bond one carbon away. The singlet oxygen attack on linoleate produces four hydroperoxides as shown in Fig. 2.41. Photooxidation has no induction period, but the reaction can be quenched by carotenoids that effectively compete for the singlet oxygen and bring it back to the ground state.

Phenolic antioxidants do not protect fats from oxidation by singlet oxidation (Yasaei et al. 1996). However, the antioxidant ascorbyl palmitate is an effective singlet oxygen quencher (Lee et al. 1997). Carotenoids are widely used as quenchers. Rahmani and Csallany (1998) reported that in the photooxidation of virgin olive oil, pheophytin A functioned as sensitizer, while β-carotene acted as a quencher.

The combination of light and sensitizers is present in many foods displayed in transparent containers in brightly lit supermarkets. The light-induced deterioration of milk has been studied extensively. Sattar et al. (1976) reported on the light-induced flavor deterioration of several oils and fats. Of the five fats examined, milk fat and

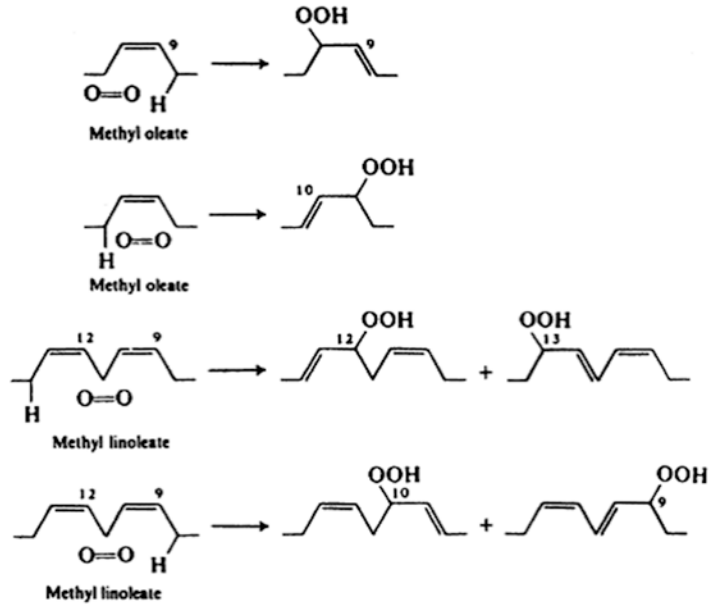


Fig. 2.41 Photooxidation. Singlet-oxygen attack on oleate produces two hydroperoxides; linoleate yields four hydroperoxides

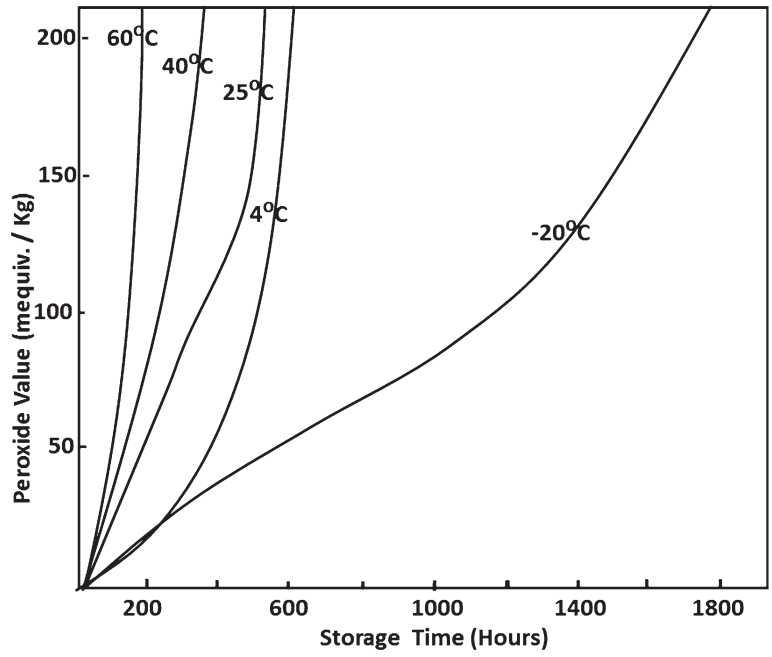


Fig. 2.42 Effect of temperature on rate of oxidation of illuminated com oil. *Adapted from:* M.H. Chahine and J.M. deMan, Autoxidation of Com Oil under the Influence of Fluorescent Light, *Can. Inst. Food Sci. Technol. J.*, Vol. 4, pp. 24–28,1971

soybean oil were most susceptible and corn oil least susceptible to singlet oxygen attack. The effect of temperature on the rate of oxidation of illuminated corn oil was reported by Chahine and deMan (1971) (Fig. 2.42). They found that temperature has an important effect on photooxidation rates, but even freezing does not completely prevent oxidation.

Heated Fats: Frying

Fats and oils are heated during commercial processing and during frying. Heating during processing mainly involves hydrogenation, physical refining, and deodorization. Temperature used in these processes may range from 120 °C to 270 °C. The oil is not in contact with air, which eliminates the possibility of oxidation. At the high temperatures used in physical refining and deodorization, several chemical changes may take place (Erickson and Frey 1994). These include randomization of the glyceride structure, dimer formation, *cis-trans* isomerization, and formation of conjugated fatty acids (positional isomerization) of polyunsaturated fatty acids (Hoffmann 1989). Heating oils during frying can result in three types of chemical modification of the oil. They are hydrolysis, oxidation and thermal degradation. Table 2.29 summarizes the principal degradation products form these reactions.

Hydrolysis is the splitting of the triacylglycerol molecule, with formation of diacylglycerols and fatty acids resulting in nonvolatile fatty acids with molecular weight significantly lower than that of the parent triacylglycerols. Analysis of glycerides and free fatty acids in frying oils with high levels of free fatty acids demonstrated that the formation of monoacylglycerols is minimal and also that the reaction is not selective and consequently, is independent of the fatty acid composition of the frying oil. Hydrolysis is a relatively simple reaction but many consider it to be one of the most important reactions during frying. Pokorny et al. (1998) reported that used frying oils from fast food operations exhibited high contents of both diacylglycerols and fatty acids. In well-controlled frying operations of potatoes substrate had a very high content of water over a wide range of conditions, hydrolytic products were minor compounds within the total recovered degradation compounds (Dobarganes et al. 1993).

Table 2.29 Principal degradation products compounds formed during frying

Reaction	Cause	Reaction Products found in oil
Hydrolysis	Moisture	Fatty acids
		Diacylglycerols
Oxidation	Air	Oxidized monomeric triacylglycerols
		Oxidized dimeric and oligomeric triacylglycerols
		Volatile compounds (aldehydes, ketones, alcohols, hydrocarbons, etc.)
Thermal degradation	High Temperature	Cyclic monomeric triacylglycerols
		Isomeric monomeric triacylglycerols
		Nonpolar dimeric and oligomeric triacylglycerols

Lipid oxidation at high temperatures such as those encountered in baking and frying is very complex because both oxidative and thermal reactions are proceeding simultaneously. Although oxygen is highly soluble in cooking oils, the oil solubility decreases at high temperature, however, the high temperatures cause the oxidation reactions to increase (Velasco et al. 2008; Frankel 1997).

Figure 2.43 illustrates the oxidation process that occurs during frying. The oxidation proceeds via a free radical mechanism with chain reactions, where RH represents here the triacylglycerol molecule undergoing oxidation in one of its unsaturated fatty acyl groups. In the initiation stage, an alkyl radical is formed by abstraction of a hydrogen radical from an allylic or bis allylic position of an unsaturated fatty acid. In the propagation step, the alkyl radical reacts with oxygen at rates controlled by diffusion to form peroxyl radicals that in turn react with new triacylglycerol molecules giving rise to hydroperoxides as the primary oxidation products and new alkyl radicals that propagate the reaction chain. Finally, in the termination stage, radicals react between them to yield relatively stable non-radical species. (http://lipidlibrary.aocs.org/frying/frying.html).

The oxygen content becomes depleted at frying temperatures thus the alkylperoxyl radicals ($ROO^{\bullet}$ are diminished and the concentration of alkyl radicals ($R^{\bullet}$) increases. These changes result in the formation of more polymeric products through reactions mainly involving alkyl ($R^{\bullet}$) and

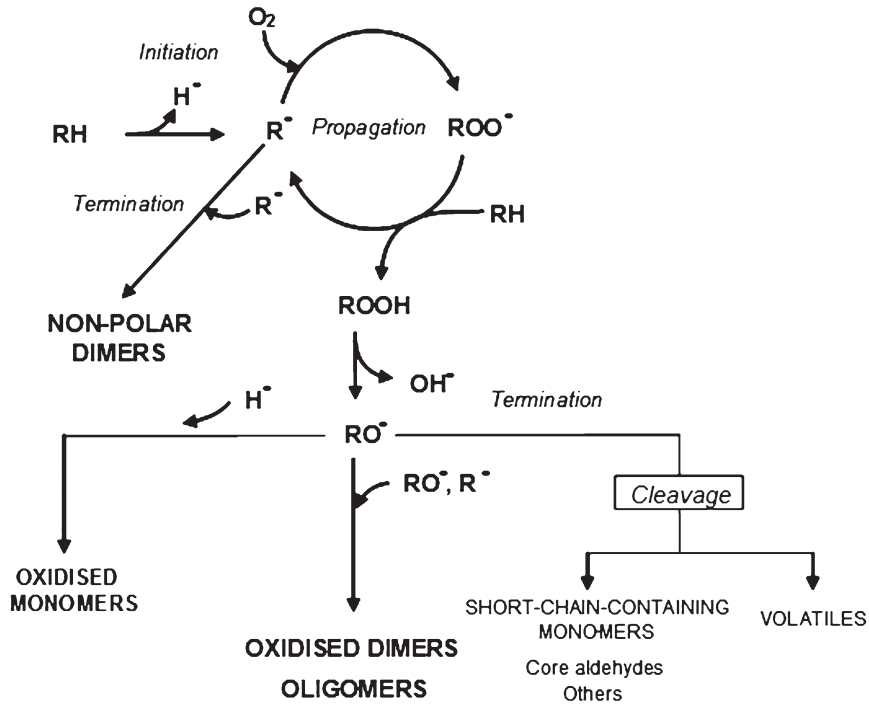


Fig. 2.43 Simplified scheme of thermal oxidation. http://lipidlibrary.aocs.org/frying/frying.html

alkoxyl (RO•) radicals. Polymerization compounds become significant in the accelerated stage of oxidation after the end of the induction period (Márquez-Ruiz and Dobarganes 2005). At high temperatures, formation of new compounds is very rapid, ROOH are practically absent above 150 °C, indicating that the rate of ROOH decomposition becomes higher than that of their formation, and polymeric compounds are formed from the very early stages of heating. Also, the formation of significant amounts of non-polar triacylglycerol dimers (R-R), typical compounds formed in the absence of oxygen through interaction of alkyl radicals, is a clear indication of the low oxygen concentration (Dobarganes and Pérez-Camino 1987). Dimers and oligomers: are the major compounds in used frying fats and are formed through interaction between triglyceride radicals.

Conditions prevailing during frying are less favorable than those encountered in the above-mentioned processes. Deep frying, where the food is heated by immersion in hot oil, is practiced in commercial frying as well as in food service operations. The temperatures used are in the range of 160–195 °C. At lower temperatures frying takes longer, and at higher temperatures deterioration of the oil is the limiting factor. Deep frying is a complex process involving both the oil and the food to be fried. The reactions taking place are schematically presented in Fig. 2.44. Steam is given off during the frying, which removes volatile antioxidants, free fatty acids, and other volátiles. Contact with the air leads to autoxidation and the formation of a large number of degradation products. The presence of steam results in hydrolysis, with the production of free fatty acids and partial glycerides. At lower frying temperatures the food has to be fried longer to reach the desirable color, and this results in higher oil uptake. Oil absorption by fried foods may range from 10 to 40%, depending on conditions of frying and the nature and size of the food.

Oils used in deep frying must be of high quality because of the harsh conditions during deep frying and to provide satisfactory shelf life in fried foods. The suitability of an oil for frying is directly related to its content of unsaturated fatty acids, especially linolenic acid. This has been

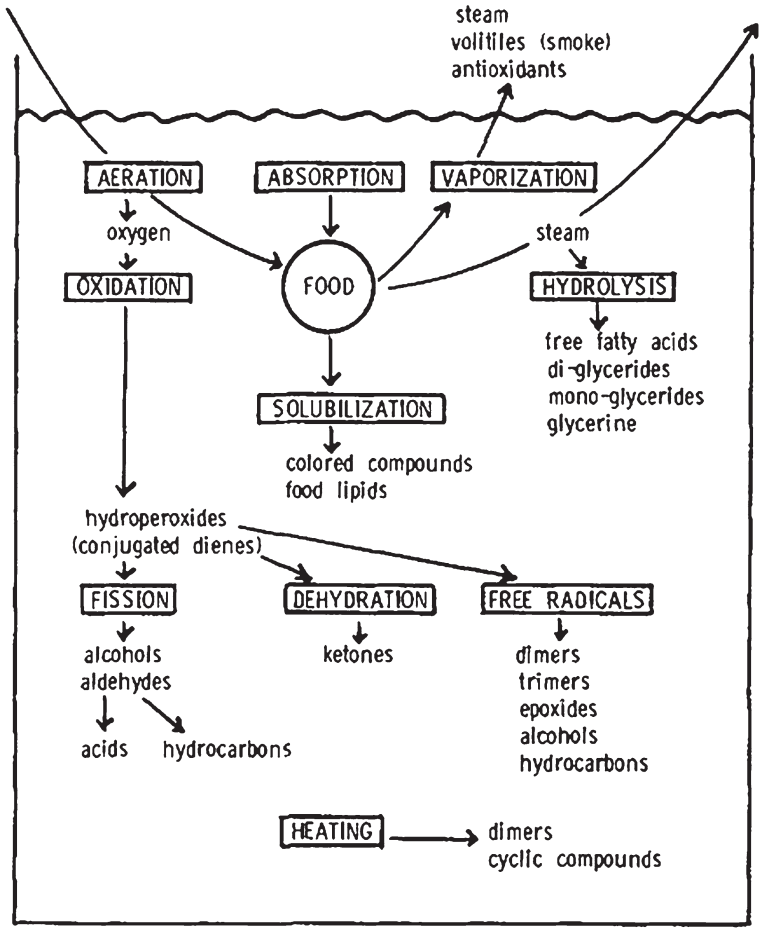


Fig. 2.44 Summary of chemical reactions occurring during deep frying. *Source:* Reprinted with permission from F.T. Orthoefer, S. Gurkin, and K. Lui, Dynamics of Frying in Deep Frying, in *Chemistry, Nutrition and Practical Applications,* E.G. Perkins and M.D. Erickson, eds., p. 224. © 1996, AOCS Press

described by Erickson (1996) as "inherent stability" calculated from the level of each of the unsaturated fatty acids (oleic, linoleic, and linolenic) and their relative reaction rate with oxygen. The inherent stability calculated for a number of oils is given in Table 2.30. The higher the inherent stability, the less suitable the oil is for frying. The liquid seed oils, such as soybean and sunflower oil, are not suitable for deep frying and are usually partially hydrogenated for this purpose. Such hydrogenated oils can take the form of shortenings, which may be plastic solids or pourable suspensions. Through plant breeding and genetic engineering, oils with higher inherent stability can be obtained, such as high-oleic sunflower oil, low-linolenic canola oil, and low-linolenic soybean oil.

The stability of frying oils and fats is usually measured by an accelerated test known as the active oxygen method (AOM). In this test, air is bubbled through an oil sample maintained at 95 °C and the peroxide value is measured at intervals. At the end point the peroxide value shows a sharp increase, and this represents the AOM value in hours. Typical AOM values for liquid seed oils range from 10 to 30 h; heavy-duty frying shortenings range from 200 to 300 h. AOM values of some oils and fats determined by measuring the peroxide value and using an automatic recording of volatile acids produced during the test are given in Table 2.31 (deMan et al. 1987).

As shown in Fig. 2.44, oil breakdown during frying can be caused by oxidation and thermal alteration. Oxidation can result in the formation

Table 2.30 Inherent stability of oils for use in frying

Oil	Iodine value	Inherent stability[a]
Soybean	130	7.4
Sunflower	120	7.7
High-oleic sunflower	90	2.0
Corn	110	6.2
Cottonseed	98	5.2
Canola	110	5.4
Peanut	92	4.5
Lard	60	1.4
Olive	88	1.8
Palm	55	1.4
Palm olein	58	1.6
Palm stearin	35	1.0
Tallow	50	0.7
Palm kernel	17	0.5
Coconut	9	0.4

[a]Inherent stability calculated from decimal fraction of fatty acids multiplied by relative reaction rates with oxygen, assuming rate for oleic acid = 1, linoleic acid = 10, and linolenic acid = 25

Table 2.31 Active oxygen method (AOM) time of several oils and fats as determined by peroxide value and conductivity measurements

Oil	AOM Time (POV)[a]	AOM time (conductivity)[b]
Sunflower	6.2	7.1
Canola	14.0	15.8
Olive	17.8	17.8
Corn	12.4	13.8
Peanut	21.1	21.5
Soybean	11.0	10.4
Triolein	8.1	7.4
Lard	42.7	43.2
Butterfat	2.8	2.0

Source: Reprinted with permission from J.M. deMan, et al., Formation of Short Chain Volatile Organic Acids in the Automated AOM Method, *J.A.O.C.S.*, Vol. 64, p. 996, © 1987, American Oil Chemists' Society

[a]At peroxide value 100

[b]At intercept of conductivity curve and time axis

of oxidized monomeric, dimeric, and oligomeric triglycerides as well as volatile compounds including aldehydes, ketones, alcohols, and hydrocarbons. In addition, oxidized sterols may be formed. Thermal degradation can result in cyclic monomeric triglycerides and nonpolar dimeric and oligomeric triglycerides. The polymerization reaction may take place by conversion of part of the *cis-cis*-1,4 diene system of linoleates to the *trans-trans* conjugated diene. The 1,4 and dienes can combine in a Diels-Alder type addition reaction to produce a dimer as shown in Fig. 2.45. Other possible routes for dimer formation are through free radical reactions. As shown in Fig. 2.46, this may involve combination of radicals, intermolecular addition, and intramolecular addition. From dimers, higher oligomers can be produced; the structure of these is still relatively unknown.

Another class of compounds formed during frying is cyclic monomers of fatty acids. Linoleic acid can react at either the C9 or C12 double bonds to give rings between carbons 5 and 9, 5 and 10, 8 and 12, 12 and 17, and 13 and 17. Cyclic monomers with a cyclopentenyl ring have been isolated from heated sunflower oil, and their structure is illustrated in Fig. 2.47 (Le Quéré and Sébédio 1996).

Some countries such as France require that frying oils contain less than 2% linolenic acid. Several European countries have set maximum limits for the level of polar compounds or for the level of free fatty acids beyond which the fat is considered unfit for human consumption. In continuous industrial frying, oil is constantly being removed from the fryer with the fried food and replenished with fresh oil so that the quality of the oil can remain satisfactory. This is more difficult in intermittent frying operations.

Flavor Reversion

Soybean oil and other fats and oils containing linolenic acid show the reversion phenomenon when exposed to air. Reversion flavor is a particular type of oxidized flavor that develops at comparatively low levels of oxidation. The off-flavors may develop in oils that have a peroxide value of as little as 1 or 2. Other oils may not become rancid until the peroxide value reaches 100. Linolenic acid is generally recognized as the determining factor of inversion flavors. These off-flavors are variously described as grassy,

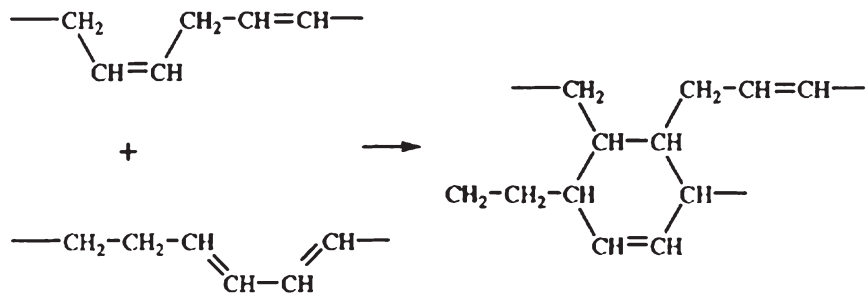
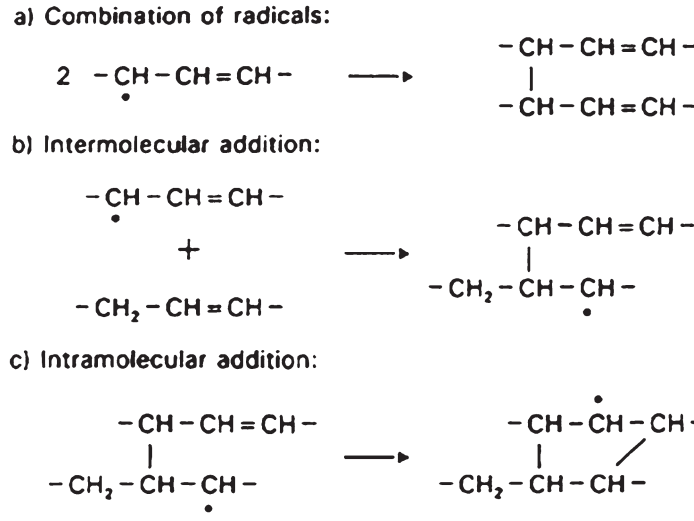


Fig. 2.45 Polymerization of Diene systems to form dimers

Fig. 2.46 Nonpolar dimer formation through free radical reactions



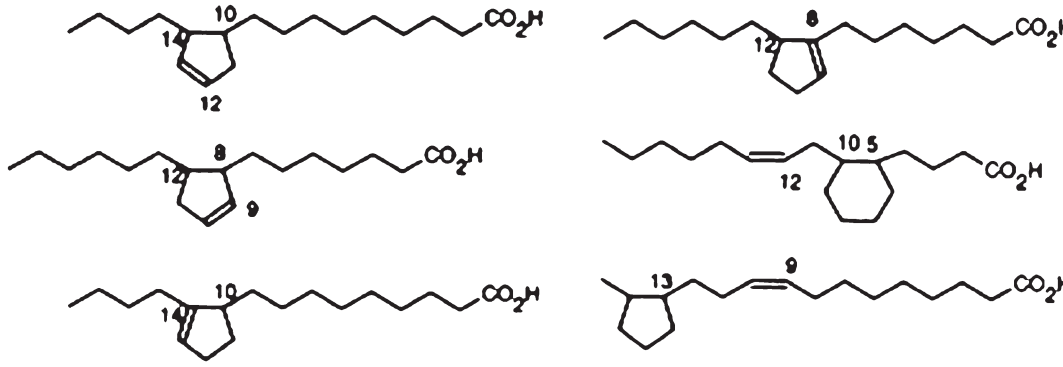


Fig. 2.47 Cyclic fatty acid monomers formed from linoleic acid in heated sunflower oil

fishy, and painty (Cowan and Evans 1961). The origin of these flavors appears to be the volatile oxidation products resulting from the terminal pentene radical of linolenic acid, CH_3–CH_2–CH=CH–CH_2–. Hoffmann (1962) has listed the flavor descriptions of reverted soybean oil (Table 2.32) and the volatile decomposition products isolated from reverted or oxidized soybean oil (Table 2.33).

The first perceptible reversion flavor was found to be caused by 3-*cis*-hexenal, which has a pronounced green bean odor. Other flavorful aldehydes isolated were 2-*trans*-hexenal (green, grassy), 2-*trans*-nonenal (rancid), and 2-*trans*-6-*cis*-nonadienal (cucumber flavor). These findings illustrate the complexity of the reversion flavor. Similar problems occur with other polyunsaturated oils such as fish oil and rapeseed oil.

Table 2.32 Flavor descriptions used for crude, processed, and reverted soybean oil

State	Flavor
Crude	Grassy, beany
Freshly processed	Sweet, pleasant, nutty
Reverted	Grassy, bany, buttery, melony, tallowy, painty, fishy

Source: From G. Hoffmann, Vegetable Oils, in *Lipids and Their Oxidation*, H.W. Schultz et al., eds., 1962, AVI Publishing Co.

Physical Properties

Fats and oils are mixtures of mixed triglycerides. Fats are semisolid at room temperature; they are known as plastic fats. The solid character of fats is the result of the presence of a certain proportion of crystallized triglycerides. Most fats contain a range of triglycerides of different melting points from very high to very low. When a fat is liquefied by heating, all glycerides are in the liquid state; upon cooling, some of the higher melting fractions become insoluble and crystallize. As the temperature is lowered, more glycerides become insoluble and crystallize, thereby increasing the solid fat content.

Crystallization of a fat is a slow process, whereas melting is instantaneous. When a fat crystallizes, the latent heat of crystallization is liberated and a volume contraction takes place. When a fat melts, the heat effect is negative and volume expands (Fig. 2.48). These changes have been used for the measurement of some fat properties such as melting point, solidification temperature, and solid fat content. Because fats contain a range of glycerides of different melting points, there is no distinct melting point but rather a melting range. Nevertheless, melting points of fats are often determined. These are not real melting points, but some arbitrary temperature at which virtually all of the fat has become liquid. The value of these melting points depends on the

Table 2.33 Volatile compounds isolated from reverted or oxidized soybean oil

Aldehydes			
Saturated	Δ2 Unsaturated Δ2,4 Unsaturated		Δ3 Unsaturated
$C_1C_2C_3^*$ $C_4C_5C_6^*$	$C_4C_5C_6C_7^*C_8$	$C_6\underbrace{C_7C_7}^{*}$ $C_8{}^*C_9$	$\underbrace{C_6C_6}$
$C_7C_8C_9$	$C_9{}^*C_{10}C_{11}$	$\underbrace{C_{10}C_{10}}^{*}$ C_{12}	
Ketones and Dicarb.	Alcohols and Esters		Acids
Methyl-pentyl ketone	1-octen-3-ol		Saturated
Di-n-propyl ketone	Ethanol		C_1–C_9 or C_{10}
Malondialdehyde	Ethyl formate		
	Ethyl acetate		

*Main products
⌣ Stereo-isomers

Source: From G. Hoffmann, Vegetable Oils, in *Lipids and Their Oxidation,* H.W. Schultz et al., eds., 1962, AVI Publishing Co.

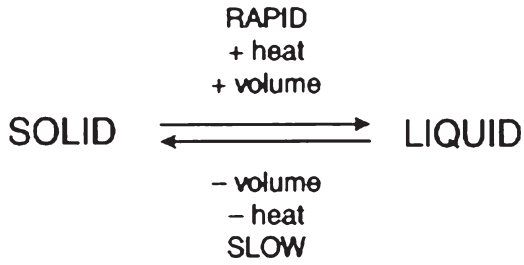


Fig. 2.48 Melting and solidification of fats

Table 2.34 Melting points of some fatty acids

Fatty Acid	MP(°C)
Oleic (*cis*)	13
Elaidic (*trans*)	44
Stearic	70
Linoleic (*cis-cis*)	–5
Linelaidic (*trans-trans*)	28
Butyric	–8

Table 2.35 Melting points of some triglycerides

Triglycerides		MP (°C)
Trisaturated	S-S-S	72
	P-P-P	65
	S-P-P	62
Disaturated	P-0-P	37
	O-P-P	34
	S-0-P	35
	S-P-O	39
	P-S-O	36
Diunsaturated	O-O-P	19
	O-O-S	23
Triunsaturated	O-O-O	5
	E-E-E	42
	L-L-L	−10

S stearic, *P* palmitic, *O* oleic, *E* elaidic, *L* linoleic

measurement technique employed (Mertens and deMan 1972). The melting point of a fat is basically determined by the melting points of its constituent fatty acids. As shown in Table 2.34, chain length and unsaturation of fatty acids affect melting point. In addition, the configuration around the double bond is important. The arrangement of the fatty acids in different glyceride types also affects the melting point, as shown in Table 2.35. One of the most important properties of fats is the

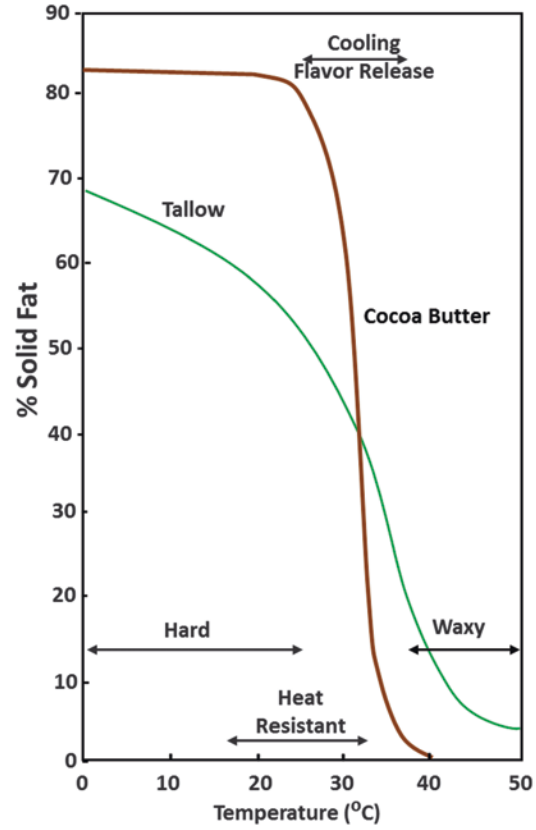


Fig. 2.49 Physical properties of tallow and cocoa butter as influenced by solid fat profile. *Adapted* from": U. Bracco, Effect of Triglyceride Structure on Fat Absorption, *American Journal of Clinical Nutrition,* Vol. 60, (Suppl.) p. 1008S, © 1994, American Society for Clinical Nutrition

solid fat content. Because the solid fat content is dependent on temperature, it is common to determine the solid fat profile, which is a graph representing the relationship between solid fat content and temperature. Earlier methods for measuring solid fat were based on dilatometry, a technique using the melting expansion of fats on heating. Modem methods employ pulsed nuclear magnetic resonance. The dilatometer technique gives an approximation of the true solid fat content and is reported as solid fat index (SFI). The nuclear magnetic resonance results are true solid fat measurements and reported as solid fat content (SFC). The relationship between solid fat content and physical properties is demonstrated in Fig. 2.49 (Bracco 1994), where the difference in properties

of cocoa butter and tallow is demonstrated. The steep solid fat curve of cocoa butter provides coolness and flavor release, and its high solids content at room temperature ensures hardness and heat resistance. Tallow has a less steep solids curve with solids beyond 37 °C, which gives rise to a waxy mouthfeel.

The rate at which a liquid fat is cooled is important in establishing solid fat content and crystal size. In contrast to the glassy state forming systems described in Chap. 1, fats do not form a glassy state. The rate of supercooling determines whether nucleation or crystal growth will predominate. At low supercooling, nucleation is at a minimum and large crystals are formed. At high supercooling, nucleation is high and small crystals result. Another result of high supercooling is the formation of solid solutions or mixed crystals. When fats are cooled quickly to well below their melting point (e.g., 0 °C), the high melting glycerides crystallize together with some of the lower melting ones into mixed crystals. When the fat is subsequently tempered at a temperature close to the melting point, the mixed crystals recrystallize into crystals of more uniform composition. This means that a rapidly cooled fat contains more solid fat than the same fat that has been tempered after the same cooling treatment (Table 2.36).

In many food products and processing operations, control of lipid crystallization is important to obtain the desired number of fat crystals, their size distribution, polymorphic form, and dispersion of the crystalline phase. Crystallization of triacylglycerols (TAG) is the most important factor, however crystallization of other lipid components (i.e., monoacylglycerols, diacylglycerols, phospholipids, etc.) can impact product quality.

Table 2.36 Solid fat content of a soft margarine fat with and without tempering at °C

	Solid fat content	
Temperature (°C)	Tempered	Not tempered
10	22.3	30.7
20	11.7	16.0
30	4.1	6.3
35	2.8	1.9

Control of the crystalline microstructure in lipid systems provides control of the physical properties of the food. For example, to obtain the desired polymorphic form of chocolate tempering prior to molding or enrobing is applied to control crystallization of the cocoa butter into a large number of very small crystals. The cocoa butter crystals in chocolate contribute to the desired appearance (shine or gloss), snap, and flavor release, melting in the mouth, and stability during shelf life. Control of fat crystallization is also important in butter, margarine, whipped cream, ice cream, shortening, peanut butter, and other foods.

Fats crystallization is often used to modify the properties of the fat. For example, winterization of vegetable oils removes fractions that crystallize at higher temperatures assuring that oil remains a clear liquid even when stored at low temperatures for extended time periods. Many fats, including palm oil, palm-kernel oil, milk fat, and tallow, are fractionated by crystallization to produce different functional fats.

Generally about 98% of the content of fats are present as triglycerides (TAGs), the remainder lipids like diacylglycerols (DAGs), monoacylglycerols (MAGs), free fatty acids (FFAs), phospholipids, glycolipids, sterols, and other trace components. These minor lipids are partially removed resulting in lower concentrations than in unrefined fats. Although the TAGs form the main crystalline phase, the minor components, or impurities, can influence how crystallization occurs and as a result crystallization may be different in refined oil than in the unrefined starting material.

It is important to understand the nature of the liquid phase prior to crystallization to understand how crystals form. Lipids retain some degree of order in the liquid phase, with temperatures well above the meltin. When melting fats, this liquid ordering is termed a crystalline memory effect, where subsequent recooling leads to formation of a different (usually more stable) phase than would occur if the fat was heated to higher temperatures to destroy the liquid memory (Larsson 1972; Hernqvist 1984).

Nucleation, refers to the organization of molecules leading to the formation of the crystalline phase from the liquid. The inherent ordering of the liquid phase in lipids leads to crystal formation. Rapid cooling of liquid lipids results in the formation of a diffuse crystalline phase (low-energy polymorph) because of the ordering structure in the liquid phase. This effect differs from rapid cooling of other systems, most notably sugars and starches which results in the formation of a glassy state where molecules that are randomly organized together with no long-term ordering.

Slower cooling from the liquid, the lipid molecules have time to organize into lamellae (1) and eventually can form coherent, three-dimensional crystals (shown schematically in Fig. 2.50). The arrangement of the molecules into the crystalline state depends on such factors as the cooling rate, the temperature at which crystallization occurs, the agitation rate, and the composition of the lipid phase.

Polymorphism is the ability of a molecule to take more than one crystalline form depending on its arrangement within the crystal lattice. The polymorphic forms in lipids are determined by the differences in hydrocarbon chain packing and variations in the angle of tilt of the hydrocarbon chain. The crystallization behavior of TAG, including crystallization rate, crystal size, morphology, and total crystallinity, are affected by polymorphism. The molecular structure of the TAG and several external factors like temperature, pressure, rate of crystallization, impurities, and shear rate influence polymorphism (Sato 2001).

Polymorphism is described a s the ability of a chemical compound to exist in differing crystalline or liquid crystalline forms. Table 2.37 describes the basic physical properties of the three main polymorphic forms found in fats. The three forms are α, β' and β. Polymorph α is the least stable is readily transformed to either β or β' forms. Polymorph β' is a meta-stable form which is found in margarine and shortening because the fat networks provide the desired physical and rheological properties. The β form is the most stable, forming large plate like structures which result in poor margarine or shortening qualities.

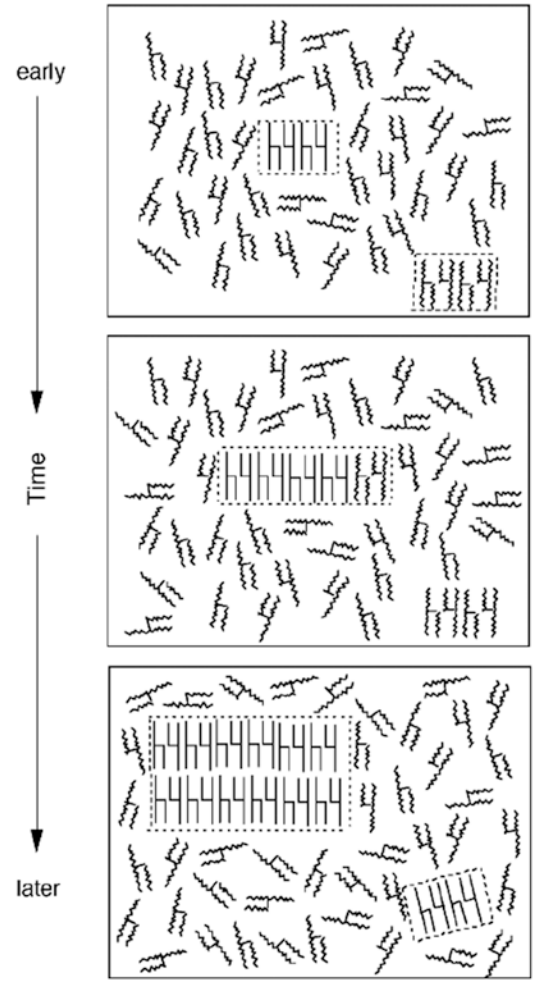


Fig. 2.50 Proposed mechanism (highly schematic) for nucleation of triacylglycerols (TAGs). *Straight chains* indicate crystallized TAGs, whereas *bent chains* indicate fluid TAGs (Hartel 2001)

TAGs are oriented in a chair or tuning fork configuration in the crystalline lattice. The TAG can take either a double or triple chain-length structure as seen in Fig. 2.51 The fatty acids of TAG pairs overlap in a double chain-length structure whereas in triple chain packing, the fatty acids do not overlap. The height of these chair structures and the distance between the molecules in the chair structures are found by using the X-ray spectra as the long and short spacings, respectively.

The polymorphic forms of fats are often simply classified into three categories, α, β′, and β, in

Table 2.37 Physical properties of the three principal polymorphic forms found in fat

Form	Stability	Density	Melting point	Morphology	Unit cell
α	Least	Lowest	Lowest	Amorphous	Hexagonal
β'	Metastable	Intermediate	Intermediate	Rectangular	Orthorhombic
β	Most	Highest	Highest	Needle-shaped	Triclinic

Adopted from Sato and Ueno 2014

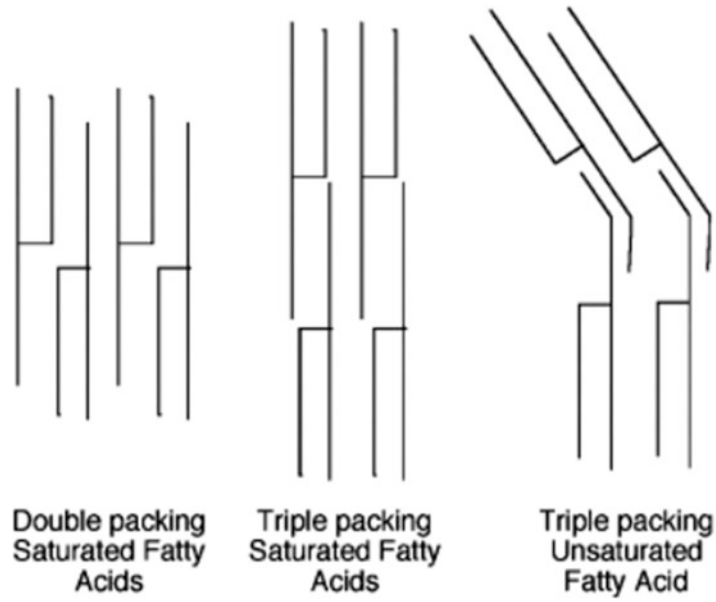


Fig. 2.51 Packing arrangements of triacylglycerol molecules in the crystal lattice

increasing order of stability. The α form is the least stable polymorph with the lowest melting point and latent heat of fusion. The β form is the most stable, with the highest melting point and latent heat. Each polymorphic form has distinct short spacings (the distances between parallel acyl groups on the TAG) that are used to distinguish the polymorphic forms based on their X-ray diffraction patterns, as summarized in Table 2.37 Based on the unique configuration of the molecules within the crystal lattice, each polymorph has a different crystallographic unit cell, also shown in Table 2.37.

Applying measuring X-ray diffraction (XRD) short spacing patterns of poly-crystalline samples the subcellular structures of the three polymorphs can be determined. The three fat polymorphs, α, β' and β of fats, exhibit differing subcell structures: α polymorphs are hexagonal sub-cell (H); β' polymorphs are orthorhombic–perpendicular subcell (O⊥); and β polymorphs are triclinic–parallel subcell (T//) (Larsson 1966;) as illustrated in Fig. 2.52a.

Figure 2.52b illustrates the chain-length structure, applying the repetitive sequence of the acyl chains involved in a unit cell lamella along the long-chain axis (Larsson 1972). A double chain-length structure is formed when the chemical properties of the three acid moieties are the same or very similar as in tripalmitin or a homologus fat with high content of long chain fatty acids or trans fatty acids. Conversely, when the chemical properties of one or two fatty acid side chains are different, a triple chain-length (TCL) structure is formed because of chain sorting. TAGs with three saturated fatty acids crystallize in double chain-length packing, whereas triple chain-length packing is obtained if the TAG contains fatty acids with different chain lengths and unsaturation). Lutton (1950) stated that if the fatty acids of a

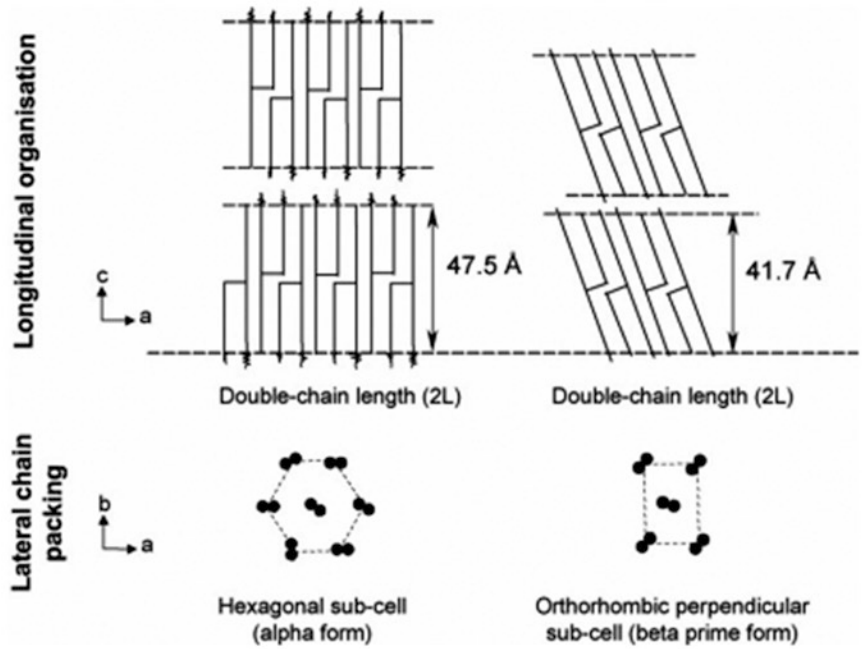


Fig. 2.52 Proposed structure of the form and form of triglycerides with double chain length longitudinal organizations as seen in the ca projection. For the form, the methyl end-group regions are somewhat disordered, as in liquid crystals. (Lopez et al. 2006) (Permission requested)

TAG differ in length by more than four carbons, it forms a triple chain-length structure. Triple chain-length packing is also observed in TAG containing a cis-unsaturated fatty acid because this causes a kink in the structure, as seen in Fig. 2.52. Because the fatty acids are not linear, cis-unsaturated fatty acids do not mix in one layer with the more linear saturated fatty acids, and triple chain-length crystals are formed. Trans-unsaturated fatty acids incorporate into a crystal structure in the same way as the saturated fatty acids. This is why oils are hydrogenated to produce solid components in the fat for margarines and shortenings. The chain-length structure influences the mixing-phase behavior of different types of TAGs in solid phases (Sato 2001). The triple chain-length structure has greater long spacings than does the double chain-length structure. (Metin and Hartel 1998)

The relevance of the chain-length structure is revealed in the mixing phase behavior of the different types of the TAGs in the solid phase: when the fatty acids with different chain lengths or degrees of unsaturation are mixed with the TCL fats, phase separation readily occurs. The chain length structures can be determined solely by measuring the XRD long spacing patterns of the poly-crystalline samples. In food fats, transformation from polymorph β' to polymorph β can result in deterioration of the end product resulting from changes in the crystal morphology and network, as shown in Table 2.37. The β-type polymorph is found in confectionery fats made of cocoa butter (Timms 2003). There are two β-type crystals: a meta-stable β2 form is more useful than the more stable β1 form (Sato and Koyano 2001; Van Mechelen et al. 2006a, b; Sato and Ueno 2014).

Monotropic polymorphism occurs in lipids where unstable forms are the first to crystallize in a subcooled fat, because of their lower energy state. Subsequently these unstable forms transform into more stable forms. The changes continue to occur until the most stable polymorph for a given lipid is reached. The transformations occur at temperatures slightly above the melting temperature of the less stable form. The increase in temperature first causes the melting of the unstable forms followed by solidification in a more stable form. Transformation to a more stable form can also take place without melting. The

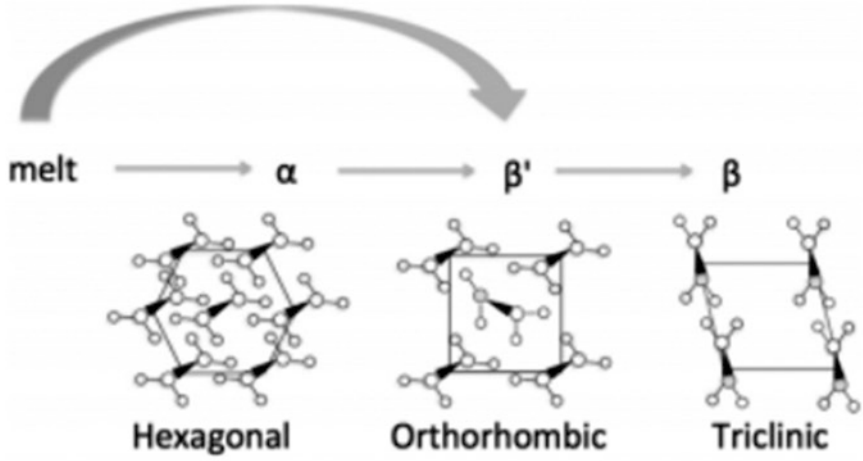


Fig. 2.53 Illustration of fat crystallization in milk fat. α-crystals (hexagonal subcell structure), forms directly from the melt, while β'-crystals (orthorhombic subcell) forms either via recrystallization of α to β∋ or directly from the melt. β-crystals (triclinic subcell) are formed via recrystallization from β'. Representation of the Packing of Triacylglycerols in the Three Main Polymorphic Forms. (Rønholt et al. 2014)

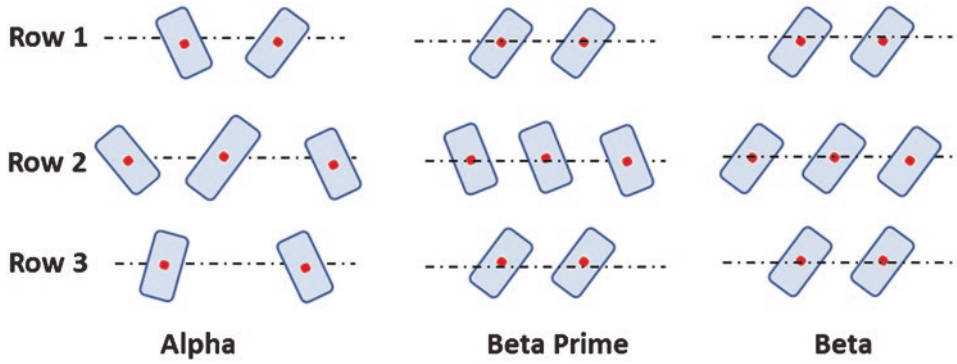


Fig. 2.54 Cross-sectional structures of long-chain compounds (Lutton 1972)

polymorphic state becomes more stable and higher melting as the molecules become more tightly arranged in the crystal lattice. Figure 2.53 represents the differences in packing of the three polymorphic forms. It is assumed that the chair structure is maintained during polymorphic transformations (Larsson 1966). The layer arrangement of the α polymorph does not change when it is transformed to the β′ polymorph, although its lateral chain packing and angle of tilt changes during polymorphic transformation.

The hydrocarbon chain packing of the β polymorph is denser than that of the α polymorph. The denser chain packing in the β polymorph gives increased stability compared with the α polymorph. The different polymorphic forms typically crystallize at rates in order of their stability ($\alpha < \beta' < \beta$). Thus, the least-stable polymorphic form typically crystallizes first in a strongly subcooled molten fat because of the lower surface energy (Bailey 1950).

Assignment of the polymorphic form of a fat can be done unequivocally only by X-ray diffraction, sometimes supported by differential scanning calorimetry. The characteristics of the three polymorphs are as follows:

- α *form*—one strong, short spacing in the X-ray diffraction pattern at 0.42 nm. The chains are arranged in a hexagonal crystal structure (H) with no order of the zigzag chain planes. The chains have no angle of tilt (Lutton 1972) (Fig. 2.54).

- β′ *form*—two strong, short spacings in the X-ray diffraction pattem at 0.38 and 0.42 nm. The chains are arranged in an orthorhombic crystal structure. The zigzag planes are arranged in a perpendicular crystal structure. The chains have an angle of tilt between 50 and 70°.
- β *form*—one strong, short spacing at 0.46 nm and two spacings at 0.37 and 0.39 nm. This form is the most densely packed and is in a triclinic structure. The zigzag planes are in a parallel arrangement, and the chains have an angle of tilt of 50–70°.

The rate of polymorphic transformation depends on the length of the fatty acid chain. The rate of change is longest for TAGs with short-chain fatty acids such as butterfat (Bailey 1950). Natural fats generally contain a large number of different TAGs. This heterogeneity results in very slow transition form unstable to stable forms is often very slow. As mentioned previously, the α- form is generally formed first in a rapidly cooled liquid fat, but it is usually very unstable and rapidly transforms to the β′ form. The β′ form may remain for hours to days. In many fats the β′ However, in many natural fats, the β′ polymorph can exist for long periods of time because the complex mixtures result in the development of a solid solution (Walstra et al. 1987). In some mixed-acid TAGs, no β polymorph may form and β′ is the most stable. In some systems both β forms may be present (Sato 2001).

More than one subtype within the main polymorphic grouping has been identified in some fats. For example, six different polymorphic forms have been identified in cocoa butter, although there is still some debate whether they are all truly unique polymorphs (Table 2.38). Two β′ and two β forms have been identified for cocoa butter (Loisel et al. 1998). These polymorphs have slightly different melting points, but they have X-ray spectra that fit within the definition of that polymorph (Metin and Hartel 1998; van Malssen et al. 1996a, b, c).

Different nomenclatures for cocoa butter have been used to describe the polymorphic forms as seen in Table 2.39. In the Greek nomenclature, where polymorphs are given a Greek letter, the most stable form within a polymorph type is given the subscript 1, and other polymorphs within that form are ordered in decreasing stability or melting temperature. For example, cocoa butter has two β′ forms, with the β′1 form having the highest melting point (most stable) (Mertin and Hartel 1998).

Table 2.38 Polymorphic forms of cocoa butter melting temperature (°C)

Form		Wille and Lutton (13)	Davis and Dimick (13)
I	γ	17.3	13.1
II	α	23.3	17.7
III	β'_2	25.5	22.4
IV	β'_1	27.5	26.4
V	β_2	33.8	30.7
VI	β	36.3	33.8

Chocolate is first melted to eliminate existing crystalline structures. The molten chocolate is then cooled and allowed for seeds to form or frequently type V seeds are added to initiate crystalline growth. The chocolate is then warmed slightly and maintained at that temperature to allow conversion of most of the structure to Type V crystals. The chocolate is then cooled to room temperature and allowed to solidify. This product will then have appropriate shine, snap in biting and cooling in the mouth. The temperature chocolate is also resistant to bloom. Bloom is the migration of fat to the surface of eh product leaving a unsightly white residue on the surface.

The polymorphs of chocolate exhibit differing characteristics as shown in Table 2.39 (Beckett 2007).

Form		Melting temperature	Typical properties
I	γ	17 °C	Produced when liquid chocolate is cooled rapidly. Unstable form results in soft crumbly product that will bloom.
II	α	21 °C	After storing rapidly cooled form chocolate morphs to this form. Soft, crumbly and tends to boom
III	β'_2	26 °C	Form II crystals become form III after storage at low temperatures

Form		Melting temperature	Typical properties
IV	β'_1	28 °C	Produced by cooling chocolate to room temperature with no further tempering. Product will be firm but not snap, Melts at too low a temperature
V	β_2	34 °C	Produced by properly tempering chocolate. Very stable, high glows, good snap, resistant to bloom
VI	β	36 °C	Produced when well-tempered chocolate is stored at room temperature for many months. Product will be hard and difficult to melt

The tendency of fats to remain stable in the β′ form for a long time or to quickly convert to the β form is more difficult to predict than that of pure triglycerides. In margarines and shortenings it is desirable that the fat crystals are in the β′ form. In margarines this results in a smooth texture, shiny surface, and good meltdown properties. The β′ crystals in shortenings give good aeration in cakes and creams. The solids or fat crystals in margarines and shortenings consist of triglycerides with melting points considerably higher than those of the whole fat. These triglycerides are referred to as high melting glycerides (HMGs) and can be obtained by fractionation from acetone (D'Souza et al. 1991). When the HMGs consist of a high level of the same fatty acid such as palmitic acid or stearic acid or a mixture of stearic and any *trans* isomeric form 18:1, the fat crystals quickly transform from β′ to β form. This happens when canola oil or sunflower oil is hydrogenated and used as a sole hardstock in margarines and shortenings (Hernqvist 1984, 1988). The fatty acid composition of canola oil (see Table 2.13) consists mainly of 18-carbon fatty acids such as 18:0, 18:1, 18:2, and 18:3; it contains only 4% palmitic acid. When canola oil is hydrogenated, the solids that are produced consist of triglycerides of 18:0 or 18:1 *trans* fatty acid, which results in a high level of 54 (3 × 18) carbon triglycerides. These triglycerides are liable to undergo polymorphic transition to the β form. Triglycerides consisting of only palmitic acid or tripalmitin (48-carbon triglycerides) are equally likely to convert to the β form. This can

Table 2.39 Characteristics of various crystalline forms of cocoa butter

Polymorph of cocoa butter	Melting point °F	Properties
Form I	61–67	Produced by rapid cooling such as placing liquid cocoa butter n freezer
Beta prime 2		Most unstable form
γ		Soft, crumbly, blooms and melts at low temperature
Form II	70–73	Produced by rapid cooling placing chocolate in refrigerator of freezer
Alpha		Alpha crystals form after short time storage at freezing temperatures
α		Melts at low temperature, contains many polymorps, soft crumbly and blooms heavily
Form III	77–78	Produced by too rapid cooling in freezer
Mixture of Alpha and Beta-prime I		Form after storage at cool temperatures above freezing
Form IV	81–84	Cooling at room temperature with no further tempering
Beta-prime		Crystals are firm but do not have snap
β'		Product melts below body temperature and blooms
Form V	92–95	Produced by tempering. After tempering cool slowly at room temperature
Beta 2		Highly desirable polymorph
β		Very stable, smooth shiny surface, good snap, melts readily when eaten
Form VI	97	Formed on long term storage of tempered chocolate at room temperature
Beta 1		Very hard, does not melt easily
β		Can be reversed by retempering

Table 2.40 Triacylglycerol (TAG) composition (%) by carbon number of common vegetable oils

	TAG (Carbon Number)				
Oil	48	50	52	54	56
Canola	–	1.1	13.0	76.8	5.6
Soybean	–	3.3	27.6	66.7	1.7
Sunflower	–	2.8	20.2	75.1	0.7
Sunflower high olein	–	2.0	15.0	80.6	1.0
Corn	–	4.6	30.4	64.2	0.8
Olive	–	4.7	27.7	66.7	0.9
Peanut	–	5.5	30.9	54.2	5.3
Cottonseed	0.9	13.6	43.5	40.5	1.3
Palm	8.0	42.5	40.5	9.0	–

Table 2.41 Major triacylglycerols (TAGs) of palm oil and their melting points

			Melting Point (°C)	
TAG	Carbon Number	%	β′	β
[a]PPP	48	6	56.7	66.2
[a]PPS	50	1	59.9	62.9
[a]PSP	50	0.5	68.8	–
POP	50	26	30.5	35.3
[a]PPO	50	6	35.4	40.4
PLP	50	7	18.6	NA
POO	52	19	14.2	19.2
POS	52	3	33.2	38.2
PLO	52	4	NA	NA
OOO	54	3	−11.8	5.1

[a]Likely to be in the palm stearin fraction
NA Not available

happen when palm stearin, which is obtained from fractionated palm oil, is used as the sole hardstock for a margarine or shortening (deMan and deMan 1995). Soybean oil contains 11% palmitic acid (see Table 2.13). When soybean oil is hydrogenated, the solids are more diverse in fatty acid chain length than those of hydrogenated canola or sunflower oil of similar consistency. The triglyceride composition (carbon number) of common vegetable oils is displayed in Table 2.40. Canola and sunflower oil contain high levels of 54-carbon triacylglycerol (TAG), while palm oil has the lowest level. When these oils are hydrogenated, the solids in the early stages of hydrogenation contain less 54-carbon and more 50-carbon triglycerides than the original oils, because the 50-carbon triglycerides already contain two saturated fatty acids and are likely to be included in the solids at the early stage. The 50-carbon triglycerides of the solids consist mainly of PSP or PEP (E stands for elaidic acid or any form of isomeric 18:1 *trans*). These triglycerides are very stable in the β′ form, as mentioned earlier, and preferably should be part of the solids. The solid 52-carbon triglycerides are mainly made up of PSS or PEE. Palmitic acid in vegetable oils is mainly located in the 1 or the 3 position. Palm oil has a high level of 50- and 52-carbon triglycerides and a low level of 54-carbon TAG (Table 2.41). The 50-carbon TAG consist mainly of POP (26%) and PLP (8%). When palm oil is hydrogenated, POP and PLP change to PSP or PEP, which provides a high level of desirable β′ triglycerides. In addition, the solid 52-carbon triglycerides that are produced are also β′ tending. The β′ stability of hydrogenated canola oil can be greatly enhanced by incorporation of hydrogenated palm oil at a level of 10%. For margarines where it is desirable to have low levels of *trans* fatty acids, palm mid-fraction that contains very high levels of POP and PLP (up to 60%) can be hydrogenated and incorporated at lower levels to secure a desirable level of PSP and PEP.

Diversification of the fatty acid composition of the solids can also be achieved by including lauric oils such as palm kernel or coconut oil, preferably in the fully hydrogenated form (Nor Aini et al. 1996, 1997). A β′ stable hardstock can, upon dilution with liquid oil, crystallize in the β form, as is done in soft margarines and to a certain extent in stick margarines. Elevated storage temperatures can also induce a polymorphic transition. β crystals are initially small but upon storage can grow into large, needle-like agglomerates that make the product grainy, make the texture brittle, and give the surface a dull appearance. Shortenings always contain about 10–12% hydrogenated palm oil—usually in the fully hydrogenated form, which extends the plastic range so that the product is still plastic at higher temperatures. The melting point of shortenings is in the low range of 40–45 °C.

Fractionation

Fats can be separated into fractions with different physical characteristics by fractional crystallization from a solvent or by fractionation from the melt. The former process gives sharply defined fractions but is only used for production of high-value fats; the latter process is much simpler and more cost-effective. Fractionation from the melt or dry fractionation is carried out on a very large scale with palm oil and other fats including beef tallow, lard, and milk fat.

There are several reasons for employing fractional crystallization (Hamm 1995):

- To remove small quantities of high melting components that might result in cloudiness of an oil. This can be either a triacylglycerol fraction or non-triacylglycerol compound. The former happens when soybean oil is lightly hydrogenated to convert it to a more stable oil. The resulting solid triglycerides have to be removed to yield a clear oil. The latter occurs when waxes crystallize from oils such as sunflower oil. This type of fractionation is known as winterization.
- To change a fat or oil into two or more fractions with different melting characteristics. In simple dry fractionation, a hard fraction (stearin) and a liquid fraction (olein) are obtained. This is by far the most common application of fractionation.
- To produce well-defined fractions with special physical properties that can be used as specialty fats or confectionery fats. This is often done by solvent fractionation.

The process of fractionation involves the controlled and limited crystallization of a melted fat. By careful management of the rate of cooling and the intensity of agitation, it is possible to produce a slurry of relatively large crystals that can be separated from the remaining liquid oil by filtration. The major application of fractionation is with palm oil; many millions of tons of palm oil are fractionated into palm stearin and palm olein every year. Palm oil is unusual among vegetable oils. It has a high level of palmitic acid; contains a substantial amount of a trisaturated simple glyceride, tripalmitin; and has a high level of SUS glycerides.

Table 2.42 Composition and slip melting point (SMP) of palm oil and its fractions

	Fatty Acid (Wt %)				
Product	16:0	18:0	18:1	18:2	SMP (°C)
Palm oil	44.1	4.4	39.0	10.6	36.7
Palm olein	40.9	4.2	41.5	11.6	21.5
Palm stearin	56.8	4.9	29.0	7.2	51.4

Source: Reprinted with permission from W.L. Siew, et al., Identity Characteristics of Malaysian Palm Oil Products: Fatty Acid and Triglyceride Composition and Solid Fat Content, *E.L.A.E.I.S.*, Vol. 5, No. 1, p. 40, © 1993, Palm Oil Research Institute of Malaysia

Table 2.43 Composition of palm oil and palm olein

Triglyceride	Palm oil	Palm olein
SSS (mainly PPP)	8	0.5
SUS (mainly POP)	50	48
SUU (mainly POO)	37	44
UUU	5	7
Iodine value	51–53	57–58

Source: Reprinted with permission from W.L. Siew, et al., Identity Characteristics of Malaysian Palm Oil Products: Fatty Acid and Triglyceride Composition and Solid Fat Content, *E.L.A.E.I.S.*, Vol. 4, No. 1, p. 40, ©1993, Palm Oil Research Institute of Malaysia

The major glycerides present in palm oil are listed in Table 2.42, together with their melting points. When a liquid fat is cooled, a crystalline solid is formed; its composition and yield are determined by the final temperature of the oil. From Table 2.43 it is obvious that the glycerides most likely to crystallize are PPP, PPS, and PSP. It is theoretically impossible to separate these glycerides sharply from the rest of the glycerides. There are two reasons: (1) the formation of solid solutions and (2) the problem of entrainment.

The formation of solid solutions can be explained by the phase diagram of Fig. 2.55 (Timms 1984, 1995). This relates to two triglycerides A and B, which form a solid solution. In other words, they crystallize together into mixed crystals. At temperature T_1 the solid phase has the composition indicated by *c* and the liquid phase composition is *a*. The fraction of the solid phase is equal to *ab/ac*. As the crystallization temperature differs from T_1, the composition of the solid and liquid phases will also differ. Solid solutions are formed when two or more solutes have melting points that are not very different. If the solute and solvent molecules have widely differing melting

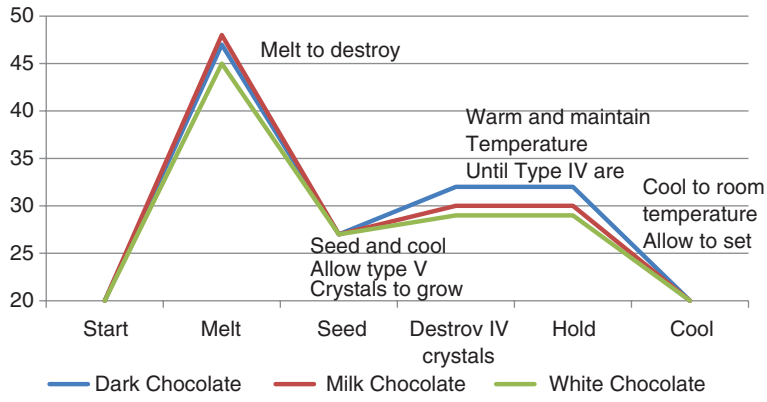


Fig. 2.55 Typical temperature profiles to temper chocolate

points, no solid solutions will be formed and the solubility is dependent only on the solute. This is known as ideal solubility. A plot of the solubility of tripalmitin in 2-oleo-dipalmitin is presented in Fig. 2.56. At all temperatures the actual solubility is higher than the ideal solubility, and the resulting solid phase that crystallizes is composed not only of tripalmitin but a solid solution of tripalmitin and 2-oleo-dipalmitin (Fig. 2.57).

Entrainment results from mechanical entrapment of liquid phase in the crystal cake obtained by filtration. The composition of the filter cake is highly dependent on the degree of pressure applied in the filtration process. The composition of palm oil and its main fractions are given in Table 2.42 (Siew et al. 1992, 1993). The triglycerides in palm oil and palm olein are listed in Table 2.43 Depending on the process used, the stearin can be obtained in yields of 20–40%, with iodine values ranging from 29 to 47. This is important because the olein is considered the more valuable commodity. Palm olein is widely used as a liquid oil in tropical climates. However, in moderate climates, it crystallizes at lower temperatures, just as olive oil and peanut oil do. Palm stearin is finding increasing application as a nonhydrogenated hard fat and as a component in interesterification for the production of no-*trans* margarines.

The fractionation of palm oil can be carried out in a number of ways to yield a variety of products as shown in Fig. 2.58. In this multistage process, a palm midfraction is obtained that can be further fractionated to yield a cocoa butter equivalent (Kellens et al. 1994). Palm oil can be double fractionated to yield a so-called super olein with iodine value of 65.

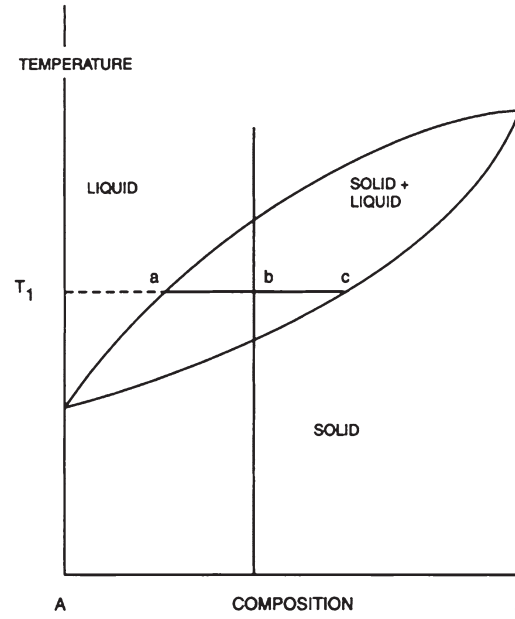


Fig. 2.56 Phase diagram of mixture of triglycerides A and B, showing a continuous solid solution. *Source:* Reprinted from R.E. Timms, Crystallization of Fats, in *Developments in Oils and Fats*, R.J. Hamilton, ed., p. 206, © 1995, Aspen Publishers, Inc.

Milk fat fractionation has been described by Deffense (1993). By combining multistep fractionation and blending, it is possible to produce modified milk fats with improved functional properties.

Emulsions and Emulsifiers

A satisfactory definition of emulsions has been provided by Becher (1965), who states that an emulsion is a heterogeneous system, consisting

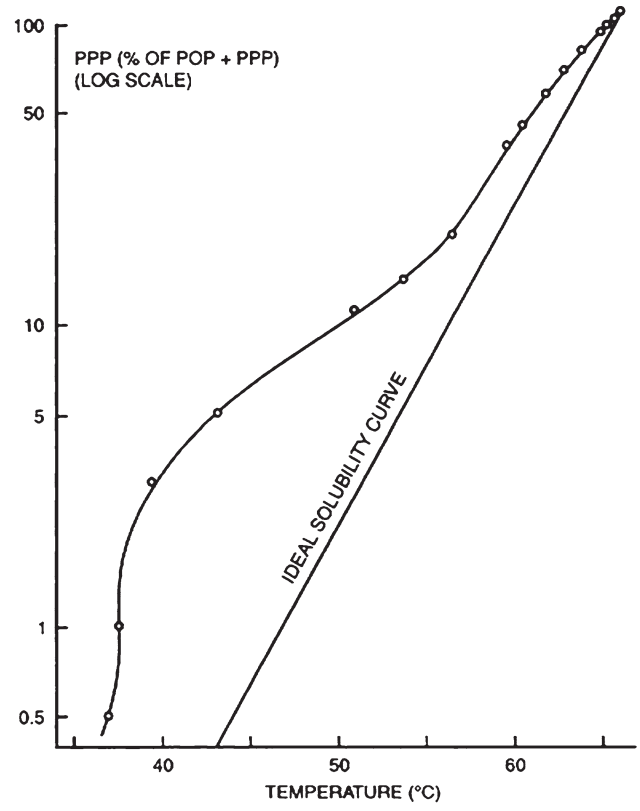


Fig. 2.57 Solubility diagram of tripalmitin in 2-oleo-dipalmitin. *Source:* Reprinted from R.E. Timms, Crystallization of Fats, in *Developments in Oils and Fats*, R.J. Hamilton, ed., p. 209, © 1995, Aspen Publishers, Inc.

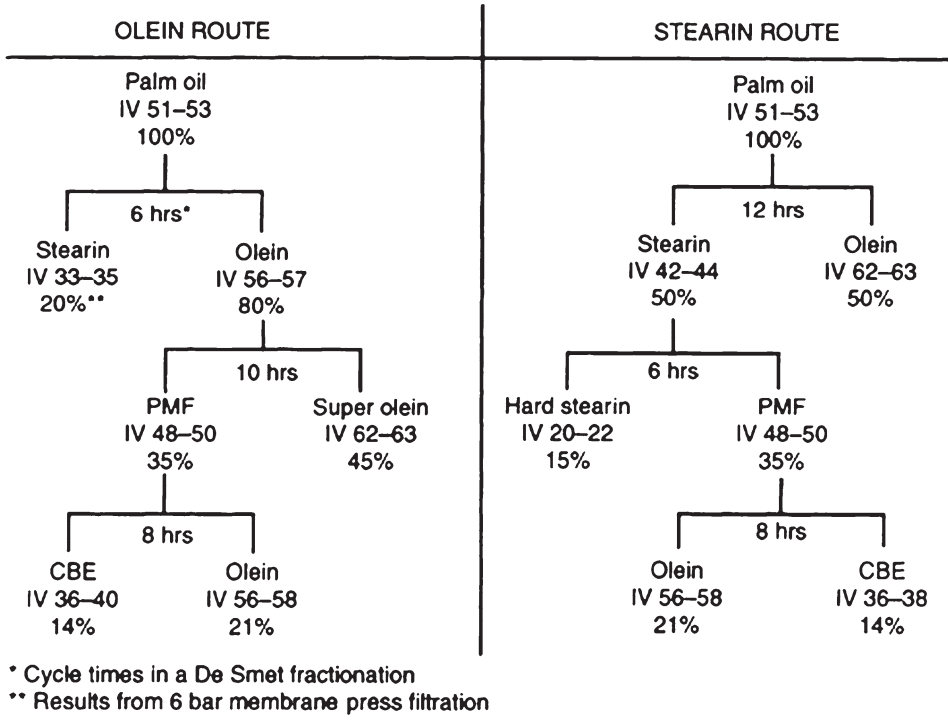


Fig. 2.58 Products obtained by multistage fractionation of palm oil. *Source:* Reprinted with permission from Developments in Fat Fractionation Technology, Paper No. 0042, Kellens et al. 1994, p. 29, © Mark Kellens, PhD

of one immiscible liquid intimately dispersed in another one, in the form of droplets with a diameter generally over 0.1 μm. These systems have a minimal stability, which can be enhanced by surface-active agents and some other substances. In foods, emulsions usually contain the two phases, oil and water. If water is the continuous phase and oil the disperse phase, the emulsion is of the oil in water (O/W) type. In the reverse case, the emulsion is of the water in oil (W/O) type. A third material or combination of several materials is required to confer stability upon the emulsion. These are surface-active agents called emulsifiers. The action of emulsifiers can be enhanced by the presence of stabilizers. Emulsifiers are surface-active compounds that have the ability to reduce the interfacial tension between air-liquid and liquid-liquid interfaces. This ability is the result of an emulsifier's molecular structure: the molecules contain two distinct sections, one having polar or hydrophilic character, the other having nonpolar or hydrophobic properties. The extent of the lowering of interfacial tension by surface-active agents is shown in Fig. 2.59. Most surface-active agents reduce the surface tension from about 50 dynes/cm to less than 10 dynes/cm when used in concentrations below 0.2%.

The relative size of the hydrophilic and hydrophobic sections of an emulsifier molecule mostly determines its behavior in emulsification. To make the selection of the proper emulsifier for a given application, the so-called HLB (hydrophile-lipophile balance) system was developed. It is a numerical expression for the relative simultaneous attraction of an emulsifier for water and for oil. The HLB of an emulsifier is an indication of how it will behave but not how efficient it is. Emulsifiers with low HLB tend to form W/O emulsions, those with intermediate HLB form OAV emulsions, and those with high HLB are solubilizing agents. HLB values can be calculated from the saponification number of the ester (S) and the acid value of the fatty acid radical (A_v) as follows:

$$\mathrm{HLB} = 20\left(1 - \frac{S}{A_v}\right)$$

In many cases the HLB value is determined experimentally and various methods have been used (Friberg 1976). The HLB scale goes from 0 to 20, in theory at least, since at each end of the scale the compounds would have little emulsifying activity. The HLB values of some commercial nonionic emulsifiers are given in Table 2.44 (Griffin 1965).

Foods contain many natural emulsifiers, of which phospholipids are the most common. Crude phospholipid mixtures obtained by degumming of soya oil are utilized extensively as food emulsifiers and are known as soya-lecithin. This product contains a variety of phospholipids, not just lecithin.

Emulsifiers can be tailor-made to serve in many food emulsion systems. Probably the most widely used is the group of monoglycerides obtained by glycerolysis of fats. Reaction of an excess of glycerol with a fat under vacuum at high temperature and in the presence of a catalyst results in the formation of so-called technical monoglycerides. These are mixtures of mono-, di-, and triglycerides. Only the 1-monoglycerides are active as emulsifiers. By molecular distillation under high vacuum, a product can be obtained in which the 1-monoglyceride content exceeds 90%.

The ability of emulsifier molecules to orient themselves at interfaces is exemplified in the phase behavior of monoglycerides. The emulsi-

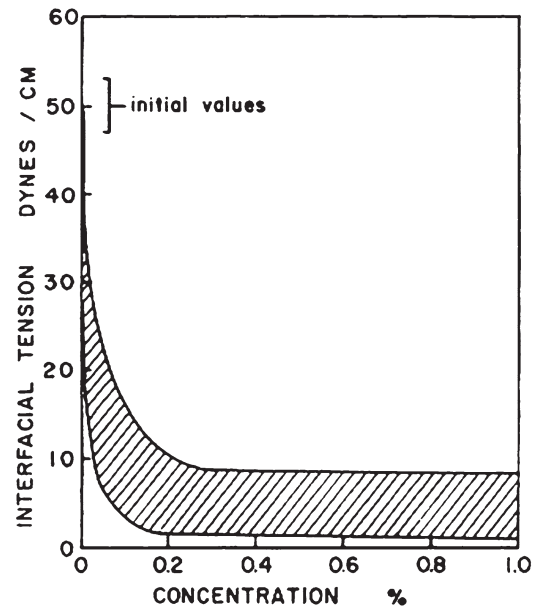


Fig. 2.59 Lowering of the surface tension of water by most surface-active compounds. *Source:* From R Becher, *Emulsions—Theory and Practice*, Becher 1965, Van Nostrand Reinhold Publishing Co.

Table 2.44 HLB values of some commercial nonionic emulsifiers

Trade name	Chemical designation	HLB
Span 85	Sorbitan trioleate	1.8
Span 65	Sorbitan tristearate	2.1
Atmos 150	Mono- and diglycerides from the glycerolysis of edible fats	3.2
Atmul 500	Mono- and diglycerides from the glycerolysis of edible fats	3.5
Atmul 84	Glycerol monostearate	3.8
Span 80	Sorbitan monooleate	4.3
Span 60	Sorbitan monostearate	4.7
Span 40	Sorbitan monopalmitate	6.7
Span 20	Sorbitan monolaurate	8.6
Tween 61	Polyoxyethylene sorbitan monostearate	9.6
Tween 81	Polyoxyethylene sorbitan monooleate	10.0
Tween 85	Polyoxyethylene sorbitan trioleate	11.0
Arlacel 165	Glycerol monostearate (acid stable, self-emulsifying)	11.0
Myrj 45	Polyoxyethylene monostearate	11.1
Atlas G-2127	Polyoxyethylene monolaurate	12.8
Myrj 49	Polyoxyethylene monostearate	15.0
Myrj 51	Polyoxyethylene monostearate	16.0

Source: From W.C. Griffin, Emulsions, in *Kirk-Othmer Encyclopedia of Chemical Technology,* 2nd ed., Vol. 8, pp. 117–154, 1965, John Wiley & Sons

fier molecules in aqueous systems show mesomorphism (formation of liquid crystalline phases). In such systems, several mesophases may exist, as Krog and Larsson (1968) have shown. When 1-monopalmitin crystals in 20–30% of water are heated to 60 °C, a mesophase, called the neat phase, is formed (Fig. 2.60a). This structure consists of bimolecular lipid layers separated by water, with the chains in a disordered state. If this phase is cooled, a gel is formed (Fig. 2.60b). The structure is lamellar with the hydrocarbon chains extended and tilted in a 54° angle toward the water layers. When the neat phase is heated, a stiff cubic phase is formed, called viscous isotropic (Fig. 2.60c). It consists of small water spheres arranged in a face-centered lattice with the polar groups pointed toward the water. On further heating, an isotropic fluid is obtained (Fig. 2.60d), in which polar groups and water form disk-like arrangements. With excess water, a dispersion is formed (Fig. 2.60e), which consists of concentric bimolecular shells of monoglyceride molecules alternating with water.

Emulsions are stabilized by a variety of compounds, mostly macromolecules such as proteins and starches.

Emulsifiers have many additional functions in foods. They form complexes with food components, resulting in modified physical properties of the food system (Larsson and Dejmek 1990). For example, consider the amylose-complexing effect of emulsifiers (Krog 1971). This effect is useful for improving the shelf life of bread (antifirming effect) and modifying the physical characteristics of potato products, pasta, and similar foods.

The amount of emulsifier required to provide a monomolecular layer for an emulsion of any particle size can be calculated from the relationship A/V = 6/D, where A, V, and D are area, volume, and diameter, respectively, of the spherical particles. The total area for 1 mL of oil dispersed as uniform spheres thus calculated is given in Table 2.45. If sodium stearate is used as the emulsifier, the amount required can be calculated, since this molecule has a surface area of about 0.2 nm^2.

$$W = \frac{\text{Total area}\left(\text{nm}^2\right) \times 306}{0.2 \times 6 \times 10^{23}}$$

where

W = weight of emulsifier

306 = molecular weight of sodium stearate

6×10^{23} = Avogadro's number

The amount of emulsifier required increases sharply with decreasing particle size of the emulsion, as shown in Table 2.46 This constitutes a practical limitation to the lower limit of particle

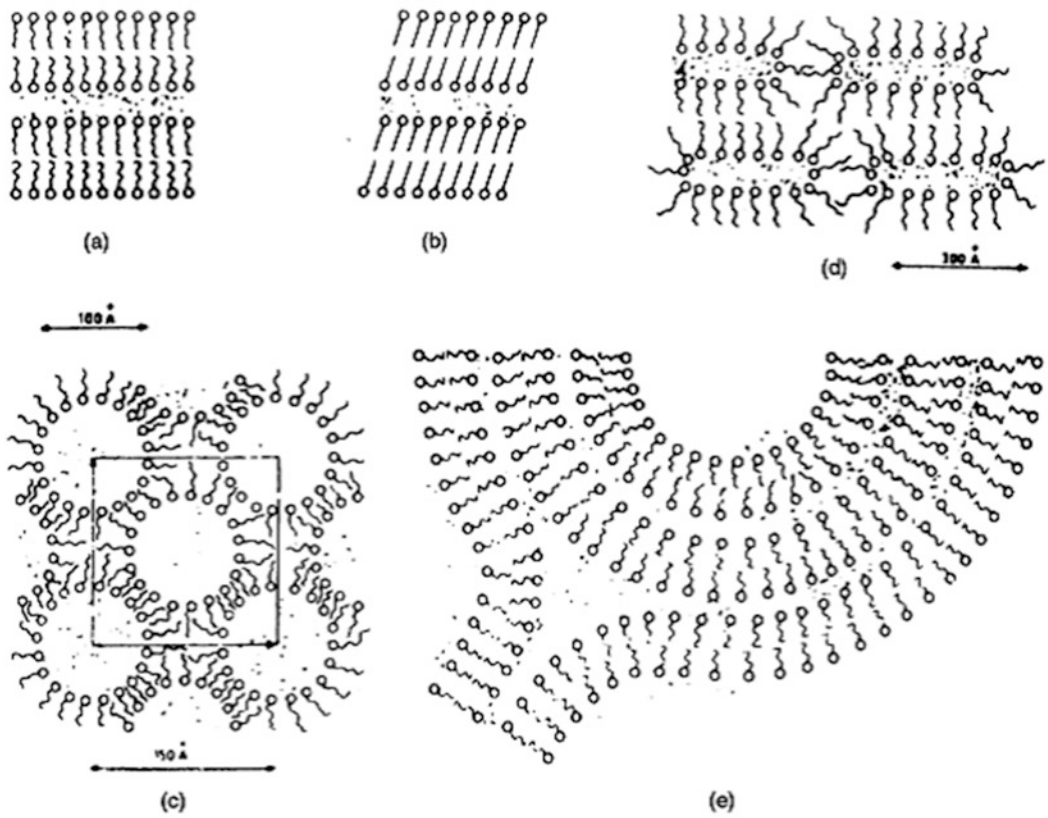


Fig. 2.60 Structure of the mesophases of monoglyceride-water systems. (**a**) Neat phase, (**b**) gel, (**c**) viscous isotropic, (**d**) fluid isotropic, and (**e**) dispersion. *Source:* From N. Krog and K. Larsson, Phase Behavior and Rheological Properties of Aqueous Systems of Industrial Distilled Monoglycerides, *Chem. Phys. Lipids,* Vol. 2, pp. 129–143, 1968

Table 2.45 Total surface area of 1 mL of oil dispersed as uniform spheres in an emulsion

Diameter		Total surface area		
μm	cm	m^2	cm^2	nm^2
10	10^{-3}	0.6	6×10^3	6×10^{17}
1	10^{-4}	6	6×10^4	6×10^{18}
0.1	10^{-5}	60	6×10^5	6×10^{19}
0.01	10^{-6}	600	6×10^6	6×10^{20}

Table 2.46 Amount of sodium stearate emulsifier required to provide a monomolecular layer in emulsions of different particle size

Particle diameter micrometer	Sodium stearate (as % of oil)
10	0.15
1	1.5
0.1	15
0.01	150

size that can be obtained in emulsification. It also indicates that a substance that consists of molecules with a large ratio of surface area to molecular weight and that yields a strong film should be an efficient emulsifier.

Fat Replacers

Fat replacers are substances that are meant to replace the calories provided by fat (9 kcal/g) either partly or completely. There are three basic groupings of fat replacers; 1- protein based. 2- carbohydrate based and 3- fat based. The fat-like substances that are either not absorbed, partially absorbed or intrinsically lower in calories. The proteinaceous or carbohydrate compounds that mimic the gustatory qualities of fats. The latter will be dealt with in Chaps. 3 and 4. Table 2.47 summarizes the fat substitutes used in food products.

Table 2.47 Fat replacers

Product	Technology	Application
Protein-based fat replacers		
Microparticulated Protein Simplesse®	Reduced-calorie (1–2 calorie/gram) ingredient made from whey protein or milk and egg protein. Digested as a protein	Ice cream, butter, sour cream, cheese, yogurt), salad dressing, margarine- and mayonnaise-type products, as well as baked goods, coffee creamer, soups and sauces
Modified Whey Protein Concentrate Dairy-Lo R®	Controlled thermal denaturation results in a functional protein with fat-like properties	Cheese, yogurt, sour cream, ice cream), baked goods, frostings, as well as salad dressing and mayonnaise-type products
Other (K-Blazer®, ULTRA-BAKE™, ULTRA-FREEZETM, Lita®)	One example a fat substitute based on egg white and milk proteins. Similar to microparticulated protein but made by a different process. Another example is a reduced-calorie fat replacer derived from a corn protein	Some blends of protein and carbohydrate can be used in frozen desserts and baked goods
Carbohydrate-based fat replacers		
Cellulose (Avicel® cellulose gel, MethocelTM, Solka-Floc®)	Non-caloric purified form of cellulose ground to microparticles which, when dispersed, form a network of particles with mouthfeel and flow properties similar to fat	Dairy-type products, sauces, frozen desserts and salad dressings
Dextrins (Amylum, N-Oil®)	Four calorie/gram fat replacers replace all or some of the fat in a variety of products.	Salad dressings, puddings, spreads, dairy-type products and frozen desserts
Fiber (Opta™, Oat Fiber, Snowite, Ultracel™, Z-Trim)	Fiber provides structural integrity, volume, moisture holding capacity, adhesiveness and shelf stability in reduced-fat foods	Baked goods, meats, spreads and extruded products
Gums (KELCOGEL®, KELTROL®, SlendidTM)	Hydrophilic colloids: guar gum, gum arabic, locust bean gum, xanthan gum, carrageenan and pectin. Non-caloric; provide thickening, sometimes gelling effect; promote creamy texture	Reduced-calorie, fat-free salad dressings, formulated foods, including desserts and processed meats
Inulin (Raftiline®, Fruitafit®, Fibruline	Reduced-calorie (1 to 1.2 calorie/gram) fat and sugar replacer, fiber and bulking agent extracted from chicory root	Yogurt, cheese, frozen desserts, baked goods, icings, fillings, whipped cream, dairy products, fiber supplements and processed meats
Maltodextrins (CrystaLean®, Lorelite, Lycadex®, MALTRIN®, Paselli®D-LITE, Paselli®EXCEL, Paselli®SA2, STAR-DRI®)	Four calorie/gram gel or powder from corn, potato, wheat and tapioca. Fat replacer, texture modifier or bulking agent	Baked goods, dairy products, salad dressings, spreads, sauces, frostings, fillings, processed meat, frozen desserts, extruded products and beverages
Nu-Trim Beta-glucan from oat or barley	Extraction process removes coarse fiber components from oat or barley	Baked goods, dairy products, salad dressings, spreads, sauces, frostings, fillings, processed meat, frozen desserts, extruded products and beverages

(continued)

Table 2.47 (continued)

Product	Technology	Application
Oatrim [Hydrolyzed oat flour] (Beta-TrimTM, TrimChoice	A water-soluble from enzyme treated oat flour containing beta-glucan soluble fiber, used as a fat replacer, bodying and texturizing ingredient. 1–4 calories/gram)	Baked goods, fillings and frostings, frozen desserts, dairy beverages, cheese, salad dressings, processed meats and confections
Polydextrose (Litesse®, Sta-LiteTM	One calorie/gram) fat replacer and bulking agent. Water-soluble polymer of dextrose containing minor amounts of sorbitol and citric acid.	Baked goods, chewing gums, confections, salad dressings, frozen dairy desserts, gelatins and puddings
Starch and Modified Food Starch (Amalean®I & II, FairnexTMVA15, & VA20, Instant StellarTM, N-Lite, OptaGrade®#, PerfectamylTMAC, AX-1, & AX-2, PURE-GEL®, STA-SLIMTM)	1–4 calories/gram as used, fat replacers, bodying agents, texture modifiers. Can be derived from potato, corn, oat, rice, wheat or tapioca starches. Used with emulsifiers, proteins, gums and other modified food starches	Processed meats, salad dressings, baked goods, fillings and frostings, sauces, condiments, frozen desserts and dairy products
Z-Trim	Zero calorie fat replacer from insoluble fiber from oat, soybean, pea and rice hulls or corn or wheat bran. It is heat stable	Baked goods, burgers, hot dogs, cheese, ice cream and yogurt
Fat-based fat replacers		
Emulsifiers (Dur-Lo®, ECT-25)	Vegetable oil mono- and diglyceride emulsifiers used with water Same caloric value as fat (9 calories/gram) but less is required. Sucrose fatty acid esters also can be used for emulsification. Emulsion systems using soybean oil or milk fat can replace fat on a one-to-one basis	Cake mixes, cookies, icings, and numerous vegetable dairy products
Salatrim	Short and long-chain acid triglyceride molecules. A five calorie-per-gram family of fats	Baked goods, confections, dairy and other applications
Benefat^{T}		
Lipid (Fat/Oil) Analogs	Reduced-calorie fat replacer. Can partially or fully replace fats and oils in all typical consumer and commercial applications	Formulated products, baking and frying
– Esterified Propoxylated Glycerol		
– Olestra (Olean®)	Calorie-free ingredient made from sucrose and edible fats and oils. Not metabolized and unabsorbed by the body. Heat stable	Fried products, Chips, dairy, confections and other food products
Sorbestrin	Low-calorie, heat stable, liquid fat substitute composed of fatty acid esters of sorbitol and sorbitol anhydrides. Approximately 1.5 calories per gram and is suitable for use in all vegetable oil applications	Fried foods, salad dressing, mayonnaise and baked goods

Based on: http://www.caloriecontrol.org/articles-and-video/feature-articles/glossary-of-fat-replacers

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Amino Acids and Proteins

3

Michael Appell, W. Jeffrey Hurst, John W. Finley, and John M. deMan

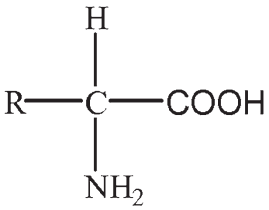
Introduction

Proteins are polymers of some 21 different amino acids joined together by peptide bonds. Because of the variety of side chains that occur when these amino acids are linked together, the different proteins may have different chemical properties and widely different secondary and tertiary structures. In peptides and proteins, the amino acids joined in a peptide chain can be grouped on the basis of the chemical nature of the side chains (Krull and Wall 1969). The side chains may be polar or nonpolar. High levels of polar amino acid residues in a protein increase water solubility. The most polar side chains are those of the basic and acidic amino acids. These amino acids are present at high levels in the soluble albumins and globulins. In contrast, the wheat proteins, gliadin and glutenin, have low levels of polar side chains and are quite insoluble in water. The acidic amino acids may also be present in proteins in the form of their amides, glutamine and asparagine. Hydroxyl groups in the side chains may become involved in ester linkages with phosphoric acid and phosphates. Sulfur amino acids may form disulfide cross-links between neighboring peptide chains or between different parts of the same chain. Proline and hydroxyproline impose significant structural limitations on the geometry of the peptide chain.

Proteins occur in animal as well as vegetable products in important quantities. In the developed countries, people obtain much of their protein from animal products. In other parts of the world, the major portion of dietary protein is derived from plant products. Many plant proteins are often deficient in one or more of the essential amino acids. Essential amino acids are defined as those that cannot be synthesized by an organism and are only obtained from the diet. The protein content of some selected foods is listed in Table 3.1.

Amino Acids

Amino acids have a range of side chains but all have an available carboxylic acid and an ammonia group in the α-position.



The structures of the amino acids can be broken into categories based on the structure and chemical characteristics of the side chains. In Fig. 3.1 the structures and groupings of the amino acids are illustrated. For convenience, amino acids are abbreviated with either a three letter

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_3

Table 3.1 Protein content of some selected foods

Product	Protein (g/100 g)
Meat	
Beef	16.5
Pork	10.2
Chicken (light meat)	23.4
Fish	
Haddock	18.3
Cod	17.6
Milk	3.6
Egg	12.9
Wheat	13.3
Bread	8.7
Soybeans	
Dry	34.1
Raw cooked	11.0
Peas	6.3
Beans	
Dry	22.3
Raw cooked	7.8
Rice	
White	6.7
Raw cooked	2.0
Cassava	1.6
Potato	2.0

abbreviation or a single letter designation which is frequently used when describing longer chains and sequences of amino acids.

There are 21 amino acids found in most proteins. An essential amino acid is an amino acid that cannot be synthesized *de novo* (*from scratch*) by the organism, and thus must be supplied in the diet. The nine amino acids humans cannot synthesize are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine (Table 3.2). Six other amino acids are considered conditionally essential in the human diet, meaning their synthesis can be limited under certain conditions, such as premature birth in the infants or individuals in severe catabolic distress. The six conditionally essential amino acids are arginine, cysteine, glycine, glutamine, proline and tyrosine. (Dietary Reference Intakes: The Essential Guide to Nutrient Requirements, published by the Institute of Medicine's Food and Nutrition Board, currently available online at http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-reports.)

The amounts of these essential amino acids present in a protein and their availability determine the nutritional quality of the protein. In general, animal proteins are of higher quality than plant proteins. Plant proteins can be upgraded nutritionally by judicious blending or by genetic modification through plant breeding. The amino acid composition of some selected animal and vegetable proteins is given in Table 3.3.

Protein digestibility-corrected amino acid score (PDCAAS) is a method of evaluating the protein quality based on both the amino acid requirements of humans and their ability to digest it. The PDCAAS rating was adopted by the US Food and Drug Administration (FDA) and the Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) in 1993 as "the preferred 'best'" method to determine protein quality.

Egg protein is one of the best quality proteins and is considered to have a biological value of 100. It is widely used as a standard, and protein efficiency ratio (PER) values sometimes use egg white as a standard. Cereal proteins are generally deficient in lysine and threonine, as indicated in Table 3.4. Soybean is a good source of lysine but is deficient in methionine. Cottonseed protein is deficient in lysine and peanut protein in methionine and lysine. The protein of potato although present in small quantity (Table 3.1) is of excellent quality and is equivalent to that of whole egg.

Peptides and Proteins

Proteins and peptides are formed from amino acids by peptide bonds. The reaction of peptide bond formation produces one peptide bond and one molecule of water. Peptides are oligomers of amino acids joined by peptide bonds and proteins can range from a few amino acids joined together to many thousands of amino acid residues in large proteins.

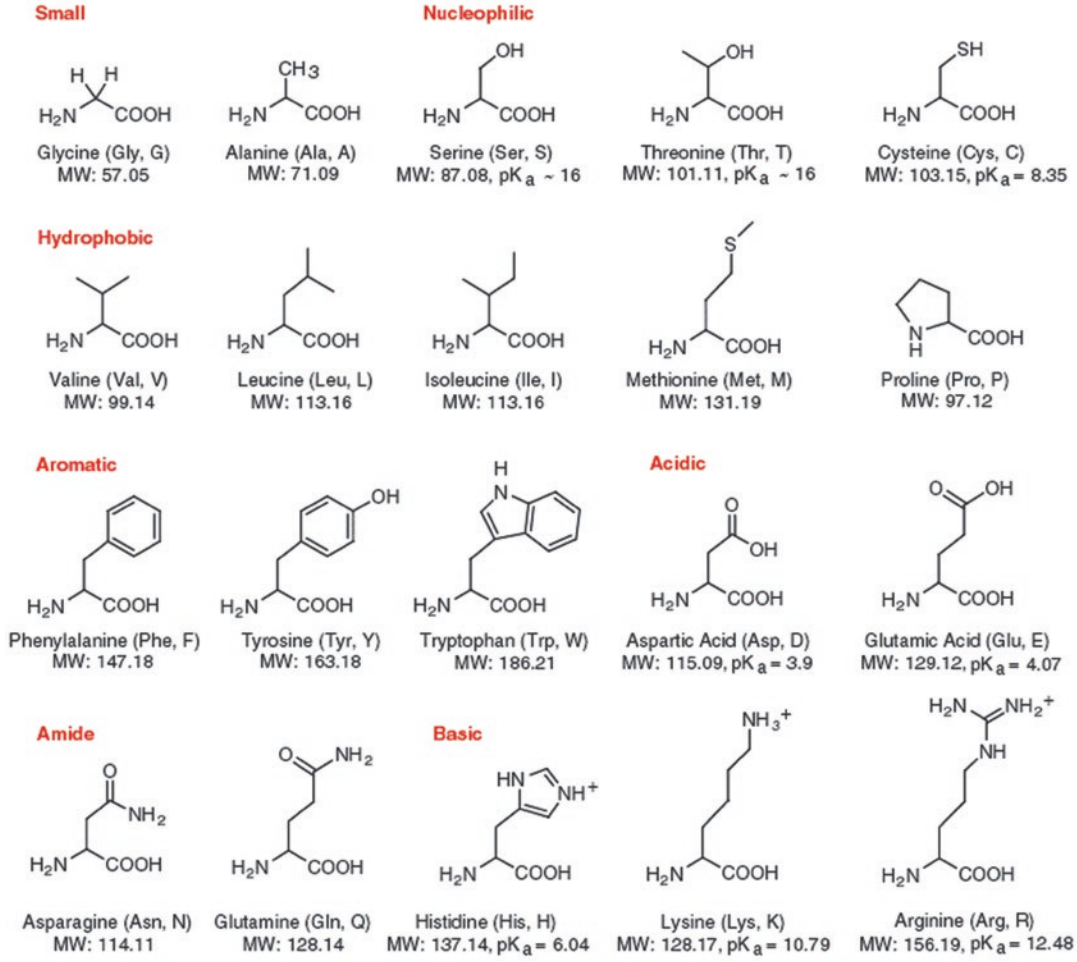
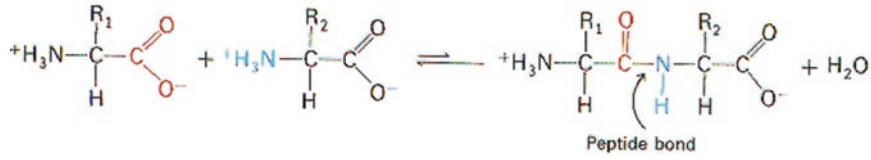


Fig. 3.1 Common amino acids found in proteins with short hand abbreviations, molecular weight and pK_a values of ionizable side chains



Proteins which make up more than half the dry weight of most cells are the most important macromolecules in all living organisms. More than half of the dry weight of a cell is made up of proteins of various shapes and sizes (Voet and Voet 2004). Proteins are based on sequences of amino acids connected by peptide bonds. The protein chains form specific folded three-dimensional shapes which enable the proteins to perform specific tasks such as structure or enzyme activity. These tasks include transporting small molecules (e.g., the hemoglobin transports oxygen in the bloodstream), catalyzing biological functions, providing structure to collagen and skin, controlling

Table 3.2 Essential and nonessential amino acids

Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Aspartic acid
Lysine	Cysteine
Methionine	Glutamic acid
Phenylalanine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
	Tyrosine
	Asparagine

sense, and regulating hormones (Pietzsch 2003). In peptides and proteins, the carboxyl terminus (known as C-terminus) of one amino acid with a free carboxyl group, the amino terminus (known as N-terminus) of another amino acid with a free amino group. The size of the protein is determined by the number of amino acids in the protein sequence. A protein sequence having n amino acids has n − 1 peptide bonds and n − 1 amide planes which are fairly rigid because of the structure of the peptide bond. The bond angle between N of amine group and alpha carbon is denoted by φ (phi) and the bond angle between C α and C of carboxyl group is denoted by ψ (psi).

There are four different levels of protein structure. These are: Primary Structure, Secondary Structure, Tertiary Structure, and Quaternary Structure as depicted in Fig. 3.2. Primary structure: The Primary structure is defined by the amino acid sequence of the polypeptide chain. The primary structure is held together by peptide bonds, which are formed during the process of protein synthesis. Secondary structure: The Secondary Structure as shown in Fig. 3.2 refers to highly regular local sub-structures such as the alpha helix in Fig. 3.2. In the secondary structure includes the alpha helix, the beta sheet, and the random coils. These secondary structures are controlled by hydrogen bonds between the main-chain peptide groups. The Tertiary structure as shown in Fig. 3.2 represents the three-dimensional structure of a single protein molecule where the alpha-helices and beta-sheets are folded into a compact globule structure.

The structure is stabilized when the parts of a protein domain are locked into place by tertiary interactions, such as salt bridges, hydrogen bonds, the packing of side chains and disulfide bridges. The Quaternary structure is a large assembly of several protein molecules or polypeptide chains generally referred to as subunits. The quaternary structure is stabilized by the same non-covalent interactions and disulfide bonds as the tertiary structure.

The native structure of a protein is its natural state in the cell, unaltered by heat, chemicals, enzyme action. In their native state proteins are folded into their 3D structures which provide maximum stability and are reached at lowest energy (Pietzsch 2003). When proteins structures are forced to be deformed they are considered to be denatured (Anfinsen 1973; Tanford 1968). The forces that cause denaturation can be chemicals or the application of heat. An example of heat denaturation is the heating of egg white where the protein is denatured resulting in a conversion form liquid to solid form.

Protein Classification

Proteins are complex molecules, and classification has been based mostly on solubility in different solvents. Increasingly, however, as more knowledge about molecular composition and structure is obtained, other criteria are being used for classification. These include behavior in the ultracentrifuge and electrophoretic properties. Proteins are divided into the following main groups: simple, conjugated, and derived proteins.

Simple Proteins

Simple proteins yield only amino acids on hydrolysis and include the following classes:

- *Albumins.* Soluble in neutral, salt-free water. Usually these are proteins of relatively low molecular weight. Examples are egg albumin, lactalbumin, and serum albumin in the whey

Table 3.3 Composition and amino acid content of some selected foods[a]

	Whole egg		Cows milk		Ground beef 15% fat		Hard red winter wheat		Soy flour (defatted)	
Energy (kcal/100 g)	143		61		215		327		327	
Composition in g/100 g										
Water	76.15		88.13		55.81		13.1		7.25	
Carbohydrates	0.72		5.26		0		71.18		33.97	
Fat	9.51		3.25		15		1.54		1.22	
Protein	12.56		3.22		18.59		12.61		51.46	
Amino acid content foods and protein g/100 g										
	AA/100 g food	**AA/100 g protein**	**AA/100 g food**	**AA/100 g protein**	**AA/100 g food**	**AA/100 g protein**	**AA/100 g food**	**AA/100 g protein**	**AA/100 g food**	**AA/100 g protein**
Tryptophan	0.17	1.33	0.04	1.24	0.09	0.51	0.16	1.27	0.68	1.33
Threonine	0.56	4.43	0.13	4.16	0.72	3.87	0.37	2.89	2.04	3.97
Isoleucine	0.67	5.34	0.16	5.06	0.82	4.42	0.46	3.63	2.28	4.43
Leucine	1.09	8.65	0.30	9.29	1.45	7.80	0.85	6.77	3.83	7.44
Lysine	0.91	7.26	0.26	8.20	1.54	8.28	0.34	2.66	3.13	6.08
Methionine	0.38	3.03	0.08	2.58	0.48	2.57	0.20	1.59	0.63	1.23
Cystine	0.27	2.17	0.02	0.59	0.19	1.03	0.32	2.55	0.76	1.47
Phenylalanine	0.68	5.41	0.16	5.06	0.75	4.05	0.59	4.69	2.45	4.77
Tyrosine	0.50	3.97	0.16	4.94	0.57	3.08	0.39	3.07	1.78	3.46
Valine	0.86	6.83	0.21	6.40	0.91	4.92	0.56	4.41	2.35	4.56
Arginine	0.74	5.85	0.09	2.80	1.21	6.51	0.60	4.72	3.65	7.09
Histidine	0.31	2.46	0.10	2.95	0.60	3.25	0.29	2.26	1.27	2.46
Alanine	0.74	5.85	0.11	3.32	1.07	5.73	0.45	3.57	2.22	4.30
Aspartic acid	1.33	10.56	0.27	8.39	1.67	9.00	0.64	5.08	5.91	11.49
Glutamic acid	1.67	13.32	0.71	21.99	2.79	15.00	4.00	31.70	9.11	17.70
Glycine	0.43	3.44	0.06	1.93	1.26	6.79	0.53	4.19	2.17	4.22
Proline	0.51	4.08	0.31	9.66	0.95	5.10	1.29	10.22	2.75	5.34
Serine	0.97	7.73	0.19	5.90	0.74	4.00	0.59	4.65	2.73	5.30
Hyroxy-proline					0.27	1.46				
PDCAAS[b]	1		1		0.92		0.42		0.91	

[a]From USDA Nutrient data base
[b]Schaafsma (2000)

proteins of milk, leucosin of cereals, and legumelin in legume seeds.

- *Globulins.* Soluble in neutral salt solutions and almost insoluble in water. Examples are serum globulins and β-lactoglobulin in milk, myosin and actin in meat, and glycinin in soybeans.
- *Glutelins.* Soluble in very dilute acid or base and insoluble in neutral solvents. These proteins occur in cereals, such as glutenin in wheat and oryzenin in rice.

Table 3.4 Limiting essential amino acids of some grain proteins

Grain	First limiting amino acid	Second limiting amino acid
Wheat	Lysine	Threonine
Corn	*Lysine*	Tryptophan
Rice	Lysine	Threonine
Sorghum	Lysine	Threonine
Millet	Lysine	Threonine

- *Prolamins.* Soluble in 50–90% ethanol and insoluble in water. These proteins have large amounts of proline and glutamic acid and occur in cereals. Examples are zein in corn, gliadin in wheat, and hordein in barley.
- *Scleroproteins.* Insoluble in water and neutral solvents and resistant to enzymic hydrolysis. These are fibrous proteins serving structural and binding purposes. Collagen of muscle tissue is included in this group, as is gelatin, which is derived from it. Other examples include elastin, a component of tendons, and keratin, a component of hair and hoofs.
- *Histones.* Basic proteins, as defined by their high content of lysine and arginine. Soluble in water and precipitated by ammonia.
- *Protamines.* Strongly basic proteins of low molecular weight (4000–8000). They are rich in arginine. Examples are clupein from herring and scombrin from mackerel.

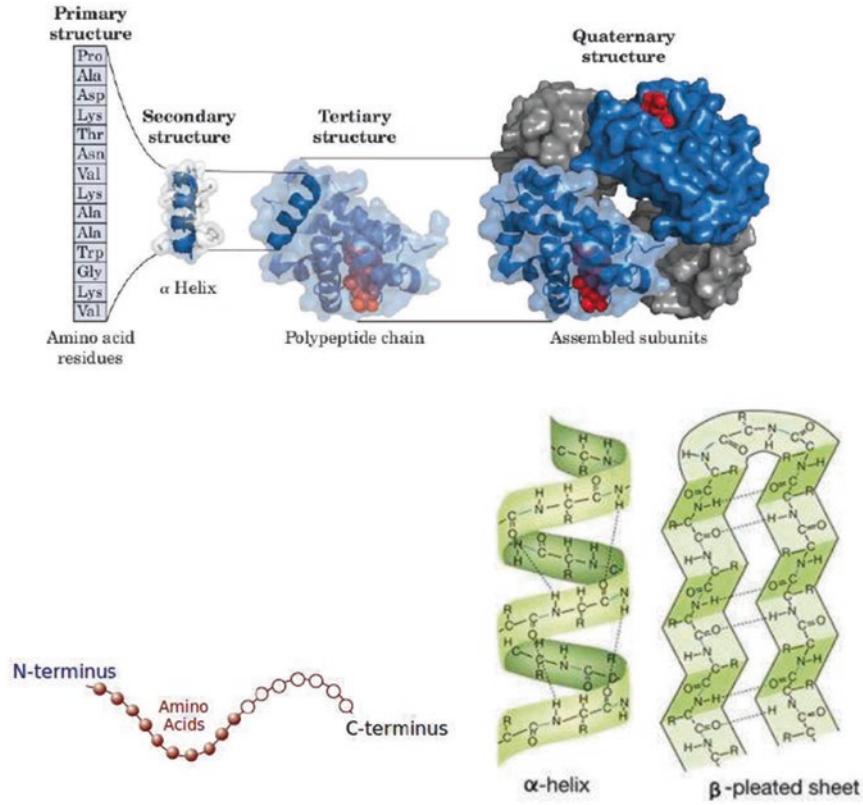


Fig. 3.2 The four levels of protein structure (Rashid et al. 2015)

Conjugated Proteins

Conjugated proteins contain an amino acid part combined with a nonprotein material such as a lipid, nucleic acid, or carbohydrate. Some of the major conjugated proteins are as follows:

- *Phosphoproteins.* An important group that includes many major food proteins. Phosphate groups are linked to the hydroxyl groups of serine and threonine. This group includes casein of milk and the phosphoproteins of egg yolk.
- *Lipoproteins.* These are combinations of lipids with protein and have excellent emulsifying capacity. Lipoproteins occur in milk and egg yolk.
- *Nucleoproteins.* These are combinations of nucleic acids with protein. These compounds are found in cell nuclei.
- *Glycoproteins.* These are combinations of carbohydrates with protein. Usually the amount of carbohydrate is small, but some glycoproteins have carbohydrate contents of 8–20%. An example of such a mucoprotein is ovomucin of egg white.
- *Chromoproteins.* These are proteins with a colored prosthetic group. There are many compounds of this type, including hemoglobin and myoglobin, chlorophyll, and flavoproteins.

Derived Proteins

These are compounds obtained by chemical or enzymatic methods and are divided into primary and secondary derivatives, depending on the extent of change that has taken place. Primary derivatives are slightly modified and are insoluble in water; rennet-coagulated casein is an example of a primary derivative. Secondary derivatives are more extensively changed and include proteoses, peptones, and peptides. The difference between these breakdown products is in size and solubility. All are soluble in water and not coagulated by heat, but proteoses can be precipitated with saturated ammonium sulfate solution. Peptides contain two or more amino acid residues. These breakdown products are formed during the processing of many foods, for example, during ripening of cheese.

Protein Structure

Proteins are macromolecules with different levels of structural organization. The primary structure of proteins relates to the peptide bonds between component amino acids and also to the amino acid sequence in the molecule. Researchers have elucidated the amino acid sequence in many proteins. For example, the amino acid composition and sequence for several milk proteins is now well established (Swaisgood 1982).

Some proteolytic enzymes have quite specific actions; they attack only a limited number of bonds, involving only particular amino acid residues in a particular sequence. This may lead to the accumulation of well-defined peptides during some enzymic proteolytic reactions in foods.

The secondary structure of proteins involves folding the primary structure. Hydrogen bonds between amide nitrogen and carbonyl oxygen are the major stabilizing force. These bonds may be formed between different areas of the same polypeptide chain or between adjacent chains. In aqueous media, the hydrogen bonds may be less significant, and van der Waals forces and hydrophobic interaction between apolar side chains may contribute to the stability of the secondary structure. The secondary structure may be either the α-helix or the sheet structure, as shown in Fig. 3.2. The helical structures are stabilized by intramolecular hydrogen bonds, the sheet structures by intermolecular hydrogen bonds. The requirements for maximum stability of the helix structure were established by Pauling et al. (1951). The helix model involves a translation of 0.54 nm per turn along the central axis. A complete turn is made for every 3.6 amino acid residues. Proteins do not necessarily have to occur in a complete α-helix configuration; rather, only parts of the peptide chains may be helical, with other areas of the chain in a more or less unordered configuration. Proteins with α-helix structure

Table 3.5 Bond and interaction energies of the interactions involved in protein structure

Bond	Bond energy[a] (kcal/mol)
Covalent C–C	83
Covalent S–S	50
Hydrogen bond	3–7
Electrostatic interactions	3–7
Hydrophobic interactions	3–5
Van der Waals forces	1–2

[a]These refer to free energy required to break the bonds: in the case of a hydrophobic bond, the free energy required to unfold a nonpolar side chain from the interior of the molecule into the aqueous medium

Table 3.6 Oligomeric food proteins

Protein	Molecular weight (Daltons)	Subunits
Lactoglobulin	35,000	2
Hemoglobin	64,500	4
Avidin	68,300	4
Lipoxygenase	108,000	2
Tyrosinase	128,000	4
Lactate dehydrogenase	140,000	4
7S soy protein	200,000	9
Invertase	210,000	4
Catalase	232,000	4
Collagen	300,000	3
11S soy protein	350,000	12
Legumin	360,000	6
Myosin	475,000	6

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may be either globular or fibrous. In the parallel sheet structure, the polypeptide chains are almost fully extended and can form hydrogen bonds between adjacent chains. Such structures are generally insoluble in aqueous solvents and are fibrous in nature.

The tertiary structure of proteins involves a pattern of folding of the chains into a compact unit that is stabilized by hydrogen bonds, van der Waals forces, disulfide bridges, and hydrophobic interactions. The tertiary structure results in the formation of a tightly packed unit with most of the polar amino acid residues located on the outside and hydrated. This leaves the internal part with most of the apolar side chains and virtually no hydration. Certain amino acids, such as proline, disrupt the α-helix, and this causes fold regions with random structure (Kinsella 1982). The nature of the tertiary structure varies among proteins as does the ratio of α-helix and random coil. Insulin is loosely folded, and its tertiary structure is stabilized by disulfide bridges. Lysozyme and glycinin have disulfide bridges but are compactly folded.

Large molecules of molecular weights above about 50,000 may form quaternary structures by association of subunits. These structures may be stabilized by hydrogen bonds, disulfide bridges, and hydrophobic interactions. The bond energies involved in forming these structures are listed in Table 3.5.

The term *subunit* denotes a protein chain possessing an internal covalent and noncovalent structure that is capable of joining with other similar subunits through noncovalent forces or disulfide bonds to form an oligomeric macromolecule (Stanley and Yada 1992). Many food proteins are oligomeric and consist of a number of subunits, usually 2 or 4, but occasionally as many as 24. A listing of some oligomeric food proteins is given in Table 3.6. The subunits of proteins are held together by various types of bonds: electrostatic bonds involving carboxyl, amino, imidazole, and guanido groups; hydrogen bonds involving hydroxyl, amide, and phenol groups; hydrophobic bonds involving long-chain aliphatic residues or aromatic groups; and covalent disulfide bonds involving cystine residues. Hydrophobic bonds are not true bonds but have been described as interactions of nonpolar groups. These nonpolar groups or areas have a tendency to orient themselves to the interior of the protein molecule. This tendency depends on the relative number of nonpolar amino acid residues and their location in the peptide chain. Many food proteins, especially plant storage proteins, are highly hydrophobic—so much so that not all of the hydrophobic areas can be oriented toward the inside and have to be located on the surface. This is a possible factor in subunits association and in some cases may result in aggregation.

The hydrophobicity values of some food proteins as reported by Stanley and Yada (1992) are listed in Table 3.7.

The well-defined secondary, tertiary, and quaternary structures are thought to arise directly from the primary structure. This means that a given combination of amino acids will automatically assume the type of structure that is most stable and possible given the considerations described by Pauling et al. (1951).

Denaturation

Denaturation is a process that changes the molecular structure without breaking any of the peptide bonds of a protein. The process is peculiar to proteins and affects different proteins to different degrees, depending on the structure of a protein. Denaturation can be brought about by a variety of agents, of which the most important are heat, pH, salts, and surface effects. Considering the complexity of many food systems, it is not surprising that denaturation is a complex process that cannot easily be described in simple terms. Denaturation usually involves loss of biological activity and significant changes in some physical or functional properties such as solubility. A simplified view of protein denaturation and the renaturation are illustrated in Fig. 3.3. Protein denaturation generally is not reversible. Thermal treatments of egg white protein are a good example of protein denaturation.

Table 3.7 Hydrophobicity values of some food proteins

Protein	Hydrophobicity (cal/residue)
Gliadin	1300
Bovine serum albumin	1120–1000
α-Lactalbumin	1050
β-Lactoglobulin	1050
Actin	1000
Ovalbumin	980
Collagen	880
Myosin	880
Casein	725
Whey protein	387
Gluten	349

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The three-dimensional structure of proteins is driven by a number of interactions in the side chain of the protein. When proteins are exposed to conditions such as extremes in pH or in the heat all these are disrupted leaving the protein structure with reduced stabilizing forces which results in the unraveling of the structure in rare instances an unraveled protein with its primary structure intact can when under carefully controlled conditions the protein will spontaneously fold and become renatured.

The destruction of enzyme activity by heat is an important operation in food processing. In most cases, denaturation is nonreversible; however, there are some exceptions, such as the recovery of some types of enzyme activity after heating. Heat denaturation is sometimes desirable—for example, the denaturation of whey proteins for the production of milk powder used in baking. The relationship among temperature,

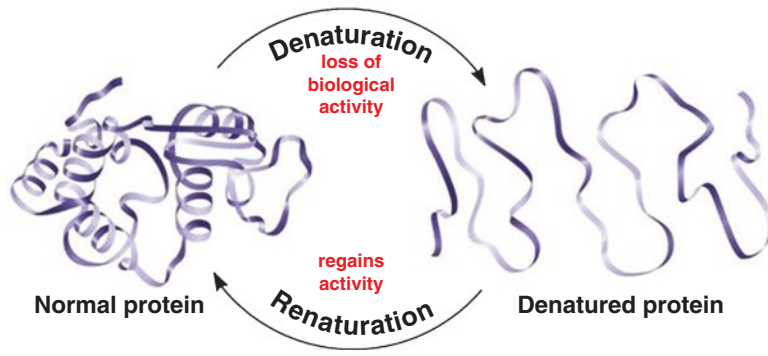


Fig. 3.3 Thermal denaturation of protein and the renaturation with loss and regain of biological activity

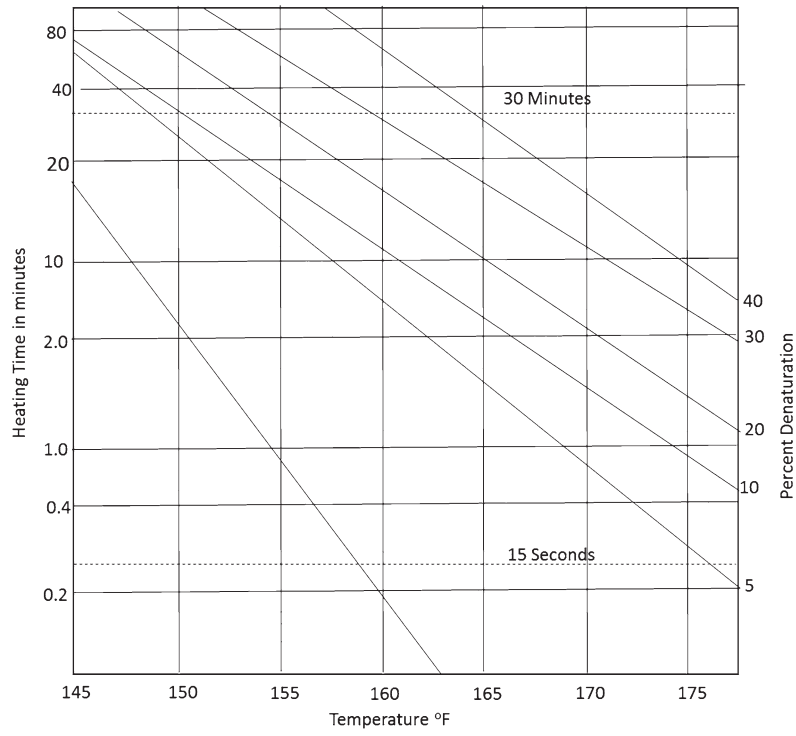


Fig. 3.4 Time-temperature relationships for the heat denaturation of whey proteins in skim milk. *Adapted from* H.A. Harland, S.T. Coulter, and R. Jenness, The Effects of Various Steps in the Manufacture on the Extent of Serum Protein Denaturation in Nonfat Dry Milk Solids. J. *Dairy Sci.* 35: 363–368, 1952

heating time, and the extent of whey protein denaturation in skim milk is demonstrated in Fig. 3.4 (Harland et al. 1952).

The proteins of egg white are readily denatured by heat and by surface forces when egg white is whipped to a foam. Meat proteins are denatured in the temperature range 57–75 °C, which has a profound effect on texture, water holding capacity, and shrinkage.

Denaturation may sometimes result in the flocculation of globular proteins but may also lead to the formation of gels. Foods may be denatured, and their proteins destabilized, during freezing and frozen storage. Fish proteins are particularly susceptible to destabilization. After freezing, fish may become tough and rubbery and lose moisture. The caseinate micelles of milk, which are quite stable to heat, may be destabilized by freezing. On frozen storage of milk, the stability of the caseinate progressively decreases, and this may lead to complete coagulation.

Protein denaturation and coagulation are aspects of heat stability that can be related to the amino acid composition and sequence of the protein. Denaturation can be defined as a major change in the native structure that does not involve alteration of the amino acid sequence. The effect of heat usually involves a change in the tertiary structure, leading to a less ordered arrangement of the polypeptide chains. The temperature range in which denaturation and coagulation of most proteins take place is about 55–75 °C, as indicated in Table 3.8. There are some notable exceptions to this general pattern.

Table 3.8 Heat coagulation temperatures of some albumins and globulins and casein

Protein	Coagulation temperature (°C)
Egg albumin	56
Serum albumin (bovine)	67
Milk albumin (bovine)	72
Legumelin (pea)	60
Serum globulin (human)	75
β-Lactoglobulin (bovine)	70–75
Fibrinogen (human)	56–64
Myosin (rabbit)	47–56
Casein (bovine)	160–200

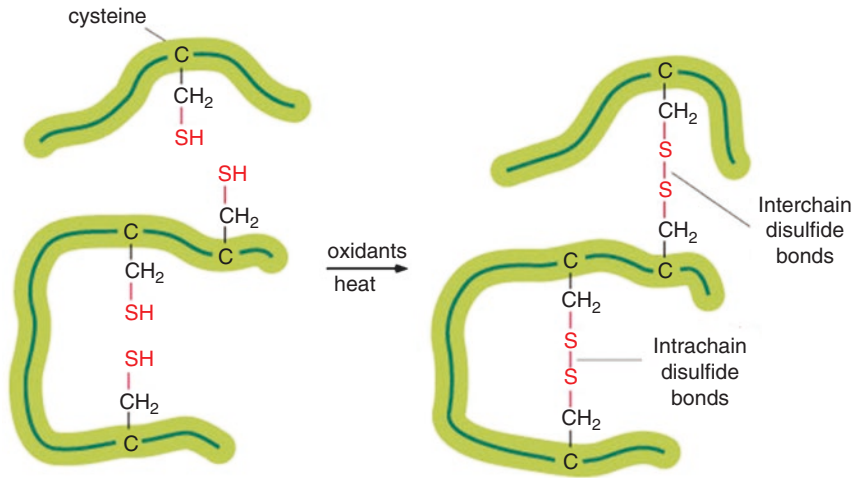


Fig. 3.5 Heat or oxidation of protein sulfhydryl groups from cysteine can form both intra- and intermolecular bonds

Table 3.9 Cysteine and cystine content of some proteins (g amino acid/100 g protein)

Protein	Cysteine (%)	Cystine (%)
Egg albumin	1.4	0.5
Serum albumin (bovine)	0.3	5.7
Milk albumin	6.4	–
β-Lactoglobulin	1.1	2.3
Fibrinogen	0.4	2.3
Casein	–	0.3

Cysteine in proteins can be involved in the denaturation process. As shown in Fig. 3.5 when cysteine is oxidized to form new disulfide bridges they can be formed within the protein or in intermolecular bridges causing aggregation of the protein crosslinked protein. Casein and gelatin are examples of proteins that can be boiled without apparent change in stability. The exceptional stability of casein makes it possible to boil, sterilize, and concentrate milk, without coagulation. The reasons for this exceptional stability have been discussed by Kirchmeier (1962). In the first place, restricted formation of disulfide bonds due to low content of cystine and cysteine results in increased stability. The relationship between coagulation temperature as a measure of stability and sulfur amino acid content is shown in Tables 3.8 and 3.9. Peptides, which are low in these particular amino acids, are less likely to become involved in the type of sulfhydryl agglomeration shown in Fig. 3.5. Casein, with its extremely low content of sulfur amino acids, exemplifies this behavior. The heat stability of casein is also explained by the restraints against forming a folded tertiary structure. These restraints are due to the relatively high content of proline and hydroxyproline in the heat stable proteins (Table 3.10). In a peptide chain free of proline, the possibility of forming inter- and intramolecular hydrogen bonds is better than in a chain containing many proline residues (Fig. 3.6). These considerations show how amino acid composition directly relates to secondary and tertiary structure of proteins; these structures are, in turn, responsible for the functionality of the protein.

Non-enzymic Browning

The non-oxyenzymic browning or *Maillard reaction* is of great importance in food manufacturing and its results can be either desirable or undesirable. For example, the brown crust formation on bread is desirable; the brown discoloration of evaporated and sterilized milk is undesirable. For products in which the browning reaction is favorable, the resulting color and flavor characteristics are generally experienced as pleasant. In other products, color and flavor may become quite unpleasant.

The browning reaction can be defined as the sequence of events that begins with the reaction of the amino group of amino acids, peptides, or proteins with a glycosidic hydroxyl group of sugars; the sequence terminates with the formation of brown nitrogenous polymers or melanoidins (Ellis 1959).

The reaction velocity and pattern are influenced by the nature of the reacting amino acid or protein and the carbohydrate. This means that each kind of food may show a different browning pattern. Generally, lysine is the most reactive amino acid because of the free ε-amino group. Since lysine is the limiting essential amino acid in many food proteins, its destruction can substantially reduce the nutritional value of the protein. Foods that are rich in reducing sugars are very reactive, and this explains why lysine in milk is destroyed more easily than in other foods (Fig. 3.7). Other factors that influence the browning reaction are temperature, pH, moisture level, oxygen, metals, phosphates, sulfur dioxide, and other inhibitors.

The browning reaction involves a number of steps. An outline of the total pathway of melanoidin formation has been given by Hodge (1953) and is shown in Fig. 3.8. According to Hurst and Ito (1972), the following five steps are involved in the process:

1. The production of an *N*-substituted glycosylamine from an aldose or ketose reacting with a primary amino group of an amino acid, peptide, or protein.

Table 3.10 Amino acid composition of serum albumin, casein, and gelatin (g amino acid/100 g protein)

Amino acid	Serum albumin	Casein	Gelatin
Glycine	1.8	1.9	27.5
Alanine	6.3	3.1	11.0
Valine	5.9	6.8	2.6
Leucine	12.3	9.2	3.3
Isoleucine	2.6	5.6	1.7
Serine	4.2	5.3	4.2
Threonine	5.8	4.4	2.2
Cystine ½	6.0	0.3	0.0
Methionine	0.8	1.8	0.9
Phenylalanine	6.6	5.3	2.2
Tyrosine	5.1	5.7	0.3
Proline	4.8	13.5	16.4
Hydroxyproline	–	–	14.1
Aspartic acid	10.9	7.6	6.7
Glutamic acid	16.5	24.5	11.4
Lysine	12.8	8.9	4.5
Arginine	5.9	3.3	8.8
Histidine	4.0	3.8	0.8

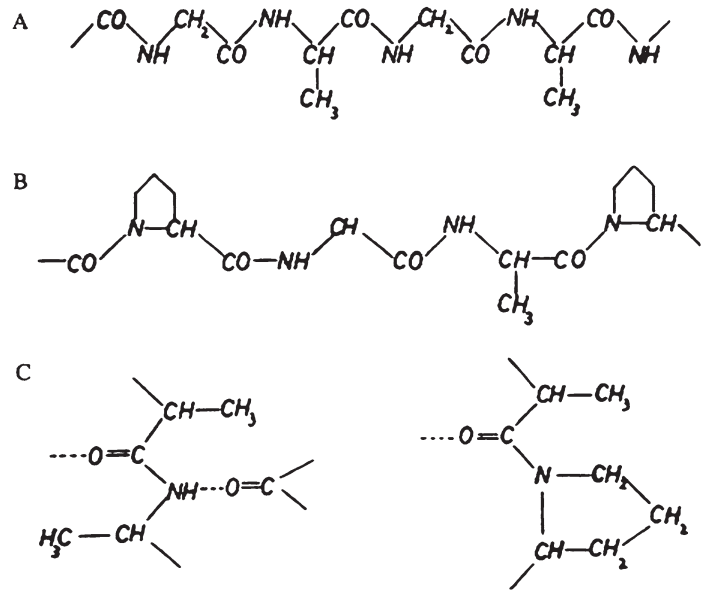


Fig. 3.6 Effect of proline residues on possible hydrogen bond formation in peptide chains. (**a**) Proline-free chain; (**b**) proline-containing chain; (**c**) hydrogen bond formation in proline-free and proline-containing chains. *Source:* From O. Kirchmeier, The Physical-Chemical Causes of the Heat Stability of Milk Proteins, *Milchwissenschaft* (German), Vol. 17, pp. 408–412, 1962

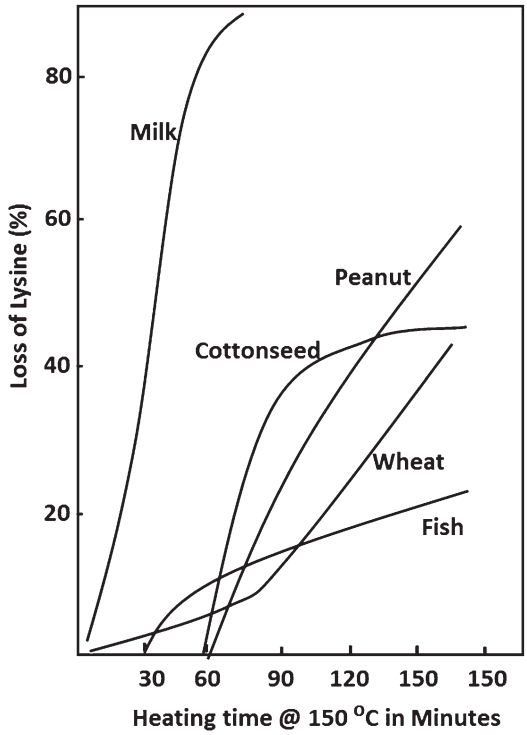


Fig. 3.7 Loss of lysine occurring as a result of heating of several foods. *Source*: Adapted from: J. Adrian, The Maillard Reaction. IV. Study on the Behavior of Some Amino Acids During Roasting of Proteinaceous Foods, *Ann. Nutr. Aliment.* (French), Vol. 21, pp. 129–147, 1967

2. Rearrangement of the glycosylamine by an Amadori rearrangement type of reaction to yield an aldoseamine or ketoseamine.
3. A second rearrangement of the ketoseamine with a second mole of aldose to form a diketoseamine, or the reaction of an aldoseamine with a second mole of amino acid to yield a diamino sugar.
4. Degradation of the amino sugars with loss of one or more molecules of water to give amino or nonamino compounds.
5. Condensation of the compounds formed in Step 4 with each other or with amino compounds to form brown pigments and polymers.

The formation of glycosylamines from the reaction of amino groups and sugars is reversible (Fig. 3.9) and the equilibrium is highly dependent on the moisture level. The mechanism as shown is thought to involve addition of the amine to the carbonyl group of the open-chain form of the sugar, elimination of a molecule of water, and closure of the ring. The rate is high at low water content; this explains the ease of browning in dried and concentrated foods.

The Amadori rearrangement of the glycosylamines involves the presence of an acid catalyst and leads to the formation of ketoseamine or 1-amino-1-deoxyketose according to the scheme shown in Fig. 3.10. In the reaction of D-glucose with glycine, the amino acid reacts as the catalyst and the compound produced is l-deoxy-1-glycino-β-D-fructose (Fig. 3.11). The ketoseamines are relatively stable compounds, which are formed in maximum yield in systems with 18% water content. A second type of rearrangement reaction is the Heyns rearrangement, which is an alternative to the Amadori rearrangement and leads to the same type of transformation. The mechanism of the Amadori rearrangement (Fig. 3.9) involves protonation of the nitrogen atom at carbon 1. The Heyns rearrangement (Fig. 3.12) involves protonation of the oxygen at carbon 6.

Secondary reactions lead to the formation of diketoseamines and diamino sugars. The formation of these compounds involves complex reactions and, in contrast to the formation of the primary products, does not occur on a mole-for-mole basis.

In Step 4, the ketoseamines are decomposed by 1,2-enolization or 2,3-enolization. The former pathway appears to be the more important one for the formation of brown color, whereas the latter results in the formation of flavor products. According to Hurst and Ito (1972), the 1,2-enolization pathway appears mainly to lead to browning but also contributes to formation of off-flavors through hydroxymethylfurfural, which may be a factor in causing off-flavors in stored, overheated, or dehydrated food products. The mechanism of this reaction is shown in Fig. 3.13 (Hurst and Ito 1972). The ketoseamine (1) is protonated in acid medium to yield (2). This is changed in a reversible reaction into the 1,2-enolamine (3) and this is assisted by the *N* substituent on carbon 1. The following steps involve the β-elimination of the hydroxyl group on carbon 3. In (4) the enolamine is in the free

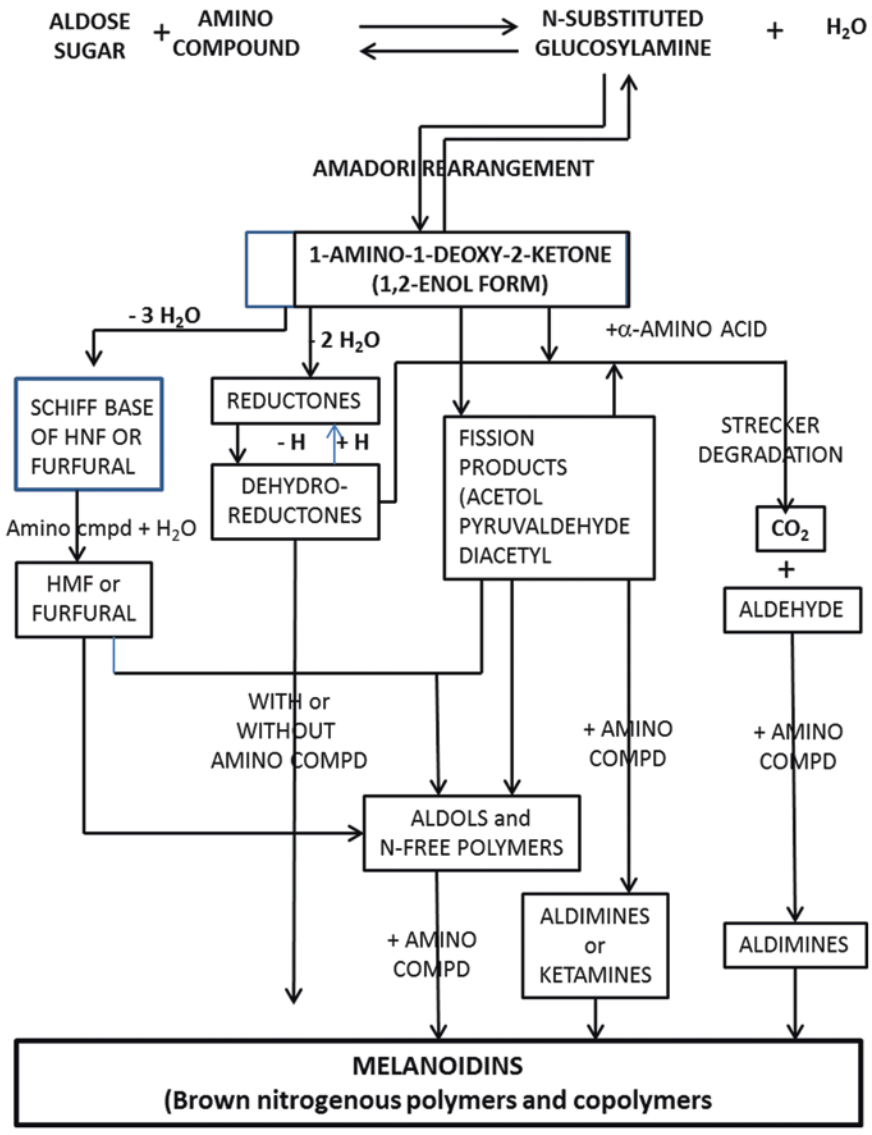


Fig. 3.8 Reaction pattern of the formation of melanoidins from aldose sugars and amino compounds. *Source:* From J.E. Hodge, Chemistry of Browning Reactions in Model Systems, *Agr. Food Chem*., VoL 1, pp. 928–943, 1953

base form and converts to the Schiff base (5). The Schiff base may undergo hydrolysis and form the enolform (7) of 3-deoxyosulose (8). In another step the Schiff base (5) may lose a proton and the hydroxyl from carbon 4 to yield a new Schiff base (6). Both this compound and the 3-deoxyosulose may be transformed into an unsaturated osulose (9), and by elimination of a proton and a hydroxyl group, hydroxymethylfurfural (10) is formed.

Following the production of 1,2-enol forms of aldose and ketose amines, a series of degradations and condensations results in the formation of melanoidins. The α-β-dicarbonyl compounds enter into aldol type condensations, which lead to the formation of polymers, initially of small size, highly hydrated, and in colloidal form. These initial products of condensation are fluorescent, and continuation of the reaction results in the formation of the brown melanoidins. These polymers

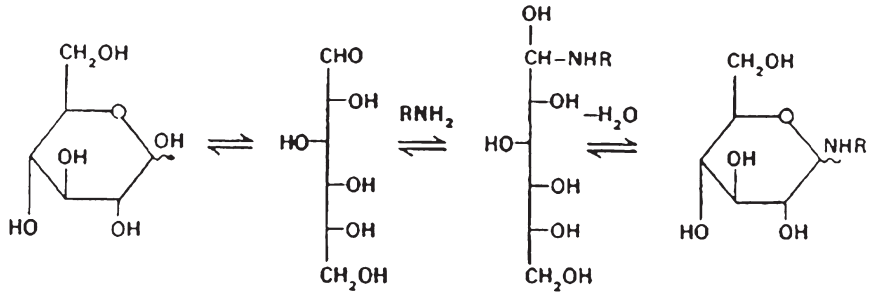


Fig. 3.9 Reversible formation of glycosylamines in the browning reaction. *Source*: From D.T. Hurst, *Recent Development in the Study of Nonenzymic Browning and Its Inhibition by Sulpher Dioxide*, BFMIRA Scientific and Technical Surveys, No. 75, Leatherhead, England, 1972

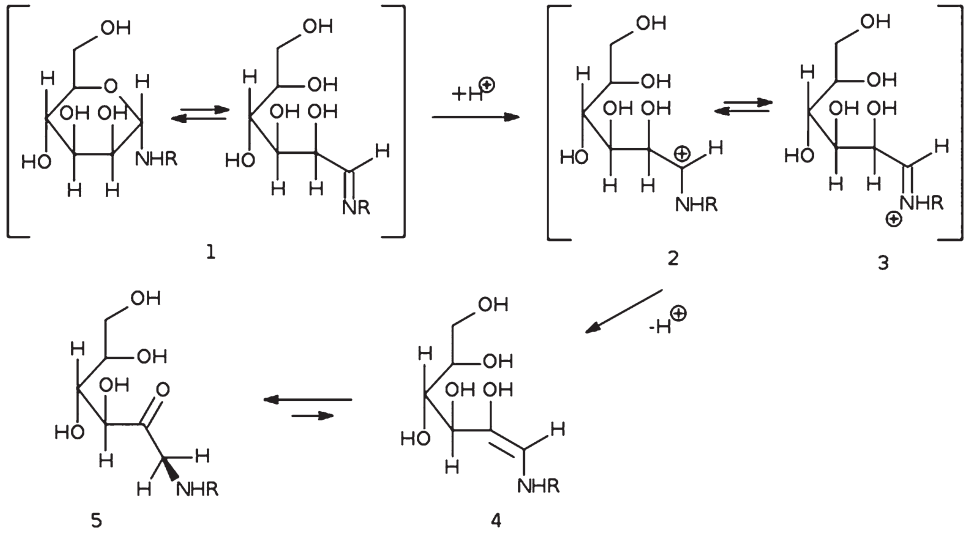
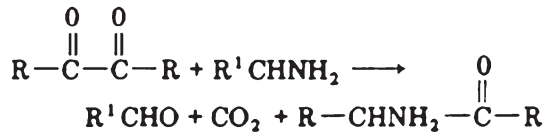


Fig. 3.10 Amadori rearrangement. *Source:* From M.J. Kort, Reactions of Free Sugars with Aqueous Ammonia, *Adv. Carbohydrate Chem. Biochem*., Vol. 25, pp. 311–349, 1970

are of nondistinct composition and contain varying levels of nitrogen. The composition varies with the nature of the reaction partners, pH, temperature, and other conditions.

The flavors produced by the Maillard reaction also vary widely. In some cases, the flavor is reminiscent of caramelization. The Strecker degradation of α-amino acids is a reaction that also significantly contributes to the formation of flavor compounds. The dicarbonyl compounds formed in the previously described schemes react in the following manner with α-amino acids:



The amino acid is converted into an aldehyde with one less carbon atom (Schönberg and Moubacher 1952). Some of the compounds of browning flavor have been described by Hodge et al. (1972). Corny, nutty, bready, and crackery aroma compounds consist of planar unsaturated heterocyclic compounds with one or two nitrogen atoms in the ring. Other important members of this group are partially saturated *N*-heterocyclics with alkyl or acetyl group substituents. Compounds that contribute to pungent, burnt aromas are listed in Table 3.11. These are mostly vicinal polycarbonyl compounds and α,β-unsaturated aldehydes. They condense rapidly to form melanoidins. The Strecker degradation aldehydes contribute to the aroma of bread, peanuts, cocoa, and other roasted foods. Although acetic, phenylacetic, isobutyric, and isovaleric aldehydes are prominent in the aromas of bread, malt, peanuts, and cocoa, they are not really characteristic of these foods (Hodge et al. 1972).

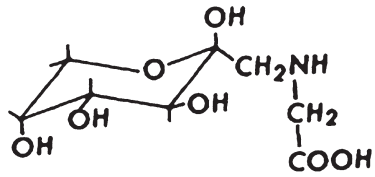


Fig. 3.11 Structure of 1-deoxy-l-glycino-β-D-fructose

A somewhat different mechanism for the browning reaction has been proposed by Burton and McWeeney (1964) and is shown in Fig. 3.14. After formation of the aldosylamine, dehydration reactions result in the production of 4- to 6-membered ring compounds. When the reaction proceeds under conditions of moderate heating, fluorescent nitrogenous compounds are formed. These react rapidly with glycine to yield melanoidins.

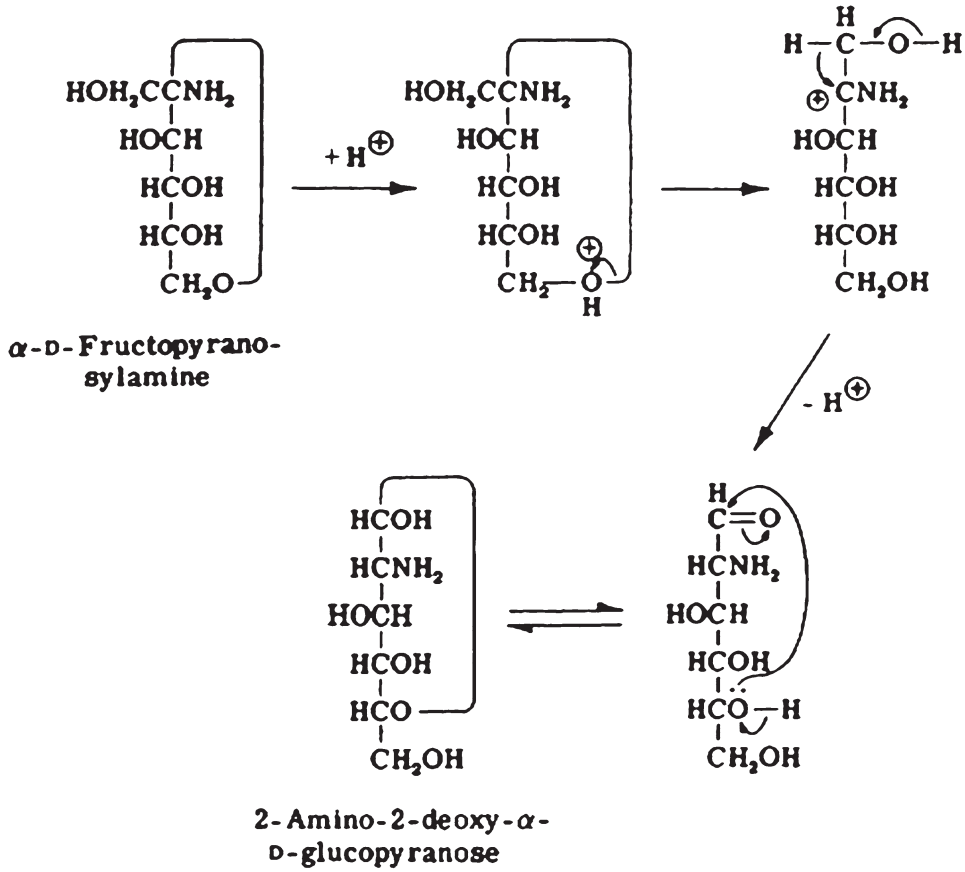


Fig. 3.12 Heyns rearrangement. *Source:* From M.J. Kort, Reactions of Free Sugars with Aqueous Ammonia, *Adv. Carbohydrate Chem. Biochem.*, Vol. 25, pp. 311–349, 1970

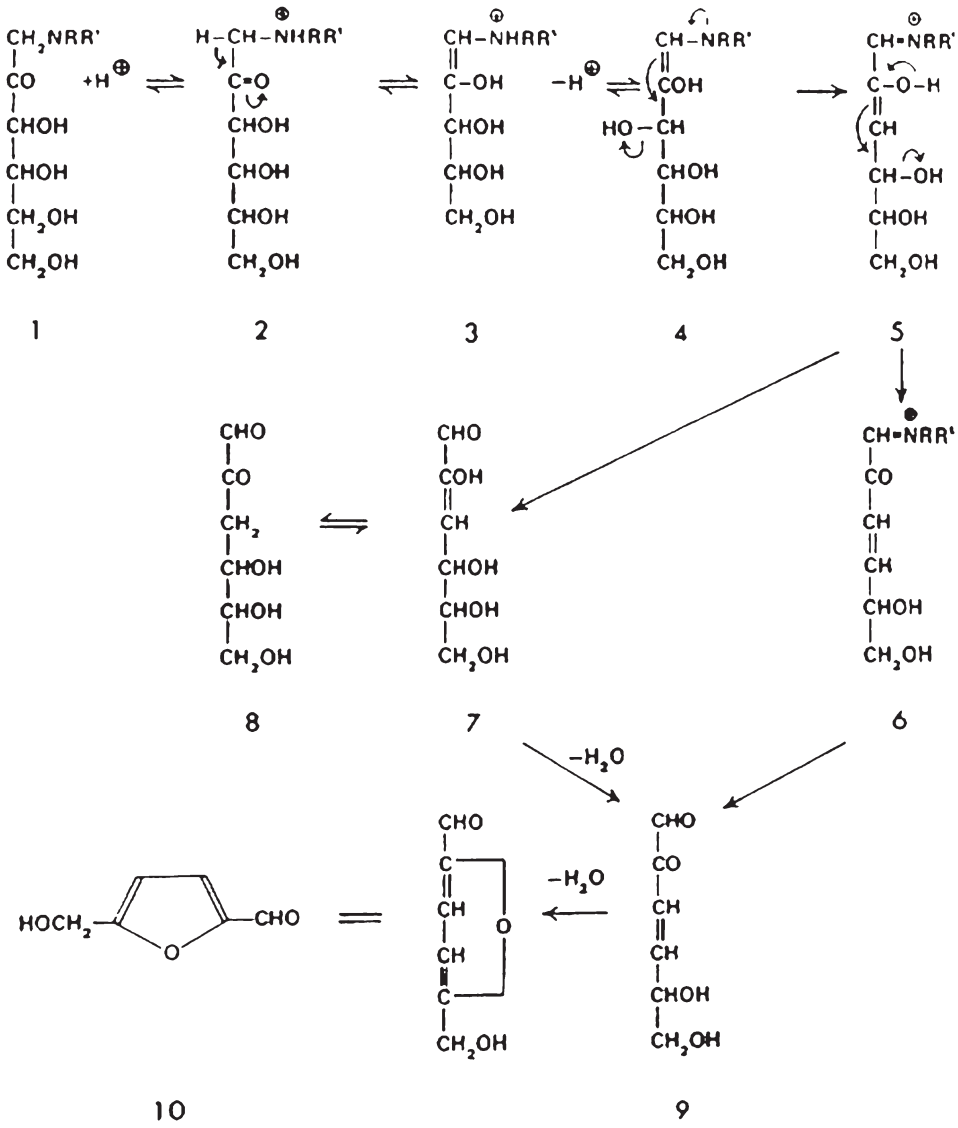


Fig. 3.13 1,2-Enolization mechanism of the browning reaction. *Source:* From D.T. Hurst, Recent Developments in the Study of Nonenzymic Browning and Its Inhibition by Sulphur Dioxide, BFMIRA Scientific and Technical Surveys, No. 75, Leatherhead, England, 1972

The influence of reaction components and reaction conditions results in a wide variety of reaction patterns. Many of these conditions are interdependent. Increasing temperature results in a rapidly increasing rate of browning; not only reaction rate, but also the pattern of the reaction may change with temperature. In model systems, the rate of browning increases 2–3 times for each 10° rise in temperature. In foods containing fructose, the increase may be 5–10 times for each 10° rise. At high sugar contents, the rate may be even more rapid. Temperature also affects the composition of the pigment formed. At higher temperatures, the carbon content of the pigment increases and more pigment is formed per mole of carbon dioxide released. Color intensity of the pigment increases with increasing temperature. The effect of temperature on the reaction rate of D-glucose with DL-leucine is illustrated in Fig. 3.15.

In the Maillard reaction, the basic amino group disappears; therefore, the initial pH or the presence of a buffer has an important effect on

Table 3.11 Aroma and structure classification of browned flavor compounds

Aromas:	Burnt (pungent, empyreumatic)	Variable (aldehydic, ketonic)
Structure:	Polycarbonyls(α,β-Unsat'd aldehydes –C:O–C:O–, =C–CHO	Monocarbonyls (R–CHO, R–C:O–CH_3)
Examples of compounds:	Glyoxal Pyruvaldehyde Diacetyl Mesoxalic dialdehyde	Strecker aldehydes Isobutyric Isovaleric Methional
	Acrolein Crotonaldehyde	2-Furaldehydes 2-Pyrrole aldehydes C_3–C_6 Methyl ketones

Source: From J.E. Hodge, F.D. Mills, and B.E. Fisher, Compounds of Browned Flavor from Sugar-Amine Reactions, *Cereal Sci. Today,* Vol. 17, pp. 34–40, 1972

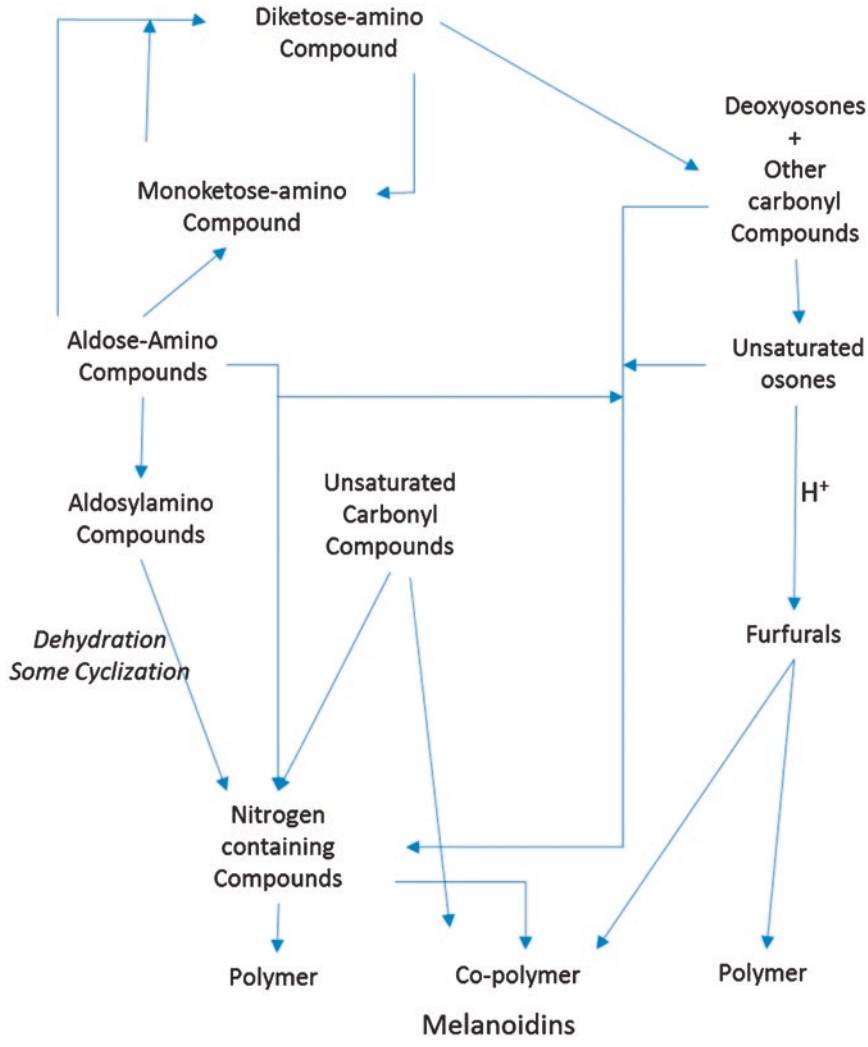


Fig. 3.14 Browning pathway leading to melanoidin formation

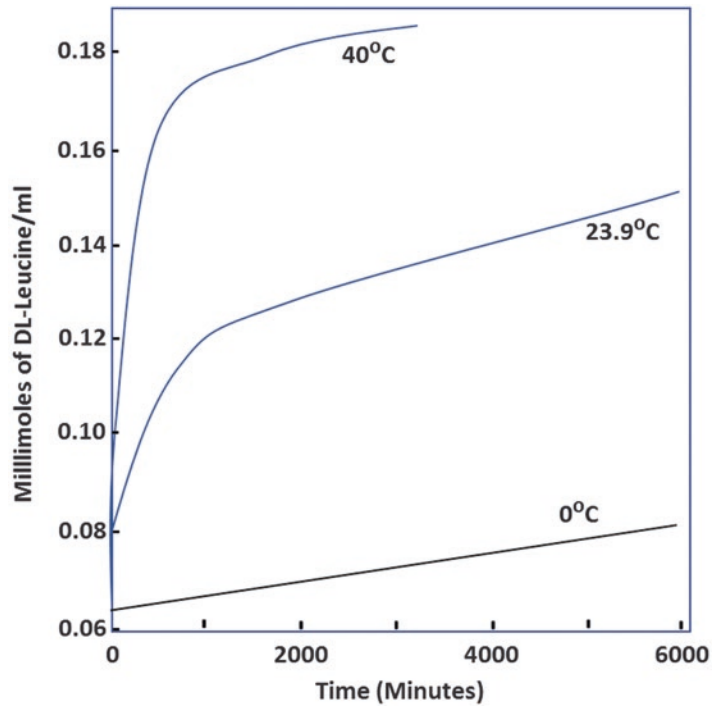


Fig. 3.15 Effect of temperature on the reaction rate of D-glucose with DL-leucine. *Adapted from:* G. Haugard, L. Tumerman, and A. Sylvestri, A Study on the Reaction of Aldoses and Amino Acids, *J. Am. Chem. Soc*., Vol. 73, pp. 4594–4600, 1951

the reaction. The browning reaction is slowed down by decreasing pH, and the browning reaction can be said to be self-inhibitory since the pH decreases with the loss of the basic amino group. The effect of pH on the reaction rate of D-glucose with DL-leucine is demonstrated in Fig. 3.16. The effect of pH on the browning reaction is highly dependent on moisture content. When a large amount of water is present, most of the browning is caused by caramelization, but at low water levels and at pH greater than 6, the Maillard reaction is predominant.

The nature of the sugars in a nonenzymic browning reaction determines their reactivity. Reactivity is related to their conformational stability or to the amount of open-chain structure present in solution. Pentoses are more reactive than hexoses, and hexoses more than reducing disaccharides. Nonreducing disaccharides only react after hydrolysis has taken place. The order of reactivity of some of the aldohexoses is: mannose is more reactive than galactose, which is more reactive than glucose.

The effect of the type of amino acid can be summarized as follows. In the α-amino acid series, glycine is the most reactive. Longer and more complex substituent groups reduce the rate of browning. In the ω-amino acid series, browning rate increases with increasing chain length. Ornithine browns more rapidly than lysine. When the reactant is a protein, particular sites in the molecule may react faster than others. In proteins, the ε-amino group of lysine is particularly vulnerable to attack by aldoses and ketoses.

Moisture content is an important factor in influencing the rate of the browning reaction. Browning occurs at low temperatures and intermediate moisture content; the rate increases with increasing water content. The rate is extremely low below the glass transition temperature, probably because of limited diffusion (Roos and Himberg 1994; Roos et al. 1996a, b).

Methods of preventing browning could consist of measures intended to slow reaction rates, such as control of moisture, temperature, or pH, or removal of an active intermediate. Generally, it is easier to use an inhibitor. One of the most effective inhibitors of browning is sulfur dioxide or sodium bisulfite. The action of sulfur dioxide is unique and no other suitable inhibitor has been

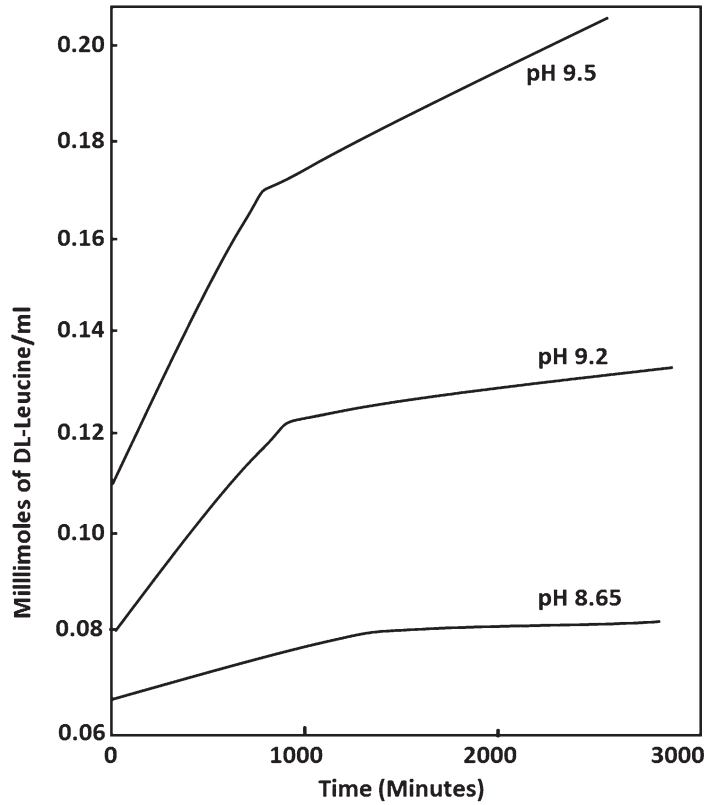


Fig. 3.16 Effect of pH on the reaction rate of D-glucose with DL-leucine. Adapted from: G. Haugard, L. Tumerman, and A. Sylvestri, A Study on the Reaction of Aldoses and Amino Acids, *J. Am. Chem. Soc*., Vol. 73, pp. 4594–4600, 1951

found. It is known that sulfite can combine with the carbonyl group of an aldose to give an addition compound:

$$NaHSO_3 + RCHO \rightarrow RCHOHSO_3Na$$

However, this reaction cannot possibly account for the inhibitory effect of sulfite. It is thought that sulfur dioxide reacts with the degradation products of the amino sugars, thus preventing these compounds from condensing into melanoidins. A serious drawback of the use of sulfur dioxide is that it reacts with thiamine and proteins, thereby reducing the nutritional value of foods. Sulfur dioxide destroys thiamine and is therefore not permitted for use in foods containing this vitamin.

Chemical Changes

During processing and storage of foods, a number of chemical changes involving proteins may occur (Hurrell 1984). Some of these may be desirable, others undesirable. Such chemical changes may lead to compounds that are not hydrolyzable by intestinal enzymes or to modifications of the peptide side chains that render certain amino acids unavailable. Mild heat treatments in the presence of water can significantly improve the protein's nutritional value in some cases. Sulfur-containing amino acids may become more available and certain antinutritional factors such as the trypsin inhibitors of soybeans may be deactivated. Excessive heat in the absence of water can be detrimental to protein quality; for example, in fish proteins, tryptophan, arginine, methionine, and lysine may be damaged. A number of chemical reactions may take place during heat treatment including decomposition, dehydration of serine and threonine, loss of sulfur from cysteine, oxidation of cysteine and methionine, cyclization of glutamic and aspartic acids and threonine (Mauron 1970; Mauron and Antener 1983).

The nonenzymic browning, or Maillard, reaction causes the decomposition of certain amino acids. For this reaction, the presence of a

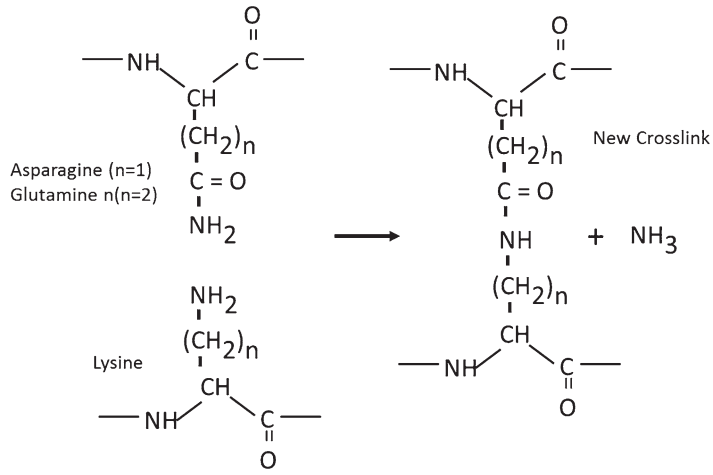
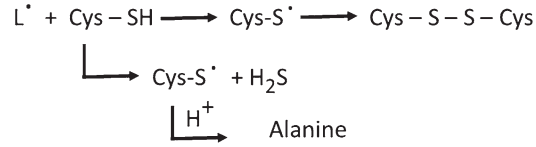


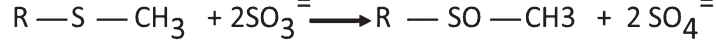
Fig. 3.17 Formation of amide-type bonds from the reaction between ε-amino groups of lysine and amide groups of asparagine ($n = 1$) glutamine ($n = 2$)

reducing sugar is required. Heat damage may also occur in the absence of sugars. Bjarnason and Carpenter (1970) demonstrated that the heating of bovine plasma albumin for 27 h at 115 °C resulted in a 50% loss of cystine and a 4% loss of lysine. These authors suggest that amide-type bonds are formed by reaction between the ε-amino group of lysine and the amide groups of asparagine or glutamine, with the reacting units present either in the same peptide chain or in neighboring ones (Fig. 3.17). The Maillard reaction leads to the formation of brown pigments, or melanoidins, which are not well defined and may result in numerous flavor and odor compounds. The browning reaction may also result in the blocking of lysine. Lysine becomes unavailable when it is involved in the Amadori reaction, the first stage of browning. Blockage of lysine is nonexistent in pasteurization of milk products, and is at 0–2% in UHT sterilization, 10–15% in conventional sterilization, and 20–50% in roller drying (Hurrell 1984).

Some amino acids may be oxidized by reacting with free radicals formed by lipid oxidation. Methionine can react with a lipid peroxide to yield methionine sulfoxide. Cysteine can be decomposed by a lipid free radical according to the following scheme:



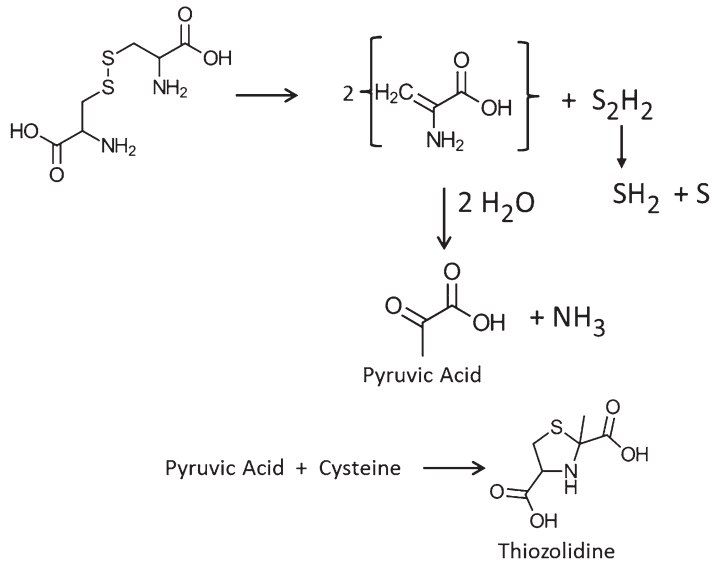
The decomposition of unsaturated fatty acids produces reactive carbonyl compounds that may lead to reactions similar to those involved in nonenzymic browning. Methionine can be oxidized under aerobic conditions in the presence of SO_2 as follows:



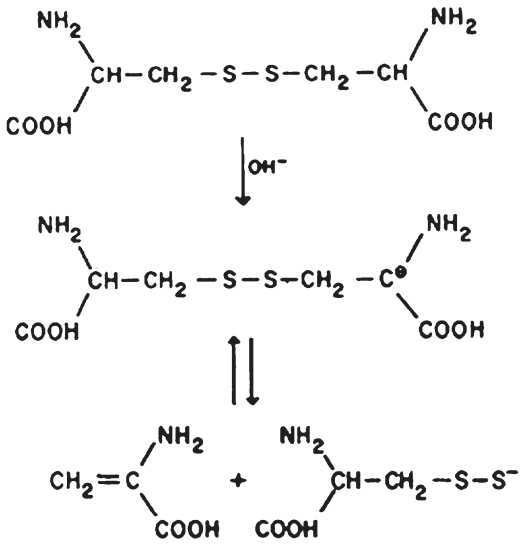
This reaction is catalyzed by manganese ions at pH values from 6 to 7.5. SO_2 can also react with cystine to yield a series of oxidation products. Some of the possible reaction products resulting from the oxidation of sulfur amino acids are listed in Table 3.12. Nielsen et al. (1985) studied the reactions between protein-bound amino acids and oxidizing lipids. Significant losses occurred of the amino acids lysine, tryptophan, and histidine. Methionine was extensively oxidized to its sulfoxide. Increasing water activity increased losses of lysine and tryptophan but had no effect on methionine oxidation.

Alkali treatment of proteins is becoming more common in the food industry and may result in several undesirable reactions. When cystine is

treated with calcium hydroxide, it is transformed into amino-acrylic acid, hydrogen sulfide, free sulfur, and 2-methyl thiazolidine-2, 4-dicarboxylic acid as follows:



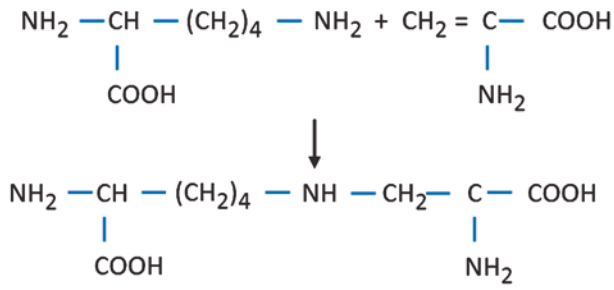
This can also occur under alkaline conditions, when cystine is changed into amino acrylic acid and thiocysteine by a β-elimination mechanism, as follows:



Amino-acrylic acid (dehydroalanine) is very reactive and can combine with the ε-amino group of lysine to yield lysinoalanine (Ziegler 1964) as shown:

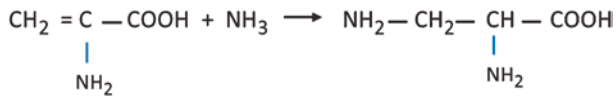
Table 3.12 Oxidation products of the sulfur-containing amino acids

Name		Formula
Methionine		R–S–CH_3
	Sulfoxide	R–SO–CH_3
	Sulfone	R–SO_2–CH_3
Cystine		R–S–S–R
	Disulfoxide	R–SO–SO–R
	Disulfone	R–SO_2–SO_2–R
Cysteine		R–SH
	Sulfenic	R–SOH
	Sulfinic	R–SO_2H
	Sulfonic (or cysteic acid)	R–SO_3H



Lysinoalanine formation is not restricted to alkaline conditions—it can also be formed by prolonged heat treatment. Any factor favoring lower pH and less drastic heat treatment will reduce the formation of lysinoalanine. Hurrell (1984) found that dried whole milk and UHT milk contained no lysinoalanine and that evaporated and sterilized milk contained 1000 ppm. More severe treatment with alkali can decompose arginine into ornithine and urea. Ornithine can combine with dehydroalanine in a reaction similar to the one giving lysinoalanine and, in this case, omithinoalanine is formed.

Treatment of proteins with ammonia can result in addition of ammonia to dehydroalanine to yield β-amino-alanine as follows:



Light-induced oxidation of proteins has been shown to lead to off-flavors and destruction of essential amino acids in milk. Patton (1954) demonstrated that sunlight attacks methionine and converts it into methional (β-methylmercaptopropionaldehyde), which can cause a typical sunlight off-flavor at a level of 0.1 ppm. It was later demonstrated by Finley and Shipe (1971) that the source of the light-induced off-flavor in milk resides in a low-density lipoprotein fraction.

Proteins react with polyphenols such as phenolic acids, flavonoids, and tannins, which occur widely in plant products. These reactions may result in the lowering of available lysine, protein digestibility, and biological value (Hurrell 1984).

Racemization is the result of heat and alkaline treatment of food proteins. The amino acids present in proteins are of the L-series. The racemization reaction starts with the abstraction of an α-proton from an amino acid residue to give a negatively charged planar carbanion. When a proton is added back to this optically inactive intermediate, either a D- or L-enantiomer may be formed (Masters and Friedman 1980). Racemization leads to reduced digestibility and protein quality.

Functional Properties

Increasing emphasis is being placed on isolating proteins from various sources and using them as food ingredients. In many applications functional properties are of great importance. Functional properties have been defined as those physical and chemical properties that affect the behavior of proteins in food systems during processing, storage, preparation, and consumption (Kinsella 1982). A summary of these properties is given in Table 3.13.

Table 3.13 Functional properties of food proteins

General property	Functional criteria
Organoleptic	Color, flavor, odor
Kinesthetic	Texture, mouth feel, smoothness, grittiness, turbidity
Hydration	Solubility, wettability, water absorption, swelling, thickening, gelling, syneresis, viscosity
Surface	Emulsification, foaming (aeration, whipping), film formation
Binding	Lipid-binding, flavor-binding
Structural	Elasticity, cohesiveness, chewiness, adhesion, network cross-binding, aggregation, dough formation, texturizability, fiber formation, extrudability
Rheological	Viscosity, gelation
Enzymatic	Coagulation (rennet), tenderization (papain), mellowing ("proteinases")
"Blendability"	Complementarity (wheat-soy, gluten-casein)
Antioxidant	Off-flavor prevention (fluid emulsions)

Source: From J.E. Kinsella, Structure and Functional Properties of Food Proteins, in *Food Proteins*, P.F. Fox and J.J. Condon, eds., 1982, Applied Science Publishers

Even when protein ingredients are added to food in relatively small amounts, they may significantly influence some of the physical properties of the food. Hermansson (1973) found that addition of 4% of a soybean protein isolate to processed meat significantly affected firmness, as measured by extrusion force, compression work, and sensory evaluation.

The emulsifying and foaming properties of proteins relate to their adsorption at interfaces and to the structure of the protein film formed there (Mitchell 1986). The emulsifying and emulsion stabilizing capacity of protein meat additives is important to the production of sausages. The emulsifying properties of proteins are also involved in the production of whipped toppings and coffee whiteners. The whipping properties of proteins are essential in the production of whipped toppings. Paulsen and Horan (1965) determined the functional characteristics of edible soya flours, especially in relation to their use in bakery products. They evaluated the measurable parameters of functional properties such as water dispersibility, wettability, solubility, and foaming characteristics as those properties affected the quality of baked products containing added soya flour.

Some typical functional properties of food proteins are listed in Table 3.14.

Surface Activity of Proteins

Proteins can act as surfactants in stabilizing emulsions and foams. To perform this function proteins must be amphiphilic just of Food Proteins like the emulsifiers discussed in Chap. 2. This is achieved when part of the protein structure contains predominantly amino acids with hydrophobic side chains and another part contains mostly hydrophilic side chains. The molecule is then able to orient itself in the oil-water interface. Thus, the ability of proteins to serve as emulsifiers varies greatly among proteins. The emulsifying capacity of a protein depends not only on its overall hydrophobicity but, more importantly, on the distribution of the hydrophobic or charged groups along the polypeptide chain and the manner in which the chain is folded (Dalgleish 1989). Hydrophobic side chains are likely to be folded into the inside of the molecule leaving the outside more hydrophilic. To be effective surfactants, proteins need to have flexible polypeptide chains, so that they are able to orient at the interface. Only proteins that have little secondary structure and are able to unfold at the interface are effective emulsifiers. Nakai and Powrie (1981) have shown the relationship between solubility, charge frequency, and hydrophobicity in graphical manner (Fig. 3.18) and in

Table 3.14 Functional properties of proteins in food systems

Functional property	Mode of action	Food system
Solubility	Protein solvation	Beverages
Water absorption and binding	Hydrogen bonding of water; entrapment of water (no drip)	Meat, sausages, bread, cakes
Viscosity	Thickening; water binding	Soups, gravies
Gelation	Protein acts as adhesive material	Meat, sausages, baked goods, pasta products
Elasticity	Hydrophobic bonding in gluten; disulfide links in gels	Meats, bakery products
Emulsification	Formation and stabilization of fat emulsion	Sausages, bologna, soup, cakes
Fat absorption	Binding of free fat	Meats, sausages, doughnuts
Flavor-binding	Adsorption, entrapment, release	Simulated meats, bakery products, etc.
Foaming	Forms stable films to entrap gas	Whipped toppings, chiffon, desserts, angel cakes

Source: From J.E. Kinsella, Structure and Functional Properties of Food Proteins, in *Food Proteins*, P.F. Fox and J.J. Condon, eds., 1982, Applied Science Publishers

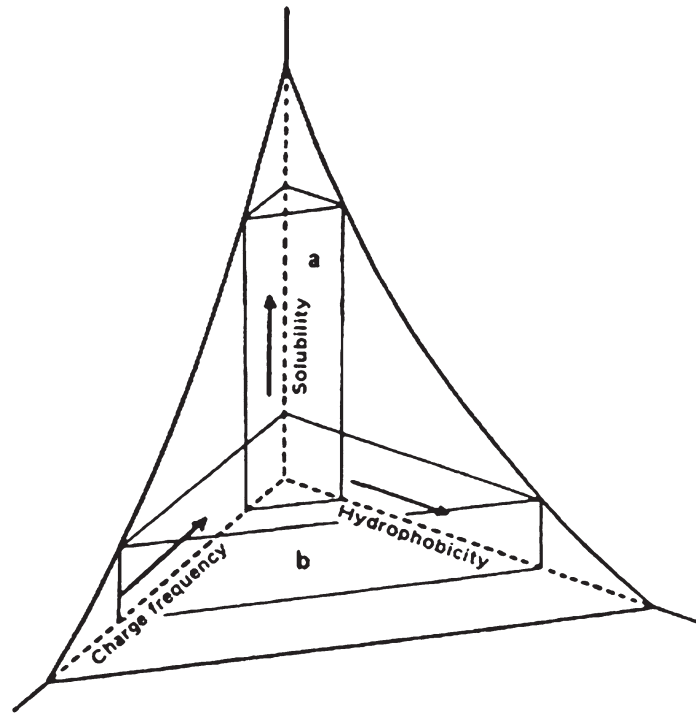


Fig. 3.18 Relationship between solubility, charge frequency, and hydrophobicity of proteins. *Source*: Reprinted with permission from S. Nakai and W.D. Powrie, Modification of Proteins for Functional and Nutritional Improvements, in *Cereals—A Renewable Resource*, *Theory and Practice*, Y. Pomeranz and L. Munck, eds., p. 225, © 1981, American Association of Cereal Chemists

the form of a table relating these parameters to the functional properties of proteins (Table 3.15).

There are two important considerations in emulsion formation: the binding of the protein to the oil-water interface and the stability of the emulsion. For an emulsion to possess stability, the proteins have to form a cohesive film. The cohesiveness of such films is stabilized by intramolecular disulfide bonds. In addition, emulsion particles can be stabilized by steric factors. This happens when disordered protein molecules at the interface prevent emulsion droplets from approaching one another closely enough to permit coagulation as a result of attraction by van der Waals forces (Dalgleish 1989).

The amount of protein required to form a stable emulsion depends on the size of emulsion droplets produced and the nature of the protein.

Table 3.15 Contribution of hydrophobicity, charge frequency, and structural parameters to functionality of proteins

	Hydrophobicity	Charge frequency	Structure
Solubility	−	+	−
Emulsification	+sur	(−)	+
Foaming	+tot	−	+
Fat binding	+sur	(−)	−
Water holding	−	+	?
Heat coagulation	+tot	−	+
Dough making	(+)	−	+

+: positive contribution; −: negative contribution; sur: surface hydrophobicity; tot: total hydrophobicity; ( ): contributes to a lesser extent

Source: Reprinted with permission from S. Nakai and W.D. Powrie, Modification of Proteins for Functional and Nutritional Improvements, *Cereals—Renewable Resource, Theory and Practice*, Y. Pomeranz and L. Munck, eds., p. 235, © 1981, American Association of Cereal Chemists

The size of the droplets, assuming the presence of sufficient protein to cover the interfacial area, is determined by the input of mechanical energy by a device such as a homogenizer or colloid mill. According to Dalgleish (1989), the protein load for monolayer coverage of the oil-water interface is in the order of a few mg/m^2. β-casein is an effective emulsifier protein and has been reported to give an interfacial loading of 2–3 mg/m^2.

Gel Formation

Proteins can form gels by acid coagulation, action of enzymes, heat, and storage. A gel is a protein network that immobilizes a large amount of water. The network is formed by protein-protein interactions. Gels are characterized by having relatively high non-Newtonian viscosity, elasticity, and plasticity. Examples of protein gels are a variety of dairy products including yogurt, soybean curd (tofu), egg protein gels including mayonnaise, and meat and fish protein gels. Some types of gel formation are reversible, especially those produced by heat. Gelatin gels are produced when a heated solution of gelatin is cooled. This sol-gel transformation is reversible. Most other types of gel formation are not reversible. Gelation has been described as a two-stage process (Pomeranz 1991). The first stage is a denaturation of the native protein into unfolded polypeptides, and the second stage is a gradual association to form the gel matrix. The type of association and, therefore, the nature of the gel depends on a variety of covalent and noncovalent interactions involving disulfide bonds, hydrogen bonds, ionic and hydrophobic interactions, or combinations of these.

Protein gels can be divided into two types: aggregated gels and clear gels (Barbut 1994). Aggregated gels are formed from casein and from egg white proteins and are opaque because of the relatively large size of the protein aggregates. Clear gels are formed from smaller particles, such as those formed from whey protein isolate, and have high water-holding capacity. The formation of such gels from ovalbumin is illustrated in Fig. 3.19 (Hatta and Koseki 1988), showing the influence of protein concentration, pH, and ionic strength. Many dairy products involve gel formation through the action of acid or by combined activity of acid and enzymes. Mayonnaise is an oil-in-water emulsion, in which egg yolk protein acts as the emulsifier. The presence of acetic acid in the form of vinegar or citric acid from lemon juice leads to interaction of the proteins covering the emulsion droplets, resulting in a gel-type emulsion.

Animal Proteins

Milk Proteins

The proteins of cow's milk can be divided into two groups: the caseins, which are phosphoproteins and comprise 78% of the total weight, and

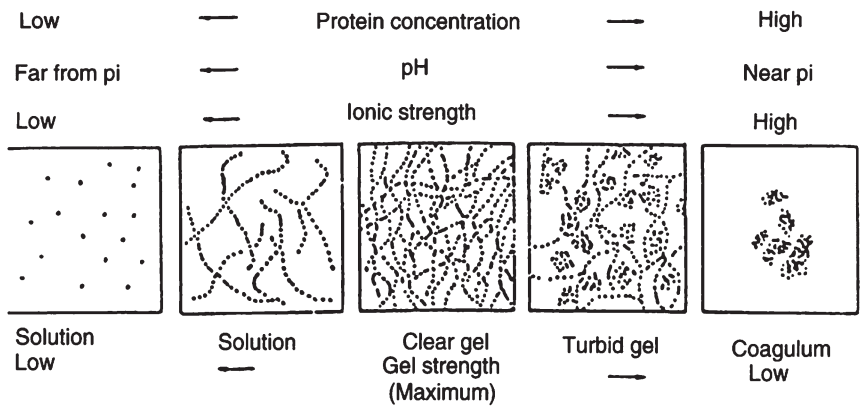


Fig. 3.19 Model for the formation of a gel network by heated ovalbumin, pi = isoelectric point. *Source*: Reprinted with permission from H. Hatta and T. Koseki, Relationship of SH Groups to Functionality of Ovalbumin, in *Food Proteins*, J.E. Kinsella and W.G. Soucie, eds., p. 265, © 1988, American Oil Chemists' Society

Table 3.16 Protein composition of mature bovine herd milk

Protein	g/L
Total protein	36
Total casein	29.5
Whey protein	6.3
α_{s1}-Casein	11.9
α_{s2}-Casein	3.1
β-Casein	9.8
κ-Casein	3.5
γ-casein	1.2
α-Lactalbumin	1.2
β-Lactoglobulin	3.2
Serum albumin	0.4
Immunoglobulin	0.8
Proteose-peptones	1.0

Source: Reprinted with permission from H.E. Swais-good, Protein and Amino Acid Composition of Bovine Milk, in *Handbook of Milk Composition*, R.G. Jensen, ed., p. 465, © 1982, Academic Press

Table 3.17 Amino acid composition of milk proteins

Amino acid	Protein (g/kg)
Essential amino acids	
Threonine	46
Valine	66
Methionine	26
Cystine	8
Isoleucine	59
Leucine	97
Phenylalanine	49
Lysine	81
Histidine	27
Arginine	35
Tryptophan	17
Nonessential amino acids	
Aspartic acid, asparagine	79
Serine	56
Glutamic acid, glutamine	219
Proline	99
Glycine	20
Alanine	34
Tyrosine	51

the milk serum proteins, which make up 17% of the total weight. The latter group includes β-lactoglobulin (8.5%), a lactalbumin (5.1%), immune globulins (1.7%), and serum albumins. In addition, about 5% of milk's total weight is nonprotein nitrogen (NPN)-containing substances, and these include peptides and amino acids. Milk also contains very small amounts of enzymes, including peroxidase, acid phosphatase, alkaline phosphatase, xanthine oxidase, and amylase. The protein composition of bovine herd milk is listed in Table 3.16 (Swaisgood 1995), and the amino acid composition of the milk proteins is shown in Table 3.17.

Casein is defined as the heterogeneous group of phosphoproteins precipitated from skim milk at pH 4.6 and 20 °C. The proteins remaining in solution, the serum or whey proteins, can be separated into the classic lactoglobulin and lactalbumin fractions by half saturation with ammonium sulfate or by full saturation with magnesium

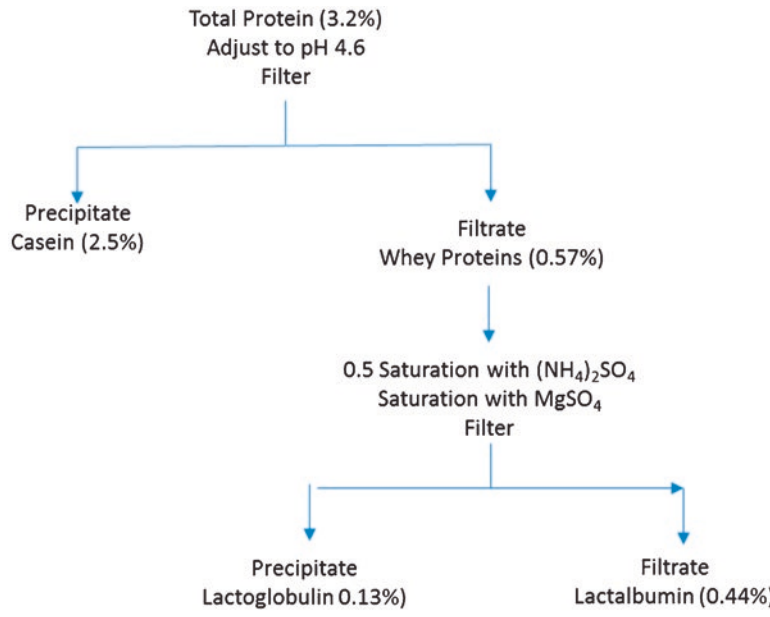


Fig. 3.20 Separation of milk proteins by precipitation

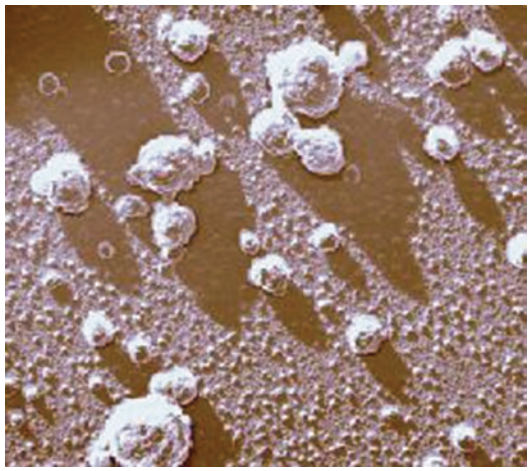


Fig. 3.21 Electron photomicrograph of casein micelles in milk (http://www.usu.edu/westcent/microstructure_food/Foods&bact.htm)

sulfate, as is shown in Fig. 3.20. However, this separation is possible only with unheated milk. After heating by, for example, boiling, about 80% of the whey proteins will precipitate with the casein at pH 4.6; this property has been used to develop a method for measuring the degree of heat exposure of milk and milk products.

Casein exists in milk as relatively large, nearly spherical particles of 30–300 nm in diameter (Fig. 3.21). In addition to acid precipitation, casein can be separated from milk by rennet action or by saturation with sodium chloride. The composition of the casein depends on the method of isolation. In the native state, the caseinate particles contain relatively large amounts of calcium and phosphorus and smaller quantities of magnesium and citrate and are usually referred to as calcium caseinatephosphate or calcium phosphocaseinate particles. When adding acid to milk, the calcium and phosphorus are progressively removed until, at the isoelectric point of pH 4.6, the casein is completely free of salts. Other methods of preparing casein yield other products; for example, salt precipitation does not remove the calcium and phosphorus, and rennet action involves limited proteolysis. The rennet casein is named paracasein.

Casein is a nonhomogeneous protein that consists of four components, identified as α_{s1}-, α_{s2}-, β-, and κ-casein. The γ-casein mentioned in the literature (see Table 3.16) has been identified as proteolytic fragments of β-casein (Wong et al. 1996). The four casein components occur as genetic variants. Such genetically determined variants or polymorphs differ from one another by one or more amino acid substitutions and/or deletions. The complete amino acid sequence has been established, and the exact nature of the genetic variants has been determined. The genetic

Table 3.18 Genetic variants of caseins

Variant	Total amino acid residues	Molecular weight
α_{s1}-CN B-8P	199	23,614
α_{s2}-CN A-11P	207	24,350
β-CN A1-5P	209	23,982
κ-CN B-1P	169	19,023

variants are described by the suffix CN (for casein) and a capital A, B, C, etc., as well as by the number of phosphorylations. Genetic variants of the four caseins are listed in Table 3.18 (Wong et al. 1996). The caseins have sites of phosphorylation that have a unique amino acid sequence. The three–amino acid sequence is Ser-X-A, with X being any amino acid and A either glutamic acid or serine-phosphate. One part of the α-casein is precipitable by calcium ions and has been designated calcium-sensitive casein or α_s. The non-calcium-sensitive fraction, κ-casein, is the protein assumed to confer stability on the casein micelle; this has been found to be removed by the action of rennin, thereby leaving the remaining casein precipitable by calcium ions, κ-casein is the fraction with the lowest phosphate content. The two α_s-caseins show strong association. The association of β-casein is temperature dependent. At 4 °C only monomers exist; at temperatures greater than 8 °C association will occur. α_{s1}-Casein has more acidic than basic amino acids and has a net negative charge of 22 at pH 6.5. The polypeptide chain contains 8.5% proline that is distributed uniformly, resulting in no apparent secondary structure. α_{s2}-Casein has the highest number of phosphorylations and a low proline content, β-casein is a single polypeptide chain with a total of 209 amino acids, and has seven genetic variants. The distribution of amino acids in the polypeptide chain is quite specific. The N-terminal segment has a high negative charge, giving it hydrophilic properties, the C-terminal portion is highly hydrophobic. This arrangement lends surfactant properties to this protein.

Casein contains 0.86% phosphorus, and it is assumed that this is present exclusively in the form of monophosphate esters with the hydroxyl groups of serine and threonine. Limited and specific hydrolysis of casein with proteolytic enzymes has produced a number of large polypeptides that resist further hydrolysis. An electrophoretically homogeneous phosphopeptone has been isolated from a trypsin digest of β-casein by Peterson et al. (1958). This phosphopeptone has a molecular weight of about 3000 and consists of 24 amino acid residues of ten different amino acids. The peptone contains five phosphate residues linked to four serine and one threonine groups. This constitutes essentially all of the phosphorus of β-casein and it appears that the phosphate residues are localized in a relatively small region of the casein molecule.

In addition to ester phosphate, casein contains calcium phosphate in the colloidal form. It appears that the presence of this colloidal calcium phosphate helps maintain the structural integrity of the casein micelle. Although the composition and structure of most of the casein fractions are now well established, the exact nature of the arrangement of the caseins and calcium phosphate into a micelle is not well known. Many models of micelle structure have been proposed (Farrell 1973; Farrell and Thompson 1974). These can be divided into three groups: coat-core models, internal structure models, and subunit models. In the most popular, the coat-core model, it is assumed that the core contains the calcium-sensitive α_s-caseins and that this core is covered by a layer of κ-casein. The function of the κ-casein coat is to protect the micelle from insolubilization by calcium ions. The κ-casein is readily attacked by the enzyme rennin, thus removing the coat and resulting in coagulation of the micelles. This model most readily accounts for the action of rennin but does not explain the position of the colloidal calcium phosphate.

It appears that micelles are formed by cross-linking of some of the ester phosphate groups by calcium. Chelation of calcium results in dissociation and solubilization of the micelles, and the rate at which this happens corresponds to the ester phosphate content of the monomers (Aoki et al. 1987).

The whey proteins of milk were originally thought to be composed of two main components, lactalbumin and lactoglobulin, as indicated

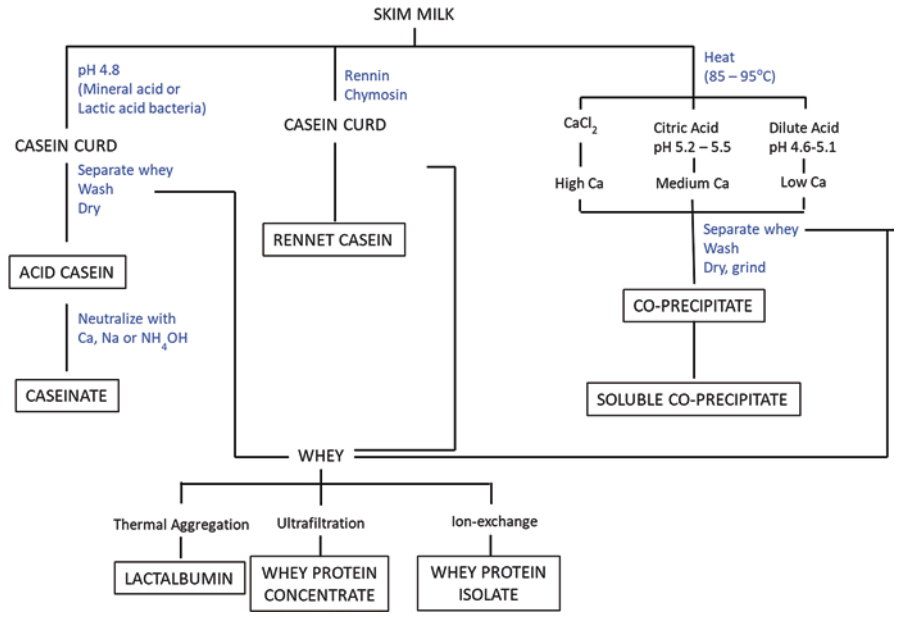


Fig. 3.22 Production of protein products from skim milk

in Fig. 3.22. Then it was found that the lactalbumin contains a protein with the characteristics of a globulin. This protein, known as β-lactoglobulin, is the most abundant of the whey proteins. It has a molecular weight of 36,000. In addition to β-lactoglobulin, the classic lactalbumin fraction contains α-lactalbumin, serum albumin, and at least two minor components.

β-lactoglobulin is rich in lysine, leucine, glutamic acid, and aspartic acid. It is a globular protein with five known genetic variants. Variants A and B have 162 amino acids and molecular weights of 18,362 and 18,276, respectively. β-lactoglobulin has a tightly packed structure and consists of eight strands of antiparallel β sheets. The interior of the molecule is hydrophobic. The molecular structure also contains a certain amount of α helix, which plays a role in the formation of the usually occurring dimer. The association is pH dependent, β-lactoglobulin A will form octamers at low temperature and high concentration and at pH values between 3.5 and 5.2. Below pH 3.5 the protein dissociates into monomers. This protein is the only milk protein containing cysteine and, therefore, contains free sulfhydryl groups, which play a role in the development of cooked flavor in heated milk. The cysteine group is also involved in thermal denaturation. At pH 6.7 and above 67 °C, β-lactoglobulin denatures, followed by aggregation. The first step in the denaturation is a series of reversible conformational changes that result in exposure of cysteine. The next step involves association through sulfhydryl-disulfide exchange.

The differences between genetic variants, although minor, may result in marked changes in some properties (Aschaffenburg 1965). The two chains of β-lactoglobulin C differ from the chains of the B variant in that a histidine residue has taken the place of a glutamic acid or glutamine residue. The A chains differ in two places from the B chains: aspartic acid replaces glycine and valine replaces alanine. Because of these minute differences, A is less soluble and more stable when heated than B. Variant A has a tendency to form tetramers at pH 4.5, whereas this tendency is absent in B. These differences are thought to be the result of differences in the three-dimensional folding or tertiary structure of the amino acid chains.

α-Lactalbumin occurs as genetic variants A and B, each with 123 amino acid residues.

The molecular weight of A is 14,147 and B is 14,175. The amino acid sequence of α-lactalbumin is very similar to that of hen egg-white lysozyme. α-Lactalbumin has a high binding capacity for calcium and some other metals. It is insoluble at the isoelectric range from pH 4 to 5. The calcium in α-lactalbumin is bound very strongly and protects the stability of the molecule against thermal denaturation.

The immune globulins were previously divided into euglobulin and pseudoglobulin. The level of these proteins in colostrum is very high and they have been shown to be transferred to the blood of the young calf, indicating that they are absorbed unchanged. The three classes of immunoglobulins in milk are designated IgM(γM), IgA(γA), and IgG(γG) (Gordon and Kalan 1974). IgG is subdivided into IgG1 and IgG2. The serum albumin has been shown to be identical to the blood serum albumin.

Nonfat milk (skim milk) is the raw material from which a number of milk protein products are manufactured. A schematic diagram of the various products obtained by processing of skim milk is given in Fig. 3.22 (Wong et al. 1996). These products are used as raw materials in many manufactured foods and include caseins, caseinates, and coprecipitates (Morr 1984). Acid casein results from isoelectric precipitation of casein at pH 4.6–4.7. The curd is recovered by centrifugation, then washed and dried. Alkali neutralization of the wet curd yields caseinate, which is spray dried. Rennet casein is made by rennet coagulation followed by washing and drying of the curd. Coprecipitates include both casein and whey proteins and are made from heated skim milk. The heating denatures the whey proteins, which can then be precipitated with acid together with the casein.

Whey protein concentrate is made from whey, the by-product of cheese making. Removal of lactose and minerals requires reverse osmosis end ultrafiltration processing (Fig. 3.23).

An up-to-date coverage of our present knowledge of milk proteins is given by Wong et al. (1996).

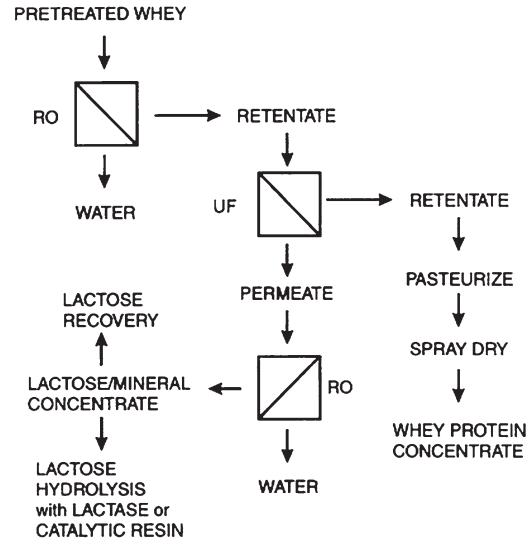


Fig. 3.23 Processing of whey to produce whey protein concentrate by reverse osmosis (RO) and ultrafiltration (UF). *Source*: From C. V. Morr, Production and Use of Milk Proteins in Food, *Food Technol*., Vol. 38, No. 7, pp. 39–18, 1984

Meat Proteins

Meromysin

The proteins of muscle consist of about 70% structural or fibrillar proteins and about 30% water-soluble proteins. The fibrillar proteins contain about 32–38% myosin, 13–17% actin, 7% tropomyosin, and 6% stroma proteins. Meat and fish proteins contribute to highly organized structures that lend particular properties to these products. Some of the other proteins discussed in this chapter are more or less globular and consist of particles that are not normally involved in an extensive structural array. Examples are milk proteins and the protein bodies in cereals and oilseeds. Extensive structure formation involving these proteins may occur in various technological processes such as making cheese from milk or texturized vegetable protein products from oilseeds.

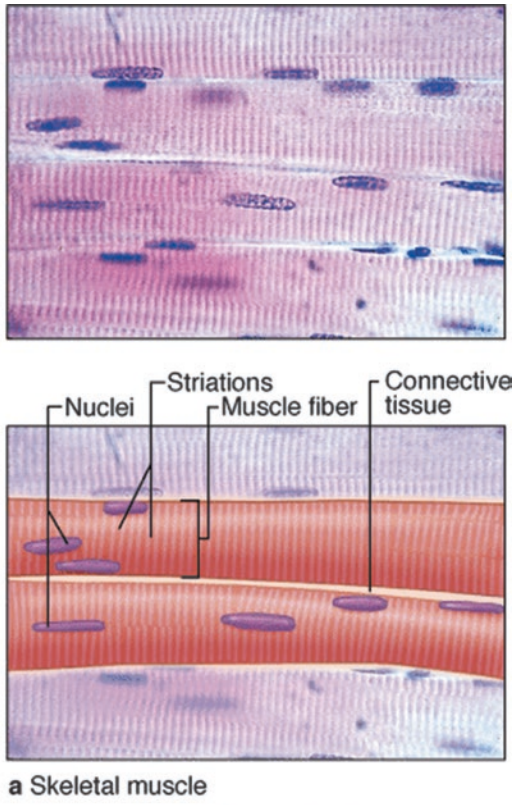


Fig. 3.24 Microscopic structure of striated muscle in longitudinal section

Muscle is made up of fibers that are several centimeters long and measure 0.01–0.1 mm in diameter. The fibers are enclosed in membranes called sarcolemma and are arranged in bundles that enclose fat and connective tissue. The fibers are cross-striated, as indicated in Fig. 3.24, and this is due to the presence of cross-striated myofibrils. The myofibrils are embedded in the cell cytoplasm called sarcoplasm. The fibers contain peripherally distributed nuclei; a diagram of the arrangement of the various constituents of a muscle fiber is given in Fig. 3.25 (Cassens 1971). In addition to the constituents mentioned, the muscle fibers contain other components including mitochondria, ribosomes, lysosomes, and glycogen granules. The fibers make up the largest part of the muscle volume, but there is from 12 to 18% of extracellular space.

The fibrils are optically nonuniform, which accounts for the striated appearance. Compounds with different refractive indexes are arranged along the fiber.

Meat contains three general types of proteins: the soluble proteins, which can easily be removed by extraction with weak salt solutions (ionic strength ≤ 0.1); the contractile proteins; and the stroma proteins of the connective tissue. The soluble proteins are classed as myogens and myoalbumins. The myogens are a heterogeneous group of metabolic enzymes. After extraction of the soluble proteins, the fibril and stroma proteins remain. They can be extracted with buffered 0.6 M potassium chloride to yield a viscous gel of actomyosin.

Myosin is the most abundant of the muscle proteins and makes up about 38% of the total. Myosin is a highly asymmetric molecule with a molecular weight of about 500,000 that contains about 60–70% α-helix structure. The molecule has a relatively high charge and contains large amounts of glutamic and aspartic acids and dibasic amino acids. Myosin has enzyme activity and can split ATP into ADP and monophosphate, thereby liberating energy that is used in muscle contraction. The myosin molecule is not a single entity. It can be separated into two subunits by means of enzymes or action of the ultracentrifuge. The subunits with the higher molecular weight, about 220,000, are called heavy meromyosin. Those with low molecular weight, about 20,000 are called light meromyosin. Only the heavy meromyosin has ATP-ase activity.

Actin makes up about 13% of the muscle protein, so the actin-myosin ratio is about 1:3. Actin occurs in two forms: G-actin and F-actin (G and F denote globular and fibrous). G-actin is a monomer that has a molecular weight of about 47,000 and is a molecule of almost spherical shape. Because of its relatively high proline content, it has only about 30% of α-helix configuration. F-actin is a large polymer and is formed when ATP is split from G-actin. The units of actin combine to form a double helix of indefinite length, and molecular weights of actin have been reported to be in the order of several millions. Bodwell and McClain (1971) indicate that actin

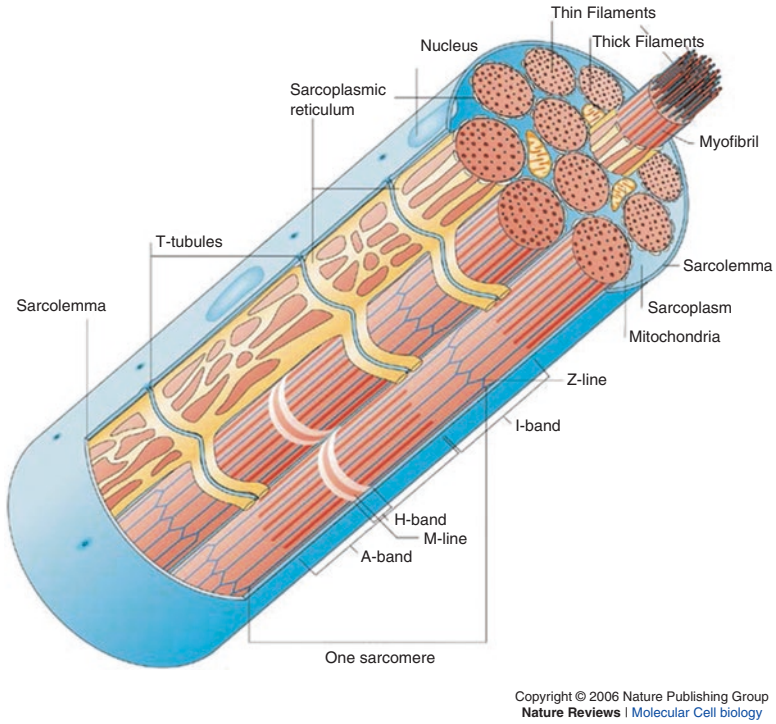


Fig. 3.25 Diagrammatic representation of a muscle fiber (http://people.eku.edu/ritchisong/301notes3.htm)

polymers may have an apparent molecular weight of over 14,000,000, with a length of 1160 nm and a diameter of 6 nm. The transformation of G-actin into F-actin is schematically represented in Fig. 3.26.

Actomyosin is a complex of F-actin and myosin and is responsible for muscle contraction and relaxation. Contraction occurs when myosin ATP-ase activity splits ATP to form phosphorylated actin and ADP. For this reaction, the presence of K^+ and Mg^{2+} is required. Relaxation of muscle depends on regeneration of ATP from ADP by phosphorylation from creatine phosphate. The precise mechanism of contraction is still unknown, although a working hypothesis is available (Bailey 1982).

The composition of various cuts of meats and their structural implications have been described by Ranken (1984).

Collagen

The contractile meat proteins are separated and surrounded by layers of connective tissues. The amount and nature of this connective tissue is an important factor in the tenderness or toughness and the resulting eating quality of meat. Collagens form the most widely occurring group of proteins in the animal body. They are part of the connective tissues in muscle and organs, skin, bone, teeth, and tendons. The collagens are a distinct class of proteins as can be demonstrated by X-ray diffraction analysis. This technique shows that collagen fibrils have regular periodicity of 64 nm, which can be increased under tension to 400 nm. Collagen exists as a triple helix; the formation of collagen into the triple helix is shown in Fig. 3.27 (Yamauchi and Sricholpech 2012). The biosynthesis of collagen is a long complicated including,

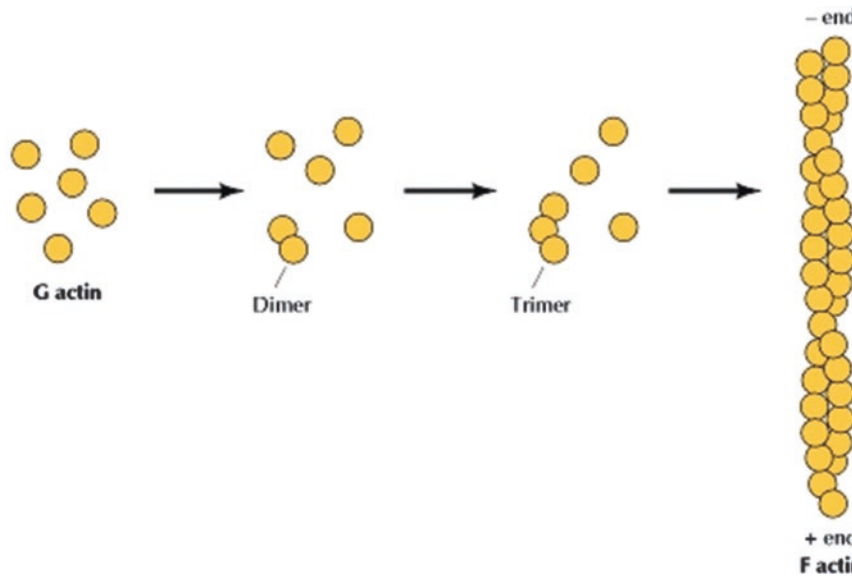


Fig. 3.26 Transformation of G-actin into F-actin from dimers to trimers to polymeric F-actin

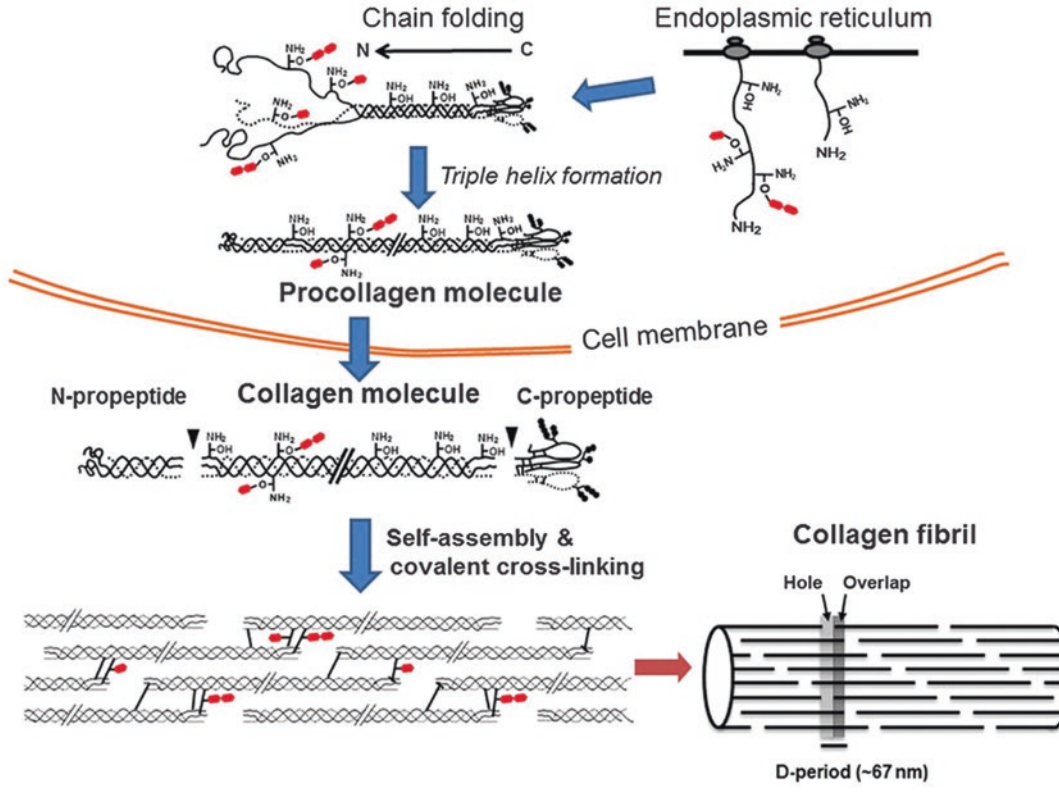


Fig. 3.27 Collagen peptides fold to form a right-handed superhelix. This is the tropocollagen molecule. Molecules line up in a staggered fashion to overlap by one-quarter length to form a fibril. *Source*: From Yamauchi M., Sricholpech (2012)

chain association and folding, secretion, procollagen processing, self-assembly and progressive cross-linking (Fig. 3.27). Type I collagen is a long (~300 nm long, ~1.5 nm thick) heterotrimeric molecule composed of two α1 chains and one α2 chain. An α1 homotrimeric form exists as a minor form. The molecule consists of three domains: the N-terminal non-triple helical domain (N-telopeptide), the central triple helical domain and the C-terminal non-triple helical domain (C-telopeptide). The single (uninterrupted) triple helical domain represents more than 95% of the molecule (Yamauchi and Sricholpech 2012).

The triple helix is the tropocollagen molecule; these are lined up in a staggered array, overlapping by one-quarter of their length to form a fibril. The fibrils are stacked in layers to form connective tissue. Important in the formation of these structures is the high content of hydroxyproline and hydroxylysine. The content of dibasic and diacidic amino acids is also high, but tryptophan and cystine are absent. As a result of this particular amino acid composition, there are few interchain cross-bonds, and collagen swells readily in acid or alkali.

Heating of collagen fibers in water to 60–70 °C shortens them by one-third or one-fourth of the original length. This temperature is characteristic of the type of collagen and is called shrink temperature (T_s). The T_s of fish skin collagen is very low, 35 °C. When the temperature is increased to about 80 °C, mammalian collagen changes into gelatin. Certain amino acid sequences are common in collagen, such as Gly-Pro-Hypro-Gly. In a triple helix, only certain sequences are permissible. The structural unit of the collagen fibrils is tropocollagen with a length of 280 nm, a diameter of 1.5 nm, and a molecular weight of 360,000. Gelatin is a soluble protein derived from insoluble collagen. Although it can be made from different animal byproducts, hide is the common source of gelatin production. The process of transforming collagen into gelatin involves the following three changes:

1. rupture of a limited number of peptide bonds to reduce the length of the chains
2. rupture or disorganization of a number of the lateral bonds between chains
3. change in chain configuration

The last of these is the only change essential for the conversion of collagen to gelatin. The conditions employed during the production of gelatin determine its characteristics. If there is extensive breakdown of peptide bonds, many lateral bonds may remain intact and soluble fragments are produced. If many lateral bonds are destroyed, the gelatin molecules may have relatively long chain lengths. Thus, there is a great variety of gelatins. In normal production, the hides or bones are extracted first, under relatively mild conditions, followed by successive extractions under more severe conditions. The first extraction yields the best-quality gelatin. The term *gelatin* is used for products derived from mammalian collagen that can be dispersed in water and show a reversible sol-gel change with temperature. The gels formed by gelatin can be considered as a partial return of the molecules to an ordered state. However, the return to the highly ordered state of collagen is not possible. High-quality gelatin has an average chain length of 60,000–80,000, whereas the value for native collagen is infinite.

The process of gel formation is probably closely associated with the presence of guanidino groups of arginine. Hypobromite has the ability to destroy guanidino groups and, when added to gelatin, it inhibits gelation.

There are three types of gelatin: alpha, with a molecular weight of 80,000–125,000; beta, with molecular weight of 160,000–250,000; and gamma, with molecular weight of 240,000–375,000 (Poppe 1992). The typical amino acid sequence in gelatin is Gly-X-Y, where X is mostly proline and Y is mostly hydroxyproline (Fig. 3.28).

When gelatin is placed in cold water it will absorb 5–10 times its own weight in water and swell. When this material is heated to above the melting point, between 27 and 34 °C, the swollen gelatin dissolves. This solgel transformation is reversible. Poppe (1992) has described the mechanism of gel formation. It involves the reversion from a random coil to a helix structure. Upon cooling, the imino acid-rich regions of different chains form a helical structure, which is stabilized by hydrogen bonding. This then forms the three-dimensional gel matrix.

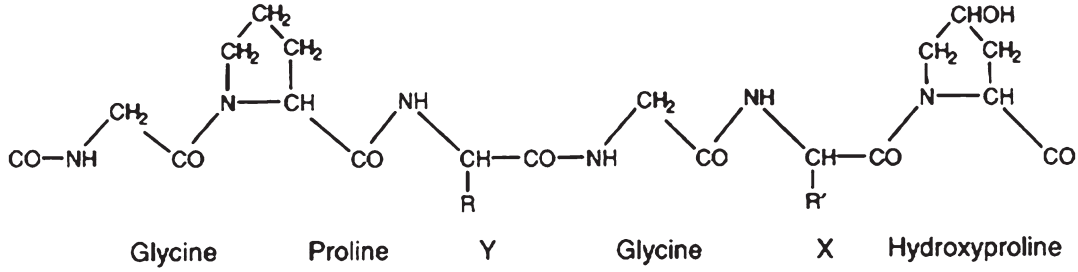


Fig. 3.28 Amino acid sequence in gelatin

Table 3.19 Division of the proteins of fish flesh according to solubility

Ionic strength at which soluble	Name of group	Location
Equal to or greater than 0	"Myogen" easily soluble	Mainly sarcoplasm, muscle cell juice
Greater than about 0.3	"Structural" less soluble	Mainly myofibrils, contractile elements
Insoluble	"Stroma"	Mainly connective tissues, cell walls, etc.

Commercial gelatin is available in two types, A and B. Type A gelatin has undergone an acid pretreatment and type B a lime pretreatment. They differ in their viscosity and their ability to combine with negatively charged hydrocolloids such as carrageenan.

Fish Proteins

The proteins of fish flesh can be divided into three groups on the basis of solubility, as indicated in Table 3.19. The skeletal muscle of fish consists of short fibers arranged between sheets of connective tissue, although the amount of connective tissue in fish muscle is less than that in mammalian tissue and the fibers are shorter. The myofibrils of fish muscle have a striated appearance similar to that of mammalian muscle and contain the same major proteins, myosin, actin, actomyosin, and tropomyosin. The soluble proteins include most of the muscle enzymes and account for about 22% of the total protein. The connective tissue of fish muscle is present in lower quantity than in mammalian muscle; the tissue has different physical properties, which result in a more tender texture of fish compared with meat. The structural proteins consist mainly of actin and myosin, and actomyosin represents about three-quarters of the total muscle protein. Fish actomyosin has been found to be quite labile and easily changed during processing and storage. During frozen storage, the actomyosin becomes progressively less soluble, and the flesh becomes increasingly tough. Connell (1962) has described the changes that may occur in cod myosin. When stored in dilute neutral solution, myosin rapidly denatures and forms aggregates in a step-wise manner as follows:

$$M \rightarrow M_D \tag{3.1}$$

$$\left.\begin{aligned} M_D + M_D &\rightarrow 2M_D \\ 2M_D + M_D &\rightarrow 3M_D \end{aligned}\right\} \tag{3.2}$$

Equation (3.1) represents the change from native to denatured protein and follows first-order reaction kinetics. In successive steps represented by Eq. (3.2), dimers, trimers, and higher polymers are formed in a concentration-dependent reaction pattern. The aggregation is assumed to be mostly in a lateral fashion with only little end-to-end aggregation. The instability of fish myosin appears to be one of the major factors causing the lability of fish muscle.

The interest in using fish for the production of fish protein concentrate has waned because the product lacks satisfactory functional properties. More promising ways of using fish resources

Table 3.20 Protein composition of egg white

Constituent	Approximate amount (%)	Approximate isoelectric point (pH)	Unique properties
Ovalbumin	54	4.6	Denatures easily, has sulfhydryls
Conalbumin	13	6.0	Complexes iron, antimicrobial
Ovomucoid	11	4.3	Inhibits enzyme trypsin
Lysozyme	3.5	10.7	Enzyme for polysaccharides antimicrobial
Ovomucin	1.5	4.5	Viscous, high sialic acid, reacts with viruses
Flavoprotein-apoprotein	0.8	4.1	Binds riboflavin
"Proteinase inhibitor"	0.1	5.2	Inhibits enzyme (bacterial proteinase)
Avidin	0.05	9.5	Binds biotin, antimicrobial
Unidentified proteins	8	5.5, 7.5, 8.0, 9.0	Mainly globulins
Nonprotein	8		Primarily half glucose and salts (poorly characterized)

Source: From R.R. Feeney and R.M. Hill, Protein Chemistry and Food Research, in *Advances in Food Research*, Vol. 10, C.O. Chichester, E.M. Mrak, and G.F. Stewart, eds., 1960, Academic Press

involve fish protein gels (surimi), which can be made into attractive consumer products (Mackie 1983).

Egg Proteins

The proteins of eggs are characterized by their high biological value and can be divided into the egg white and egg yolk proteins. The egg white contains at least eight different proteins, which are listed in Table 3.20. Some of these proteins have unusual properties, as indicated in the table; for example, lysozyme is an antibiotic, ovomucoid is a trypsin inhibitor, ovomucin inhibits hemagglutination, avidin binds biotin, and conalbumin binds iron. The antimicrobial properties help to protect the egg from bacterial invasion.

Liquid egg white contains 10–11% of protein, and the dried form contains about 83%. The most abundant protein is ovalbumin, a phosphoprotein with a molecular weight of 45,000 that contains a small proportion of carbohydrate. The carbohydrate is present as a polysaccharide composed of two glucosamine and four mannose groups. Ovalbumin can be separated by electrophoresis into two components, one component with two phosphate groups and another component with one phosphate group. Some of the diphosphate changes to monophosphate on storage. Ovalbumin is readily denatured by heat.

Conalbumin has a molecular weight of 70,000 and has iron-binding and antimicrobial properties. It can render iron unavailable to microorganisms; this property is lost after heat denaturation. Iron is bound in the ferric form by coordination. The groups involved in the binding of iron are amino, carboxyl, guanidine, and amides. When these groups are blocked, the iron-binding property is lost.

Ovomucoid is a trypsin inhibitor and a glycoprotein with a molecular weight of 27,000–29,000, containing mannose and glucosamine. This protein is highly resistant to denaturation.

Lysozyme is classed as a globulin and has the ability to cause lysis of bacterial cells. There are three fractions, designated G_1, G_2, and G_3. The activity resides in the G_1 fraction. The protein has a molecular weight of 14,000–17,000 and is a basic protein with unusually high content of histidine, arginine, and lysine. It is stable to many agents that denature other proteins, such as heat, cold, and denaturing reagents. It is also quite resistant to proteolysis by enzymes such as papain and trypsin.

Table 3.21 Protein components of egg yolk

Constituent	Approximate amount (%)	Particular properties
Livetin	5	Contains enzymes—poorly characterized
Phosvitin	7	Contains 10% phosphorus
Lipoproteins	21	Emulsifiers
(Total protein)	(33)	

Source: From R.R. Feeney and R.M. Hill, Protein Chemistry and Food Research, in *Advances in Food Research*, Vol. 10, C.O. Chichester, E.M. Mrak, and G.F. Stewart, eds., 1960, Academic Press

Ovomucin is an insoluble protein, which precipitates from egg white on dilution with water. It is not well known, has a high molecular weight (7,600,000), and is a mucoprotein. The ability of certain viruses to agglutinate red blood cells, called *hemagglutination*, is inhibited by ovomucin.

Avidin is a protein characterized by its ability to bind biotin and render it unavailable. Heat denaturation destroys this property.

Egg yolk proteins precipitate when the yolk is diluted with water. The protein components of egg yolk are listed in Table 3.21. The yolk contains a considerable amount of lipid, part of which occurs in bound form as lipoproteins. Lipoproteins are excellent emulsifiers, and egg yolk is widely used in foods for that reason. The two lipoproteins are lipovitellin, which has 17–18% lipid, and lipovitellenin, which has 36–41% lipid. The protein portions of these compounds after removal of the lipid are named vitellin and vitellenin. The former contains 1%% phosphorus, the latter 0.29%.

The membranes of eggs consist of keratins and mucins.

When fluid egg yolk is frozen, changes take place, causing the thawed yolk to form a gel (Powrie 1984). Gelation increases as the freezing temperature is lowered from −6 to −14 °C. Gradual aggregation of lipoprotein is postulated as the cause of gelation.

Plant Proteins

As with animal proteins, plant proteins occur in wide variety. Many plant proteins have until recently received much less study than the animal proteins. This is gradually changing, and more information is now becoming available on many nontraditional food proteins. Proteins can be obtained from leaves, cereals, oilseeds, and nuts. Leaf proteins have been extracted from macerated leaves and are very labile. They are readily denatured at about 50 °C and undergo surface denaturation in the pH range 4.5–6.0. Cereal seed proteins are generally low in lysine. Peanut protein is poor in lysine, tryptophan, methionine, and threonine. Legume seeds are low in cyst(e)ine and methionine. Great improvement in nutritional value can sometimes be obtained by judicious mixing of different products.

The proteins of cereal grains are very important to their physical properties, even though the protein content of grains is not very high. Protein levels vary within wide limits, depending on species, soil, fertilizer, and climate. The protein is nonuniformly distributed throughout the kernel, with the center having the lowest protein content. Wheat has a protein content of 8–14%, rye about 12%, barley 10%, and rice 9%.

Wheat Proteins

Wheat proteins are unique among plant proteins and are responsible for bread-making properties of wheat. The classic method of fractionation based on solubility characteristics indicates the presence of four main fractions (Fig. 3.29): albumin, which is water-soluble and coagulated by heat; globulin, soluble in neutral salt solution; gliadin, a prolamine soluble in 70% ethanol; and glutenin, a glutelin insoluble in alcohol but soluble in dilute acid or alkali.

The methods of gel electrophoresis and isoelectric focusing now provide highly efficient tools for separation of these proteins. By using

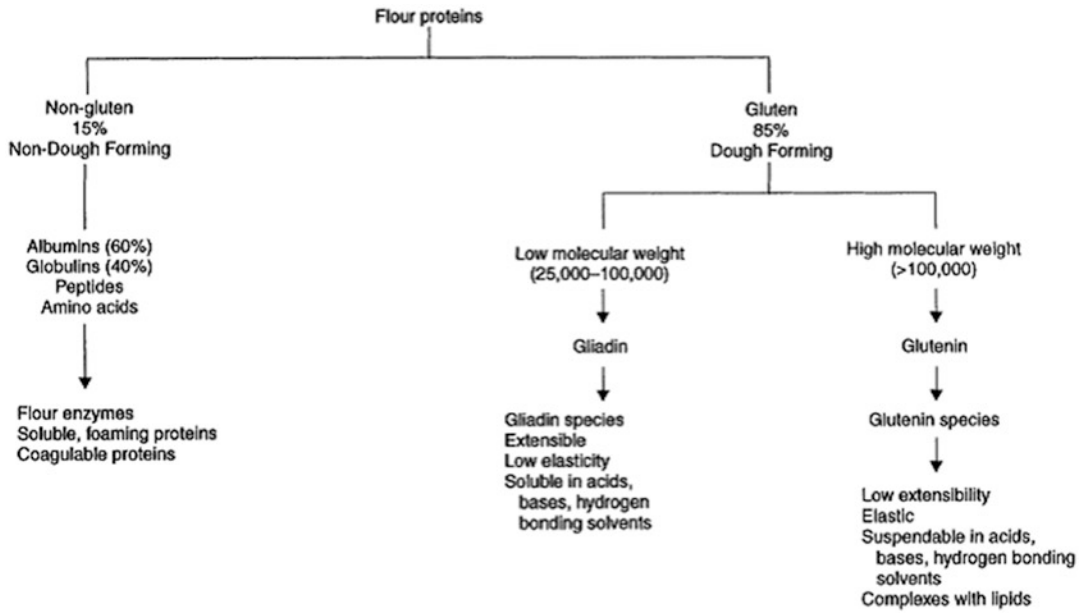


Fig. 3.29 Schematic representation of the main protein fractions of wheat flour. *Source*: From J. Holme, A Review of Wheat Flour Proteins and Their Functional Properties, *Bakers' Dig*., Vol. 40, No. 6, pp. 38–42, 78, 1966

these techniques, both gliadin and glutenin have been shown to be complex mixtures. Gliadin and glutenin are the storage, or gluten-forming, proteins of wheat. The formation of gluten takes place when flour is mixed with water. The gluten is a coherent elastic mass, which holds together other bread components such as starch and gas bubbles, thus providing the basis for the crumb structure of bread. The hydration of the gluten proteins results in the formation of fibrils (Simmonds and Orth 1973), with gliadins forming films and glutenins forming strands.

Gluten proteins have a high content of glutamine but are low in the essential amino acids lysine, methionine, and tryptophan. The insolubility of gluten proteins can be directly related to their amino acid composition. High levels of nonpolar side chains result from the presence of glutamic and aspartic acids as the amides. Because these are not ionized, there is a high level of apolar (hydrogen) bonding. This contributes to aggregation of the molecules and results in low solubility.

Heat damage to gluten can result from excessive air temperatures used in the drying of wet grain. The gluten becomes tough and is more difficult to extract. Heat-denatured wheat gives bread poor texture and loaf volume (Schofield and Booth 1983).

Gliadin and glutenin are composed of many different molecular species. Gliadin proteins consist mostly of single-chain units and have molecular weights near 36,500 (Bietz and Wall 1972). Whole gliadin also contains polypeptides of molecular weight 11,400 that may be albumins, a major polypeptide of molecular weight 44,200, and ω-gliadins of molecular weights 69,300 and 78,100. The polypeptides of 44,200 and 36,500 molecular weight are joined through disulfide bonds into higher molecular weight proteins. Glutenin consists of a series of at least 15 polypeptides with molecular weights ranging from 11,600 to 133,000. These units are bound together by disulfide bonds to form large molecules.

The nongluten albumin and globulin proteins represent from 13 to 35% of the total protein of

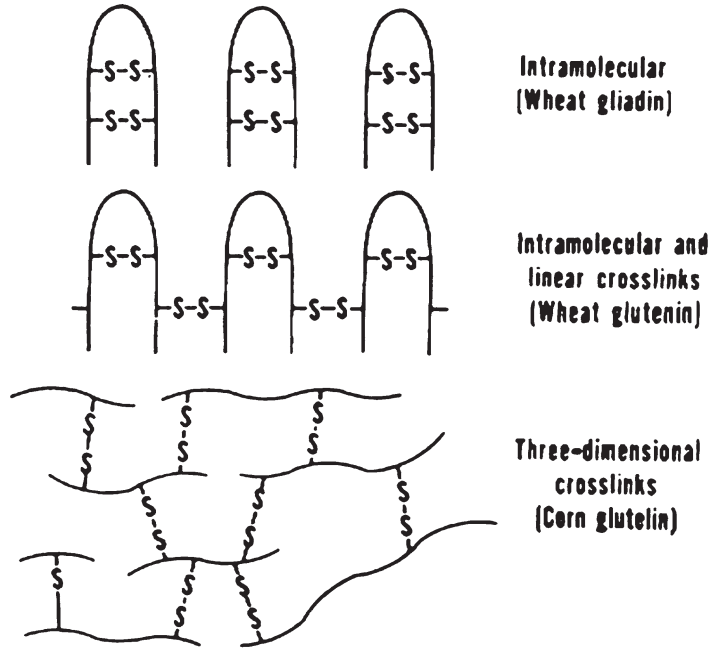
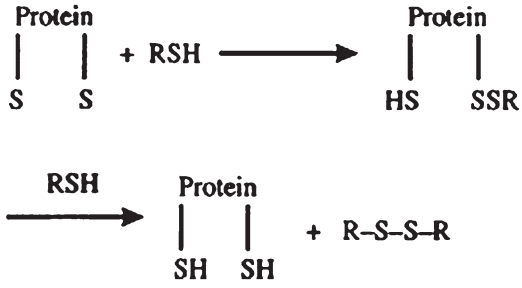


Fig. 3.30 Types of disulfide bonds in cereal proteins. *Source*: From J.S. Wall, Disulfide Bonds: Determination, Location, and Influence on Molecular Properties of Proteins, *Agr. Food Chem*., Vol. 19, pp. 619–625, 1971

cereal flours. This protein fraction contains glycoproteins, nucleoproteins, lipoproteins, and a variety of enzymes.

Chemical modification of gluten proteins plays an important role in the industrial use of cereals. Especially reactions that lead to splitting or formation of an SS bond can greatly influence solubility and rheological properties such as extensibility and elasticity.

An example of the reduction of an SS bond by means of an SH containing reagent is as follows:



The disulfide bonds in wheat gluten play an important role in cross-linking polypeptide chains. Some of the bonds present in cereal proteins are shown in Fig. 3.30 (Wall 1971). Reduction of the disulfide bonds in gliadin and glutenin results in the unfolding of the peptide chains (Krull and Wall 1969), as indicated in Fig. 3.31. This type of change has a profound effect on the rheological properties of dough (Pomeranz 1968).

Maize Proteins

Maize (corn), wheat, and rice are the three main cereal crops of the world. The protein content of maize varies widely depending on variety, climate, and other factors; it is generally in the 9–10% range. The main proteins of maize are the storage proteins of the endosperm, namely zein and glutelin. There are also minor amounts of albumin and globulin. Maize proteins are low in levels of the essential amino acids lysine and tryptophan. To overcome this problem Mertz et al. (1964) developed high-lysine corn, Opaque-2. In this mutant the synthesis of zein, the protein with the lowest lysine content, is suppressed.

The storage proteins of maize can be divided into low molecular weight (zeins) and high molecular weight (glutelins). Zein is the protein group that is soluble in alcohols. The zeins can be

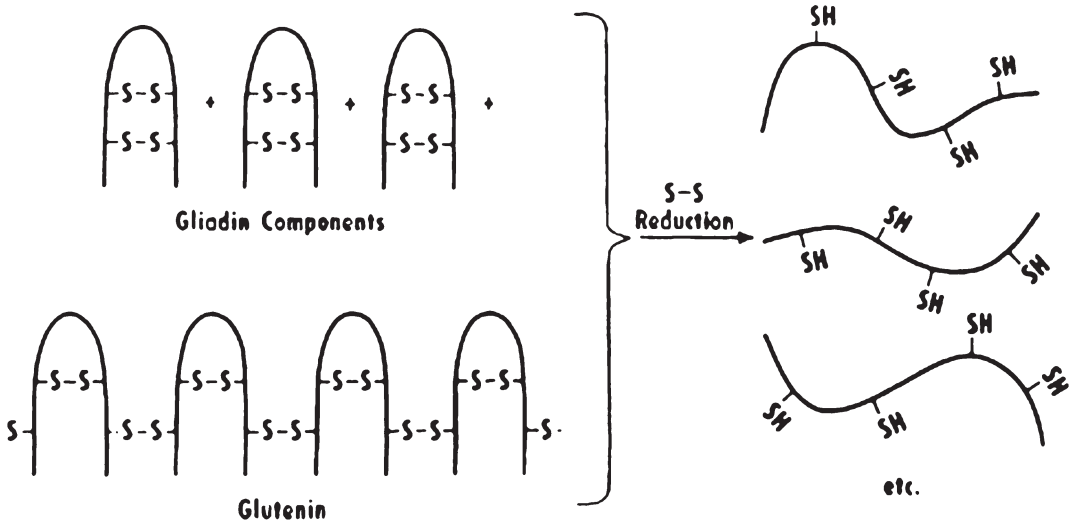


Fig. 3.31 Reduction of disulfide bonds in gliadin and glutenin. *Source*: From L.H. Krull and J.S. Wall, Relationship of Amino Acid Composition and Wheat Protein Properties, *Bakers' Dig*., Vol. 43, No. 4, pp. 30–39, 1969

separated into as many as 30 components, belonging to two major groups with molecular weights of about 22 and 24 kDa (Lasztity 1996). Zeins are asymmetric molecules containing about 45% α-helix and 15% β-sheet; the rest is aperiodic.

The high molecular weight storage proteins are the glutelins. The glutelins are less well-defined than the zeins. They have higher lysine, arginine, histidine, and tryptophan and lower glutamic acid content than the zeins. Glutelin consists of several subunits joined together by disulfide bonds.

The remaining proteins, present in amounts of about 8% each, are albumins and globulins. These proteins are soluble in water and/or salt solutions, and are characterized by higher levels of essential amino acids and lower levels of glutamic acid. Albumins have higher contents of aspartic acid, proline, glycine, and alanine, and lower levels of glutamic acid and arginine than the globulins.

Rice Proteins

Rice and wheat are the two staple cereals for much of the world population. The wheat kernel is almost never eaten as such, but only after extensive processing such as milling and baking. In contrast, rice is eaten mostly as the intact kernel after the bran has been removed. In some parts of the world, however, rice is also consumed in the form of rice noodles. The protein content of polished or white rice is lower than that of wheat. Values reported for protein content of rice range from 6 to 9%. The nutritional value of rice protein is high because of its relatively high content of lysine, the first limiting essential amino acid. Up to 18% of the protein in the rice kernel is lost in the bran and polish. In rice the main storage protein is glutelin, in contrast to wheat, whose main storage protein is gliadin. The approximate protein distribution in rice proteins is as follows: albumin 5%, globulin 10%, prolamin 5%, and glutenin 80% (Lookhart 1991).

The protein in rice is present in the form of encapsulated protein bodies, which are found throughout the endosperm. The protein bodies may be small or large with the former containing primarily glutelin and the latter containing prolamin and glutelin (Hamaker 1994). The protein bodies are insoluble and remain intact during cooking. Rice has little or no intergranular matrix protein. This characteristic is different than in most other cereals, and it may have an effect in the process of noodle making.

Glutelin is the principal protein in the whole grain as well as in milled rice and rice polish. The major proteins in rice bran are albumin and globulin. This indicates that glutelin is concentrated in the milled rice and albumin and globulin are enriched in the bran and rice polish. These byproducts are mainly used as animal feed and would constitute a valuable food source if properly processed.

Soybean Proteins

The proteins in soybeans are contained in protein bodies, or aleurone grains, which measure from 2 to 20 µm in diameter. The protein bodies can be visualized by electron microscopy (Fig. 3.32). Soy protein is a good source of all the essential amino acids except methionine and tryptophan. The high lysine content makes it a good complement to cereal proteins, which are low in lysine. Soybean proteins have neither gliadin nor glutenin, the unique proteins of wheat gluten. As a result, soy flour cannot be incorporated into bread without the use of special additives that improve loaf volume. The soy proteins have a relatively high solubility in water or dilute salt solutions at pH values below or above the isoelectric point. This means they are classified as globulins. There is as yet no generally accepted nomenclature for the soy proteins, and only some of the common terminology is used here. The complex character of the mixture of proteins in soybeans is indicated by the fact that starch gel electrophoresis of acid-precipitated globulins in 5 M urea with alkaline buffer reveals 14 protein bands, and in acid buffer 15 bands appear (Puski and Melnychyn 1968).

Generally, soybean proteins are differentiated on the basis of their behavior in the ultracentrifuge. Water-extractable proteins are separated into four fractions with approximate sedimentation rates of 2, 7, 11, and 15S. The percentage of these fractions is indicated in Table 3.22, along with their components and their molecular

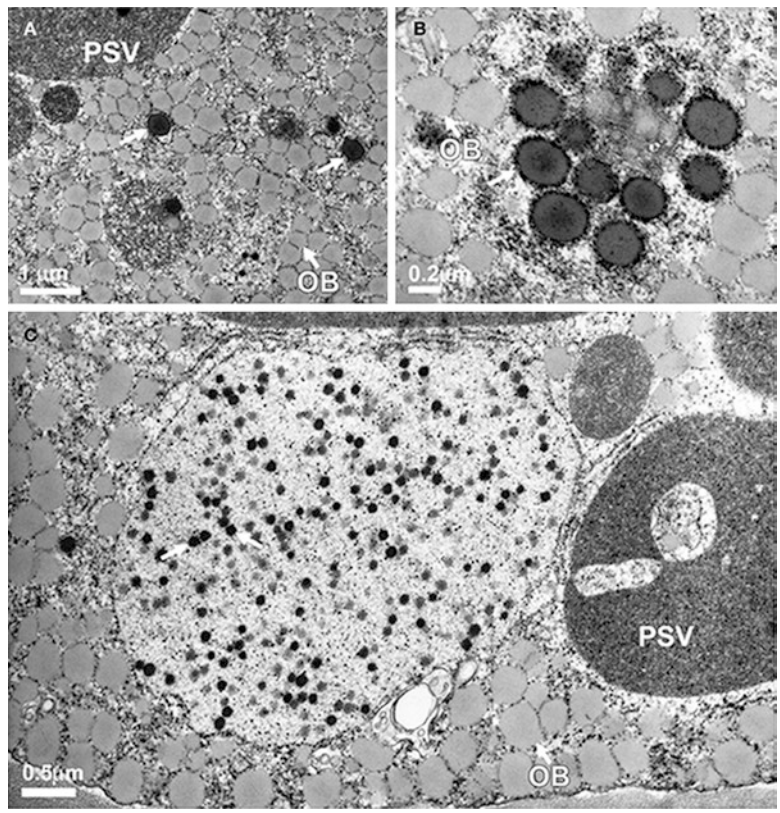


Fig. 3.32 Transmission electron microscopy observation of protein bodies in transgenic soybean seeds. Seeds grown in presence of 0.5 mM magnesium sulfate contain a few endoplasmic reticulum-derived spherical protein bodies (**a**, *arrows*) while seeds grown in presence of 2 mM magnesium sulfate reveal numerous dark staining spherical protein bodies (**b**, **c**). PSV, protein storage vacuole; *OB* oil bodies, *PB* protein body (http://journal.frontiersin.org/article/10.3389/fpls.2014.00633/full)

Table 3.22 Ultracentrifuge fractions of soybean proteins

Protein fraction	Percentage of total	Components	Molecular weight
2S	22	Trypsin inhibitors	8000
			21,500
		Cytochrome c	12,000
		2.3S Globulin	18,200
		2.8S Globulin	32,000
		Allantoinase	50,000
7S	37	Beta-amylase	61,700
		Hemagglutinins	110,000
		Lipoxygenases	108,000
		7S Globulin	186,000–210,000
11S	31	11S Globulin	350,000
15S	11	–	600,000

Source: From W.J. Wolf, What Is Soy Protein, *Food Technol.*, Vol. 26, No. 5, pp. 44–54, 1972b

weights. Several of the ultracentrifuge fractions can be further separated into a number of components. The 2S fraction contains trypsin inhibitors, cytochrome c, allantoinase, and two globulins. The 7S fraction contains β-amylase, hemagglutinin, lipoxygenase, and 7S globulin. The 11S fraction consists mainly of 11S globulin. This compound has been separated by electrophoresis into 18 bands in alkaline gels and 10 bands in acid gels. The 11S protein is usually named glycinin, and there are various proposals for naming other protein fractions conglycinin (Wolf 1969). The detailed subunit structures of 7S and 11S globulins, the most important of the soy proteins, have been described by Wolf (1972b). The 11S globulin has a quaternary structure consisting of 12 subunits. According to Catsimpoolas et al. (1967), these have the following amino-terminal residues: 8 glycine, 2 phenylalanine, and either 2 leucine or 2 isoleucine. It appears that the 11S protein is a dimer of two identical monomers, each consisting of six subunits, three of which are acidic and three basic. Interactions among these subunits may be a factor in stabilizing the molecule. The 7S globulin consists of 9 subunits of single polypeptide chains. The protein is a glycoprotein and the polysaccharide is attached as a single unit to one of the polypeptide chains. The carbohydrate consists of 38 mannose and 12 glucosamine residues.

Current information on soy protein fractionation and nomenclature has been given by Brooks and Moor (1985). The 7S globulins are classified into three major types. Type I is β-conglycinin (B_1–B_6), type II is β-conglycinin (B_0), and type III is γ-conglycinin.

Application of heat to soybeans or defatted soy meal makes the protein progressively more insoluble. Hydrogen bonds and hydrophobicity appear to be responsible for the decrease in solubility of the proteins during heating.

Both 7S and 11S proteins show a complicated pattern of association and dissociation reactions. The 7S globulin at 0.5 ionic strength and pH 7.6 is present as a monomer with molecular weight of 180,000–210,000. At 0.1 ionic strength, the molecule forms the 9S dimer. This reaction is reversible. At pH 2 and low ionic strength, the 7S globulin forms 2S and 5S compounds; these are the result of dissociation into subunits. This reaction is reversed at higher ionic strengths.

Changes in the quaternary structure of the 11S globulin have been summarized by Wolf (1972a). Secondary and tertiary structures of this protein involve no alpha helix structure but instead consist of antiparallel beta-structure and disordered regions. The structure appears to be compact and is stabilized by hydrophobic bonds. The changes in quaternary structure that occur as a function of experimental conditions are represented in Fig. 3.33.

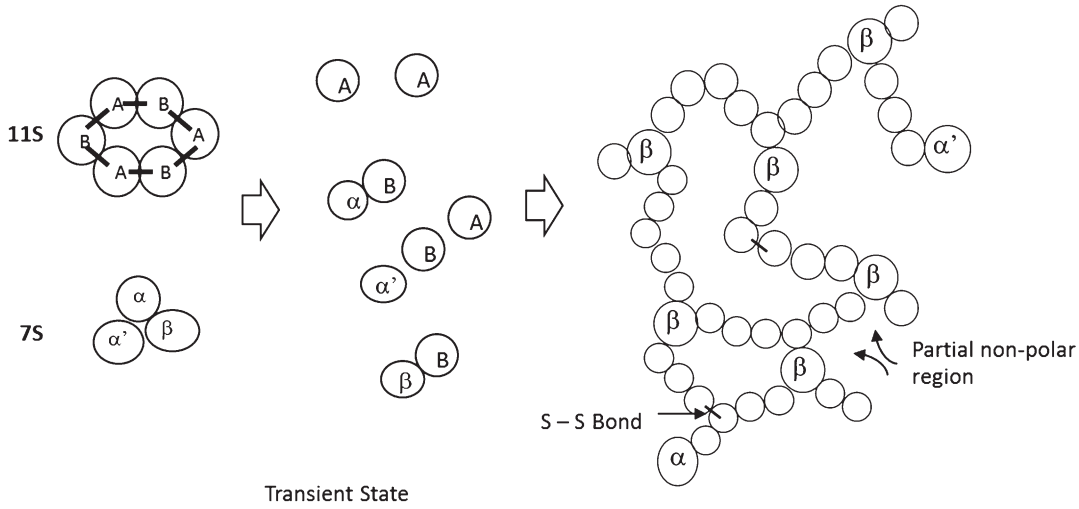


Fig. 3.33 Schematic representation of the heat induced molecular complex between the subunits of 7s and 11s globulins. α, α′, and β refer to the three subunits of 7s globulin and A and B refer to the acidic and basic subunits of 11s globulin. These are generally linked by one intermolecular disulfide (SS) as depicted by the black line linking the A and B subunits in the half 11s molecular shown. The first stage of the reaction is a heat induced dissociation at >80 "C in the presence of thiol (RSH) reagent and the initial electrostatic association of the heat-dissociated 7s subunits with the heat plus RSH dissociated basic (B) subunits of 11s. The dissociated acidic subunits remain as monomers in solution. Further heating results in gradual polymerization to a soluble macromolecular complex composed mostly of β subunits of 7s associated electrostatically with BS of 11s at contact points that are relatively nonpolar. A few a and d subunitn and some disulfide linked basic subunits occur in the complex. Modified from Utsumi et al. (1984)

At ionic strength of 0.1, the protein partially associates into agglomerates with a higher sedimentation velocity. Increasing the ionic strength reverses this reaction. Various conditions promote dissociation of the 11S protein into half-molecules with a sedimentation velocity of 7S. Further breakdown of the half-molecules may occur and will result in the formation of unfolded subunits. It has been suggested (Catsimpoolas 1969) that the 11S molecule is made up of two annular-hexagonal structures, each containing six alternating acidic and basic subunits. This feature would result in a stabilization of the structure by ionic bonds (Utsumi et al. 1984).

Soybean whey proteins are obtained in the solution left after acid precipitation of protein from an aqueous extract. The solution contains an unknown number of albumins and globulins, in addition to water-soluble carbohydrates, nonprotein nitrogen, salts, vitamins, and phytates. In the production of soy protein isolates, the whey proteins may create a disposal problem.

For direct human consumption, soybeans are mainly used as soymilk and tofu or bean curd. In the preparation of soymilk the extractability of proteins is related to the age and storage conditions of soybeans (Thomas and Robertson 1989). Adverse storage conditions result in low protein extraction and poor quality of tofu. Tofu is manufactured from soymilk with 10% of total solids by coagulation of the proteins with the following coagulants: $CaSO_4$, $MgSO_4$, $CaCl_2$, $MgCl_2$, or GDL (glucono delta lactone) (deMan et al. 1986). When using the salts, the Ca and Mg ions are the prime reactants. $CaSO_4$ is only slightly soluble in water; the reaction is slow, but the resulting curd is cohesive and, when pressed, has a soft texture. With soluble salts such as $CaCl_2$ the reaction is fast and the resulting curd is fragmented. When pressed, the tofu has a higher protein content and the texture is firm. Coagulation with GDL is a different process. In freshly prepared tofu utilizing GDL the pH

slowly decreases and coagulation occurs at the isoelectric point of the proteins. The texture of GDL tofu is very homogeneous.

Gluten Sensitivity

Gluten is a mixture of prolamin (gliadin in wheat) and glutelin proteins naturally present in wheat, rye, barley, and related grains, including those wheat varieties known by such names as durum (semolina), spelt, einkorn, emmer, khorasan (*Kamut*), club wheat, triticale, and farro. It is most commonly present in products made from wheat flour and in certain other food products in which it is used as an ingredient, providing elasticity in baked goods, for example, as well as texture, moisture retention, and flavor.

Celiac disease, also referred to as celiac sprue, is a genetic disease that is said to affect about 1% of the people in North America and Europe. The immune system of people with the disease responds to the consumption of gluten by damaging the lining of the small intestine, thus interfering with absorption of nutrients. The disease has no cure but can be managed by avoiding gluten in the diet.

The number of products marketed as gluten-free is increasing worldwide, but even with the establishment of regulations allowing such labeling, it is possible that foods labeled as gluten-free may be contaminated during processing by equipment previously used for gluten-containing foods. Because of the high prevalence of wheat in the food supply, even products that are formulated or processed to not contain it may still contain enough trace amounts of gluten to produce symptoms in gluten-sensitive individuals. Consequently, reliable tests are required for the detection of gluten in foods.

Test Methods

There are three types of methods in gluten testing with the majority of tests for gluten in food products are enzyme-linked immunosorbent assays (ELISAs). Microwell versions of ELISAs provide quantitative results. Lateral-flow devices generally provide qualitative results, indicating the presence of gluten above a threshold level, but in some instances can also provide semi-quantitative results. Other types of tests include polymerase chain reaction (PCR), which detects DNA rather than protein; adenosine triphosphate (ATP) swab tests for assessing cleanliness of equipment surfaces; and general protein swabs, which are not specific to gluten but detect all types of protein and can be used for assessing cleanliness.

The most common form of ELISA for gluten detection is the sandwich format. In sandwich ELISAs the antigen (gluten proteins in this case) binds to anti-gluten antibodies that are affixed to a surface, generally a microwell plate. Then a second gluten-specific antibody—this one linked to an enzyme—is applied over the surface and also binds to any gluten that is now affixed to the surface. Finally, a substance is added that the enzyme can convert into a detectable signal, such as a color change.

Lateral flow tests, also known as immunochromatographic assays, are usually available in dipstick format, in which the test sample flows along a solid substrate by capillary action. When the sample is applied to the strip, it mixes with a colored reagent and moves with the substrate into specific zones on the strip that contain the specific antibodies. When liquid from a sample or wet equipment surface passes over this zone, the gluten will bind to the antibody. Color also forms as a line in this zone on the strip. A control zone is also usually included that will form a color that merely indicates that the strip has worked correctly. Thus, a negative test is the formation of one line while a positive test is the formation of two lines.

Most commercially available kits for routine food allergen analysis rely on immunological methods such as enzyme-linked immunosorbent assay (ELISA) or on polymerase chain reaction (PCR) approaches. ELISA has the advantage of being relatively quick and simple to perform and can be used to detect many known allergens; however, it can only detect one allergen at a time, is susceptible to cross-reactivity, and can lead to the generation of false positive and false negative

results. PCR methods are complementary to ELISA methods, and are used to amplify and detect the DNA of an allergen. PCR approaches are highly specific, sensitive to very low levels of allergen, and can be used to detect more than two allergens at once. However, the PCR is limited because some food processing methods can destroy detectable DNA, causing false negative results. PCR methods are also highly susceptible to food matrix interferences. Liquid chromatography tandem mass spectrometry (LC–MS–MS) is an alternative method for allergen detection that is highly specific, sensitive, and has the ability to directly analyze multiple allergens in a single analysis. LC–MS–MS detection is also not as strongly influenced by food processing, and has the capability for accurate quantitation of the allergens. LC–MS–MS allows direct analysis of multiple allergenic proteins in a single preparation; is more sensitive; and allows more accurate quantitation than traditional approaches.

LC–MS–MS has grown in popularity for allergen testing because it provides quantitative capabilities for multiple allergens simultaneously. Allergenic proteins are extracted from samples and are subsequently digested into peptide fragments that are directly analyzed using their characteristic molecular masses. The analysis of multiple target peptides and their unique masses and fragmentation patterns improves the reliability of allergen detection.

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Carbohydrates

4

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Introduction

Carbohydrates or saccharides (from the Greek word *sakkharon* meaning sugar) occur in plant and animal tissues as well as in microorganisms; as macronutrients they are the human body's preferred energy source, providing fuel for the central nervous system and energy for working muscles. Carbohydrates also serve as (1) a short-term energy source for all organisms, (2) structural molecules in plants, and (3) storage forms of foods in plants and animals. Carbohydrates are technically hydrates of carbon with the empirical formula $C_m(H_2O)_n$ (where m could be different from n), but structurally they are more accurately viewed as polyhydroxy aldehydes and ketones. Carbohydrates can be divided into three chemical groups: monosaccharides, oligosaccharides, and polysaccharides, with the first being small (lower molecular weight) and commonly referred to as simple sugars. Carbohydrates in food can also be classified as simple or complex, with the difference between the two forms being the chemical structure and how quickly they are absorbed and digested. In animal organisms, the main sugar is glucose and the storage carbohydrate is glycogen; in milk, the main sugar is almost exclusively the disaccharide lactose. In plant organisms, a wide variety of monosaccharides and oligosaccharides occur, as well as storage polysaccharides such as starch, and structural polysaccharides such as cellulose and hemicellulose. Gums are a varied group of polysaccharides obtained from plants, seaweeds, and microorganisms. Because of their useful physical properties, the gums have found widespread application in food processing. The main carbohydrates that occur in a number of example food products are listed in Table 4.1.

When consumed as part of the diet, carbohydrates cause a range of physiological effects. Simple sugars are generally absorbed and rapidly utilized for energy or converted to fat for storage. The more complex carbohydrates range from those that are highly digestible, i.e., starch to those that are non-digestible, i.e., celluloses and chitins. Hemi-cellulose and some oligosaccharides are not digestible but are fermented in the colon resulting in production of beneficial short chain fatty acids. In addition to the physiological effect *in vivo* the carbohydrates can serve as bulking agents. In foods the carbohydrates have a wide range of functions. The simple sugars can add sweetness, alter the boiling point and freezing point of foods, and help stabilize proteins in solution. Carbohydrates are generally based on simple sugars and polymers of widely varying chain length. Carbohydrates are generally and broadly classified according to their size or degree of polymerization (DP). This is a rather broad definition and as you will see in this chapter there are many exceptions. We will start with a discussion of the simple sugars and advance through the larger and more complex carbohydrates later in the chapter.

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_4

Table 4.1 Carbohydrates in some example foods and food products

Product	Total sugar (%)	Mono- and disaccharides (%)	Polysaccharides (%)
Fruits			
Apple	14.5	Glucose 1.2; fructose 6.0; sucrose 3.8; mannose trace	Starch 1.5; cellulose 1.0
Grape	17.3	Glucose 5.4; fructose 5.3; sucrose 1.3; mannose 2.2	Cellulose 0.6
Strawberry	8.4	Glucose 2.1; fructose 2.4; sucrose 1.0; mannose 0.1	Cellulose 1.3
Vegetables			
Carrot	9.7	Glucose 0.9; fructose 0.9; sucrose 4.3	Starch 7.8; cellulose 1.0
Onion	8.7	Glucose 2.1; fructose 1.1; sucrose 0.9	Cellulose 0.7
Peanuts	18.6	Sucrose 4–12	Cellulose 2.4
Potato	17.1		Starch 14; cellulose 0.5
Sweet corn	22.1	Sucrose 12–17	Cellulose 0.7
Sweet potato	26.3	Glucose 0.9; sucrose 2–3	Starch 14.7; cellulose 0.7
Turnip	6.6	Glucose 1.5; fructose 1.2; sucrose 0.4	Cellulose 0.9
Others			
Sugar beet juice	18–20	Glucose 0.01; fructose 0.01; sucrose 16–18	
Sugar cane juice	14–28	Glucose + fructose 0.7–1.5; sucrose 11.2–22.4	
Maple syrup	65.5	Sucrose 58.2–65.5; hexoses 0.0–7.9	
Honey	82.3	Glucose 28–35; fructose 34–41; sucrose 1–5	
Meat		Glucose 0.01	Glycogen 0.1
Milk	4.9	Lactose 4.9	

Monosaccharides

The simplest sugars or monomeric units are called monosaccharides, which cannot be hydrolyzed into smaller carbohydrates. Chemically, they are aldehydes or ketones with two or more hydroxyl groups. The general chemical formula of an unmodified monosaccharide is $C_n(H_2O)_n$. Monosaccharides range from three to eight carbon atoms which constitute the backbone of the sugar. The carbon backbone is then appended with Hydrogen (H) or Hydroxy (−OH) groups. Monosaccharides are classified according to three different characteristics: (1) the placement of the carbonyl group, (2) the number of carbon atoms it contains, and (3) chirality. If the carbonyl group is an aldehyde then the sugar is an aldose; if the carbohyl group is a ketone the sugar is a ketose. Monosaccharides with three carbon atoms, i.e., glyceraldehyde (Fig. 4.1) are named trioses, those with four carbons are tetroses, with five carbons pentoses, with six carbons hexoses, and so on. Monosaccharides of 5 or 6 carbons, however, are the most common.

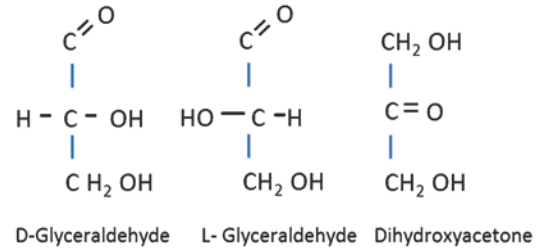


Fig. 4.1 Structures of the simplest carbohydrates: three carbon sugars (trioses) with an aldehyde (aldose) or ketone (ketose) group

Carbohydrates have a chiral center around which the OH group is either to the left (L) or to the right (D). Most natural sugars are members of the D series (see Fig. 4.1). D and L structures are non-superimposable mirror images, named enantiomers. The simplest of the sugars are the three carbon sugars and, unfortunately, their most common nomenclature is different than the larger molecules (4–6 carbons). The three carbon aldose is generally referred to as D or L-glyceraldehyde (2,3-dihydroxypropanal) and the keto version is dihydroxyacetone (dihydroxypropanone) (Fig. 4.1).

The most common sugars in food and nutritional chemistry are pentoses and hexoses. D-glucose is the most important monosaccharide and is derived from the simplest sugar, D-glyceraldehyde, an aldotriose. The designation of aldose and ketose sugars indicates the chemical character of the reducing form of a sugar and can be indicated by the simple or open-chain formula of Fischer, as shown in Fig. 4.2.

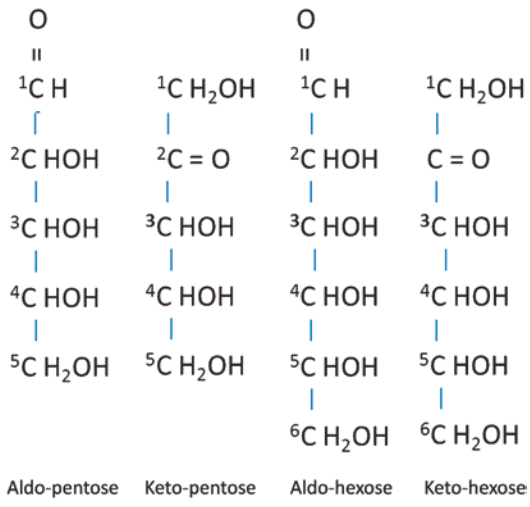


Fig. 4.2 Fischer projection of aldo- and keto structures for pentoses and hexoses

After Fischer formulas were introduced for carbohydrates, Haworth representations were developed to give a more accurate spatial view of the molecule (Fig. 4.3). Carbohydrates are either acyclic or exist as furanosides (*f*) or pyranosides (*p*), as shown in Fig. 4.3. Because the Haworth formula does not account for the actual bond angles, the modern conformational formulas (also seen in Fig. 4.3) more accurately represent the sugar molecule. Pyranose structures exist in chair conformation, with the bulky $-CH_2OH$ group on carbon 5. A number of chair conformations of pyranoside sugars are possible (Shallenberger and Birch 1975), and the two most important ones for glucose are shown in Fig. 4.3. These are named the *CI* D and the *IC* D forms (also described as *O-outside* and *O-inside,* respectively). In the *CI* D form of β-D-glucopyranose, all hydroxyls are in the equatorial position, which represents the highest thermodynamic stability.

Isomers of the same pyranosides are distinguishable from the OH in the C-1 position (also known as anomeric carbon). The two possible anomeric forms of monosaccharides are designated by Greek letter prefix α or β. In the α-anomer the hydroxyl group points to the right, according to the Fischer projection formula; the

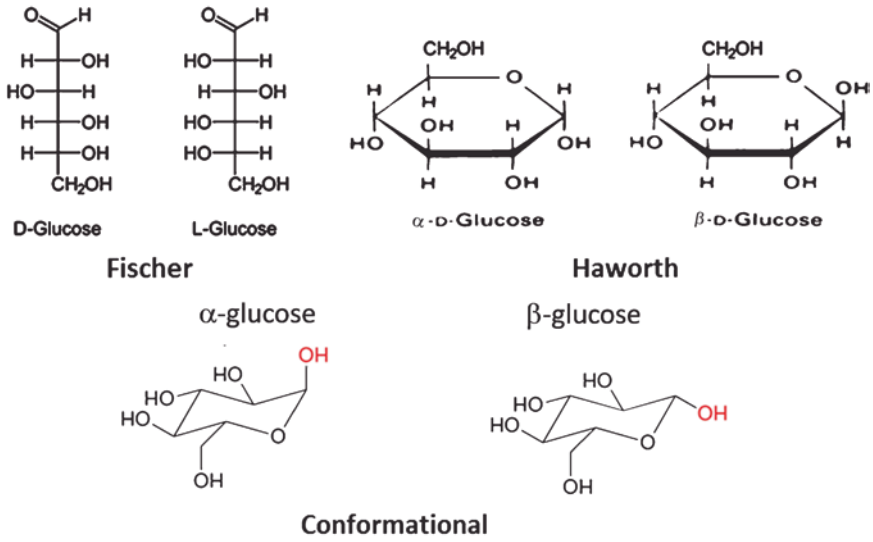


Fig. 4.3 Representation fischer, haworth, and conformational representations of α- and β- D-glucose structures

hydroxyl group points to the left in the β-anomer. In Fig. 4.3 the structure marked *C*l D represents the α-anomer, and 1*C* D represents the β-anomer. The anomeric forms of the sugars are in tautomeric equilibrium in solution; and this causes the change in optical rotation when a sugar is placed in solution. Under normal conditions, it may take several hours or longer before the equilibrium is established and the optical rotation reaches its equilibrium value. At room temperature an aqueous solution of glucose can exist in four tautomeric forms (Angyal 1984): β-furanoside—0.14%, acyclic aldehyde—0.0026%, β-pyranoside—62%, and α-pyranoside—38% (Fig. 4.4). Fructose under the same conditions also exists in four tautomeric forms as follows: α-pyranoside—trace, β-pyrano-side—75%, α-furanoside—4%, and β-furanoside—21% (Fig. 4.5) (Angyal 1976).

When the monosaccharides become involved in condensation reactions to produce di-, oligo-, and polysaccharides, the conformation of the bond on the carbon 1 becomes fixed and the different compounds have either an all-α or all-β structure at this position.

Naturally occurring sugars are mostly hexoses, but sugars with different numbers of carbons are also present in many food products. There are also sugars with different functional groups or substituents, creating diverse compounds, including aldoses, ketoses, amino sugars, deoxy sugars, sugar acids, sugar alcohols, acetylated or methylated sugars, anhydro-sugars, oligo- and polysaccharides, and glycosides. Fructose is the most widely occurring ketose and is shown in its various representations in Figs. 4.5 and 4.6. It is the sweetest known natural sugar, and occurs bound to glucose in sucrose or common table sugar. Of all the other possible hexoses, only two occur widely—D-mannose and D-galactose. Their formulas and relationship to D-glucose are given in Fig. 4.7.

Anomers are also sterioisomers and diastereomers that differ in configuration around the anomeric carbon atom as shown in Fig. 4.7. As we

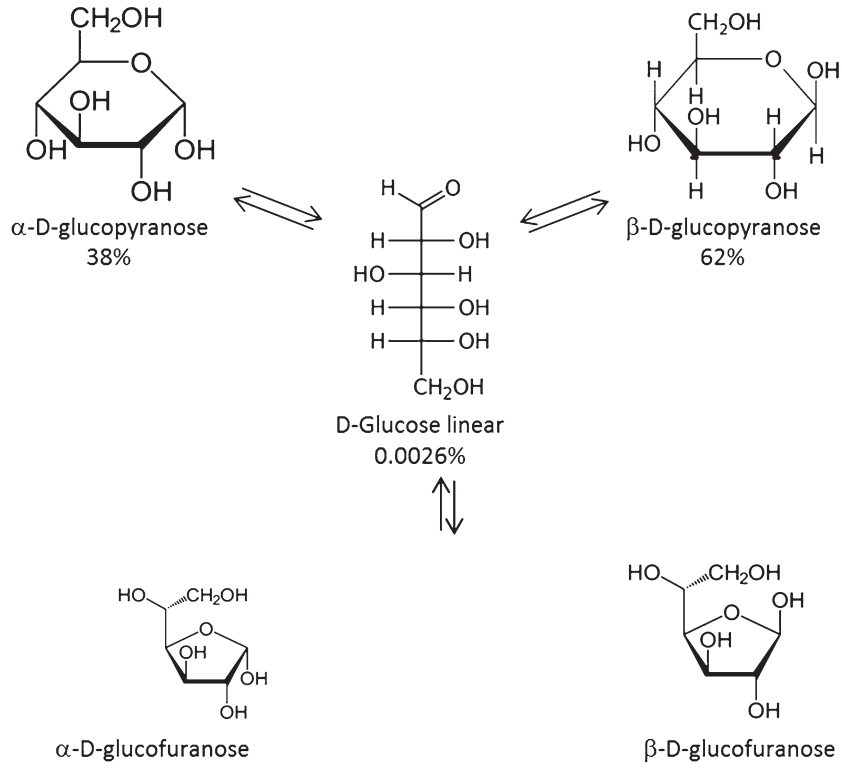


Fig. 4.4 Tautomeric forms of glucose in aqueous solution at room temperature

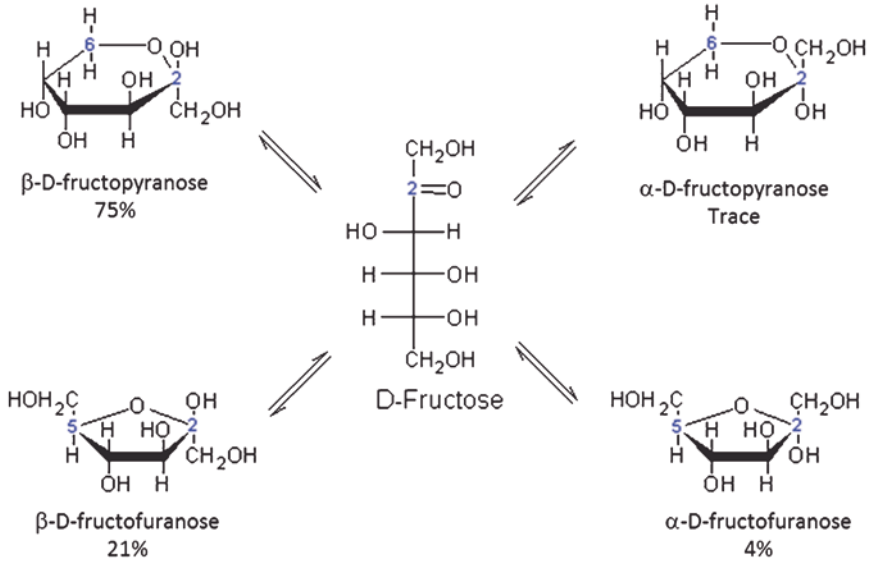


Fig. 4.5 Tautomeric forms of fructose in aqueous solution at room temperature

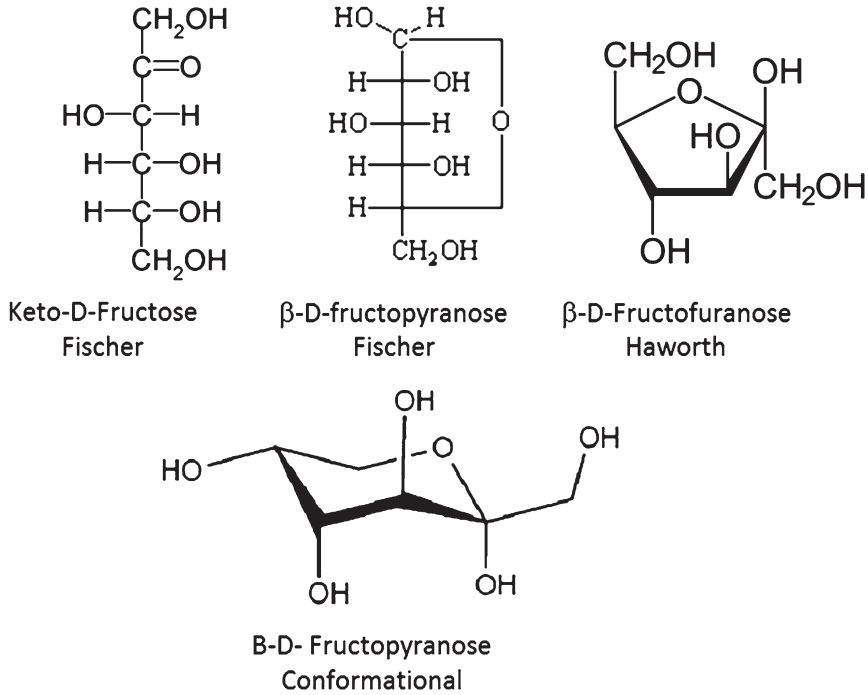


Fig. 4.6 Methods of representation of D-fructose. *Source*: From M.L. Wolfrom, Physical and Chemical Structures of Carbohydrates, in *Symposium on Foods: Carbohydrates and Their Roles*, H.W. Shultz, R.F. Cain, and R.W. Wrolstad, eds., 1969, AVI Publishing Co.

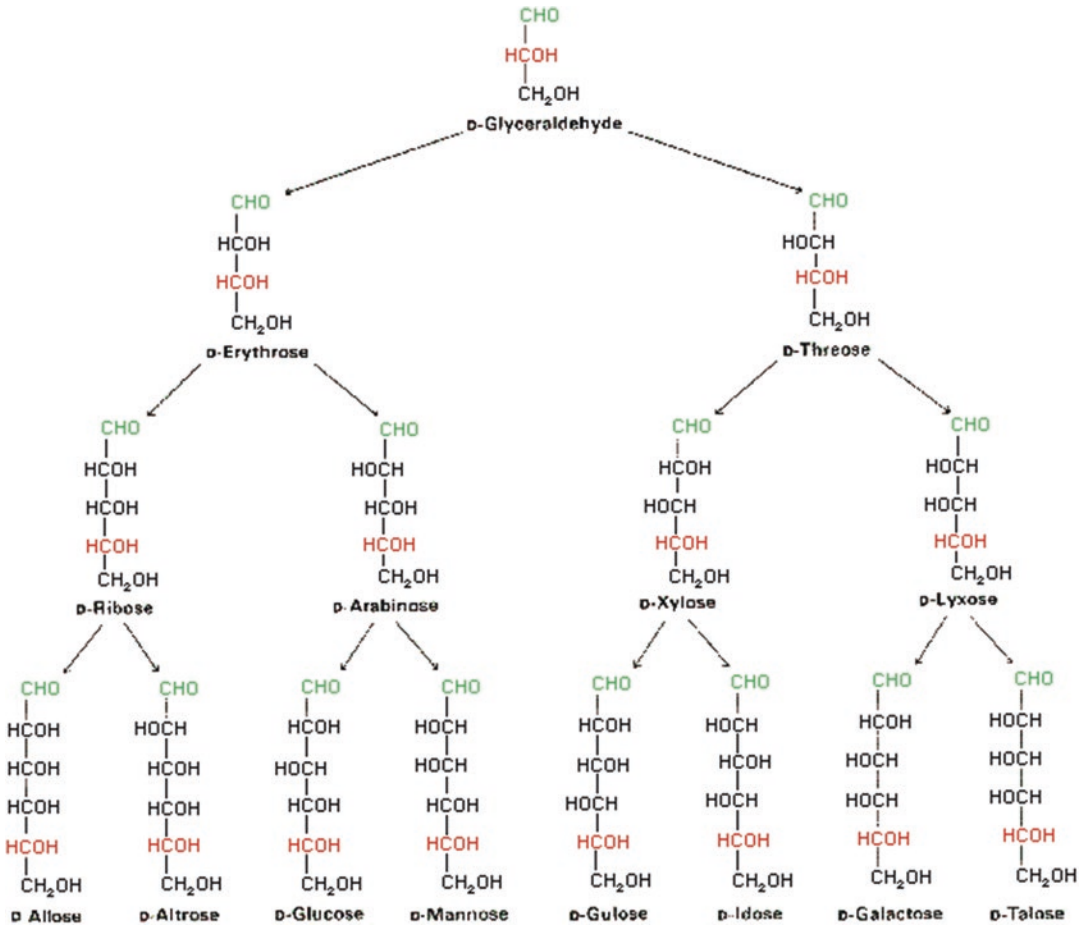


Fig. 4.7 Relationship of D-aldehyde sugars. *Source:* From M.L. Wolfrom, Physical and Chemical Structures of Carbohydrates, in *Symposium on Foods: Carbohydrates and Their Roles,* H.W. Schultz, R.F. Cain, and R.W. Wrolstad, eds., 1969, AVI Publishing Co.

will see later, the anomeric forms result in very different properties of polymers, such as starch and cellulose, which are both glucose polymers.

When sugars are in solution and only two tautomers are formed it is considered a simple mutarotation, whereas with three or more it is considered complex. The furanose and pyranose rings are generated from the straight chain intermediate of the sugar. Sugars with the gluco, manno, gulo, and allo configurations exhibit simple mutarotation. For example, when glucose is in solution (Fig. 4.8), only α-D-flucopyranose and β−D-glucopyranose are present in solution. The aldehyde content in the solution is estimated to be 0.003%. Maltose and lactose exhibit approximately the same α- and β- anomer distribution as glucose.

The thermal effects in food are very important in food chemistry, and temperature impacts the relative sweetness of sugars in solution. In Fig. 4.9, the sweetness of four simple sugars are compared relative to sucrose. It is clear that fructose is much sweeter than sucrose at low temperatures but not as sweet in hot solutions. This is because at low temperatures there of the intensely sweet β-D-fructopyranose present. At higher temperatures there are more open ring structures which are not as sweet. It, therefore, has a greater effect in a cold beverage than a hot beverage such as coffee or tea.

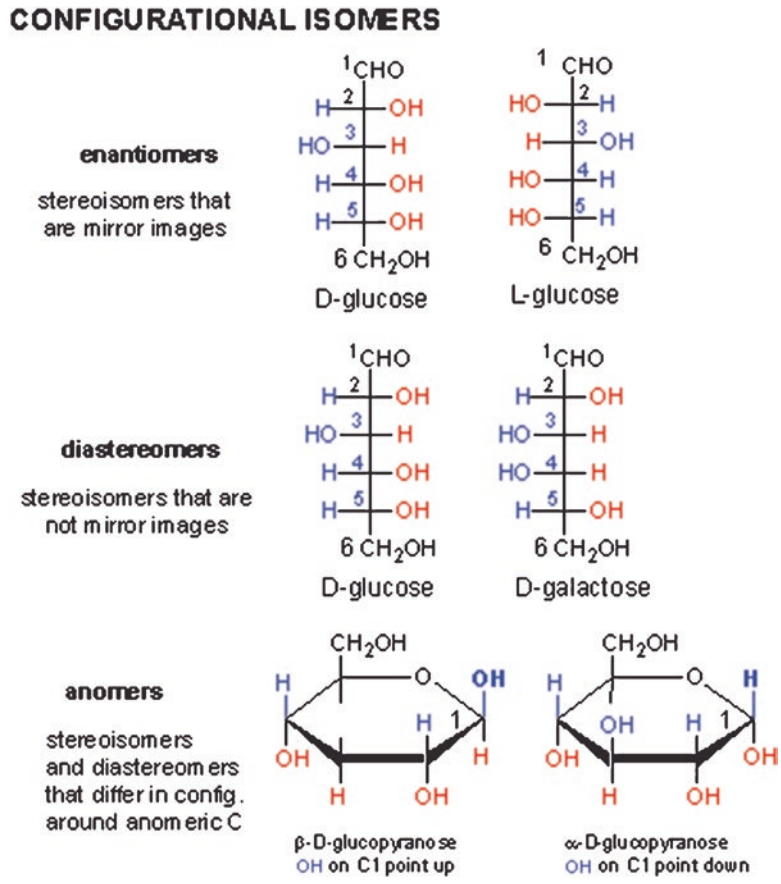


Fig. 4.8 Conformational isomers of glucose

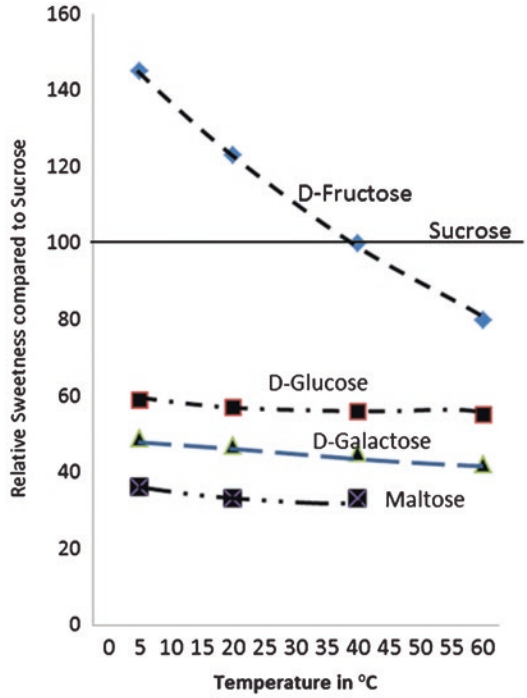


Fig. 4.9 Effect of temperature on the relative sweetness of sugars. Modified form Shallenberger and Birch (1975)

Related Compounds to Monosaccharides

Amino Sugars

Amino sugars usually contain D-glucosamine (2-deoxy-2-amino glucose). They occur as components of high molecular weight compounds such as the chitin of crustaceans and mollusks, insects, as well as in certain mushrooms and in combination with the ovomucin of egg white. Chitin can be hydrolyzed to produce chitosan which has potential as a food ingredient, and this is discussed later in this chapter. After cellulose, chitin is the second most important natural polymer in the world. Chitin is a largely inert polymer

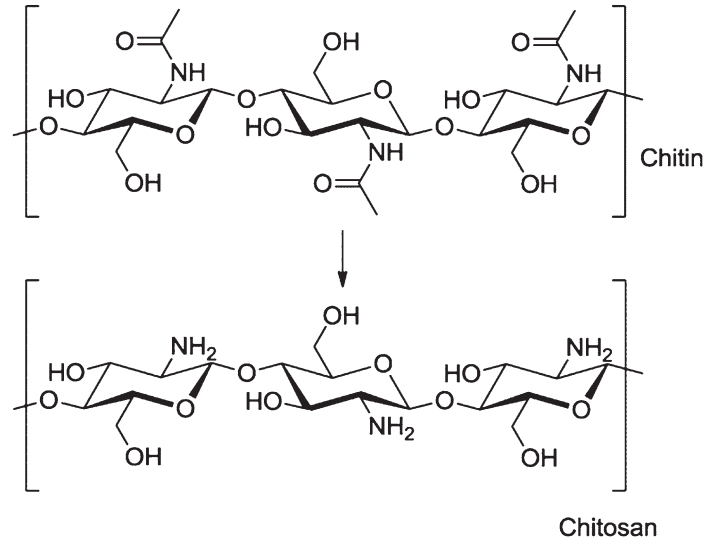


Fig. 4.10 Hydrolysis of chitin removes acetate groups yielding chitosan

composed of long chains of acetylglucosamine. Upon hydrolysis the acetates are removed from the amino group leaving a glucosamine unit. The long chain polymers are also reduced to various chain lengths of glucosamine polymer. The main sources exploited are two marine crustaceans, shrimp and crabs. Chitin can be hydrolyzed with acid or enzymatically to remove the acetate from the amino sugar leaving a free amino group in chitosan as illustrated in Fig. 4.10. Chitosan, which is soluble in acidic aqueous media, is used in many applications (food, cosmetics, biomedical, and pharmaceutical applications).

Glycosides

Glycosides are sugars in which the hydrogen of an anomeric hydroxy group has been replaced by an alkyl or aryl group to form a mixed acetal. Glycosides are hydrolyzed by acid or enzymes but are stable to alkali. Formation of the full acetal means that glycosides have no reducing power. Hydrolysis of glycosides yields sugar and the aglycone. Genistin is an isoflavone found in a number of dietary plants including soy. When genistin is hydrolyzed with either hydrochloric acid or enzymatically in the gastrointestinal tract, 1 mole each of genistein and glucose are produced as shown in Fig. 4.11. Chemically it is the 7-O-beta-D-glucoside form of genistein and is the predominant form of the isoflavone naturally occurring in plants. The aglycone, genesteine is the bioactive form that includes antiatherosclerotic, estrogenic and anticancer.

Sugar Alcohols

These are compounds obtained when aldo- and keto- groups of a sugar are reduced to hydroxyl groups. Thus, since sugars are polyhydroxy compounds, their corresponding sugar alcohols merely have one more alcohol group. Sugar alcohols are also referred to as polyols, polyalcohols, or polyhydric alcohols. (The term "polyol" could properly cover a much larger group containing any compound with three or more hydroxy groups, but common usage normally restricts the term to those compounds closely related to sugars and sugar derivatives.) Some sugar alcohols, particularly pentitols and hexitols, are widely distributed in many fruits and vegetables (Washüttl et al. 1973), while others are produced industrially from the corresponding sugar by catalytic hydrogenation as food ingredients (Table 4.2).

Chemically, physically, and biologically the sugar alcohols resemble sugars to the extent that some are even sweet to the taste and some are used as food sweeteners (see Table 4.3),

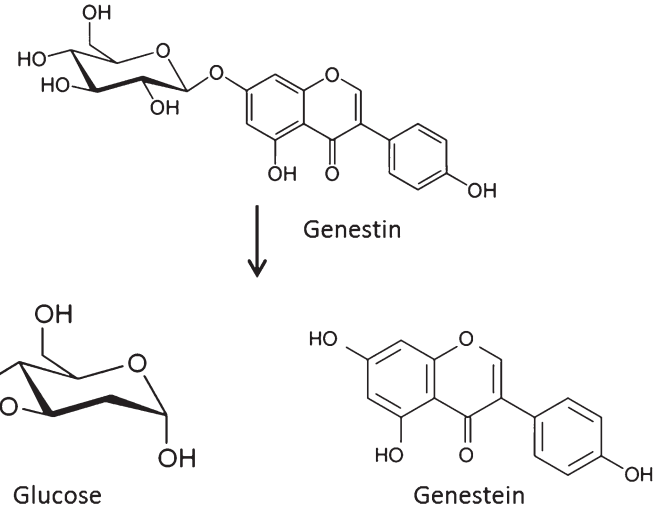


Fig. 4.11 Hydrolysis of genestin to yield genestein and glucose

Table 4.2 Occurrence of sugar-alcohols in some fruits and vegetables (expressed as mg/100 g of dry food)

Product	Arabinitol	Xylitol	Mannitol	Sorbitol	Galactitol
Bananas	–	21	–	–	–
Pears	–	–	–	4600	–
Raspberries	–	268	–	–	–
Strawberries	–	362	–	–	–
Peaches	–	–	–	960	–
Celery	–	–	4050	–	–
Cauliflower	–	300	–	–	–
White mushrooms	340	128	476	–	48

Source: From J. Washüttl, P. Reiderer, and E. Bancher, A Qualitative and Quantitative Study of Sugar-Alcohols in Several Foods: A Research Role, *J. Food Sci.,* Vol. 38, pp. 1262–1263,1973

Table 4.3 The caloric values, sweetness intensity and application of some key sugar alcohols and common carbohydrate sweeteners

Type	Calories per gram	Relative sweetness (sucrose = 100%)	Typical food applications
Sucrose	4	100	Common beverage and food sweetener
Crystalline fructose	3	180	Occurs naturally in fruits and vegetables. Common beverage and food sweetener
Fructose (5–15% solution)		115–125	Common beverage and food sweetener
High fructose corn syrup (HFCS)	~2.8	100–130	Common beverage and food sweetener; market share has decreased since 1999 due to consumer perception problems
Glucose (8–10% solution)		60–70	Common beverage and food sweetener
Invert syrup	3	105	Common beverage sweetener
Sorbitol	2.6	50–70%	Sugar-free candies, chewing gums, frozen desserts and baked goods

(continued)

Table 4.3 (continued)

Type	Calories per gram	Relative sweetness (sucrose = 100%)	Typical food applications
Xylitol	2.4	100%[a]	Chewing gum, gum drops and hard candy, pharmaceuticals and oral health products, such as throat lozenges, cough syrups, children's chewable multivitamins, toothpastes and mouthwashes; used in foods for special dietary purposes
Maltitol	2.1	68–75%	Hard candies, chewing gum, chocolates, baked goods and ice cream
Isomalt	2.0	45–65%	Candies, toffee, lollipops, fudge, wafers, cough drops, throat lozenges
Lactitol	2.0	30–40%	Chocolate, some baked goods (cookies and cakes), hard and soft candy and frozen dairy desserts
Mannitol	1.6	40–70%	Dusting powder for chewing gum, ingredient in chocolate-flavored coating agents for ice cream and confections
Erythritol	0–0.2[b]	60–80%	Bulk sweetener in low calorie foods
Hydrogenated Starch Hydrolysates (HSH)	3	25–50%	Bulk sweetener in low calorie foods, provide sweetness, texture and bulk to a variety of sugarless products

[a]Depends *on solution concentration*
[b]FDA accepts 0.2 kcal/g, but some other countries, such as Japan and the European Union, accept 0 kcal/g
American Diabetes Association. Nutrition principles and recommendations in diabetes-Position Statement. Diabetes Care, Jan.2004. Eggleston et al. (2017)

representing one type of reduced-calorie sweetener. Furthermore, sugar alcohols provide fewer calories than sugar and have less of an effect on blood glucose (blood sugar) than other carbohydrates. Examples of sugar alcohols are: glycerol, erythritol, isomalt, lactitol, mannitol, sorbitol, and xylitol. They can be made by reduction of free sugars with sodium amalgam, sodium borohydride in water, or by catalytic hydrogenation. The sugar alcohols are poorly absorbed but are fermented in the lower gastrointestinal tract which can result in discomfort. For this reason, the daily dose needs to be limited. Reduction of glucose yields glucitol (Fig. 4.12; the trivial name is sorbitol). Another commercially produced sugar alcohol is xylitol, a five-carbon compound, which is also used in diabetic foods (Fig. 4.12).

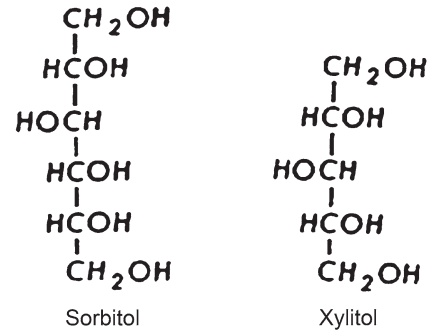


Fig. 4.12 Structures of sorbitol and xylitol

Sorbitol, the most widely distributed natural sugar alcohol, is found in many fruits such as plums, berries, cherries, apples, and pears (Table 4.2). It is also a component of fruit juices, fruit wines, and other fruit products. Sorbitol is commercially produced by catalytic hydrogenation of D-glucose. Mannitol, the reduced form of D-mannose, is found as a component of mushrooms, celery, and olives. Xylitol is obtained from saccharification of xylan-containing plant materials; it is a pentitol, being the reduced form of xylose. Sorbitol, mannitol, and xylitol are monosaccharide-derived sugar alcohols with properties that make them valuable for specific applications in foods: they are suitable for diabetics, they are noncariogenic, they possess reduced physiological

caloric value, and they are useful as sweeteners that are non-fermentable by yeasts. Sugar alcohols have also gained commercial viability as sweeteners because they are less expensive than sucrose and corn-based sweeteners, particularly high fructose corn syrup (Eggleston et al. 2017). Furthermore, sugar alcohols are not considered sugars for food labeling purposes, so foods containing them as sweeteners can be named "sugar free" or "no sugar added" (Eggleston et al. 2017). Most, sugar alcohols, however, are less sweet than sucrose. Some sugar alcohols give a cooling sensation on the tongue. Table 4.3 lists the relative sweetness of sugar alcohols and commercial sugar sweeteners.

Oligosaccharides

When the number of monosaccharides in a glycosidic chain is between 2 to 10, the resulting compound is an oligosaccharide. Oligosaccharides can be homologous or heterologous, and occur widely in plants, but can also be produced synthetically or via microbial fermentative and enzymatic processes. Plant oligosaccharides are often grouped into two distinct classes: (1) primary or (2) secondary oligosaccharides (Kandler and Hopf 1980). Primary oligosaccharides are those synthesized *in vivo* from a mono- or oligosaccharide and a glycosyl donor by the action of a glycosyl transferase enzyme (Kandler and Hopf 1980; Eggleston and Côté 2003). Sucrose is the most common primary oligosaccharide in plants. In comparison, secondary oligosaccharides are those formed *in vivo* or *in vitro* by hydrolysis of larger oligosaccharides, polysaccharides, glycoproteins, or glycolipids. Common oligosaccharides occurring in foods are listed in Table 4.4.

Disaccharides

Two chemically bonded monosaccharides are known as disaccharides. The most common disaccharides in food are sucrose (α-D-glucopyranosyl β-D-fructofuranoside; table sugar is sucrose extracted and refined from either sugarcane or sugar beets), lactose (4-O-β-D-galactopyranosyl-D-glucopyranose; milk sugar), and maltose (4-O-α-D-glucopyranosyl-D-glucopyranose; from starch). Disaccharides can be classified into two types: reducing and non-reducing. Disaccharides are formed by a glycosidic link between the reducing groups on one saccharide with the hydroxyl group of another saccharide. There are multiple forms possible even with a simple glucose-glucose disaccharide. Structures of the most abundant disaccharides are illustrated in Fig. 4.13.

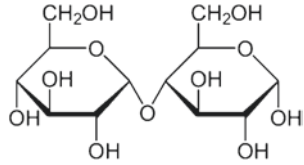
Sucrose

Sucrose, a major sweetener, is a primary oligosaccharide (or disaccharide) found widely in plants, however, the largest commercial sources are from sugarcane or sugar beet. In sucrose the reducing groups of the constituent monosaccharides (glucose and fructose) are linked by a glycosidic bond (Fig. 4.13), thus sucrose is one of the few non-reducing disaccharides. As a non-reducing disaccharide, sucrose does not reduce Fehling solution or form osazones and it does not undergo mutarotation in solution. Because of the unique carbonyl-to-carbonyl linkage, sucrose is highly labile in acid medium, and acid hydrolysis is more rapid than other di- and oligosaccharides. When sucrose is heated to 210 °C, partial decomposition takes place and caramel is formed. Sucrose is highly soluble over a wide temperature range, as is illustrated in Fig. 4.14. This property makes sucrose an excellent ingredient for syrups and other sugar-containing foods.

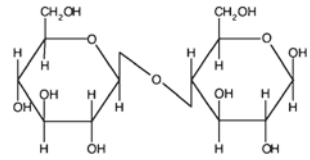
Table 4.4 Common oligosaccharides occurring in foods

Sucrose	(α-D-glucopyranosyl β-D-fructofuranoside)
α,α-Trehalose	(α-D-glucopyranosyl-α-D-glycopyranoside)
Raffinose	[O-α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside]
Stachyose	[O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside]
Verbascose	[O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside]

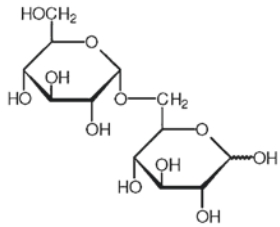
Source: From R.S. Shallenberger and G.G. Birch, *Sugar Chemistry,* 1975, AVI Publishing Co.



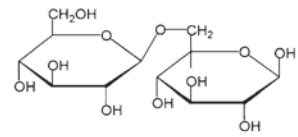
Maltose
α-D-glucopyranosyl-(1-4)-α-D-glucopyranose



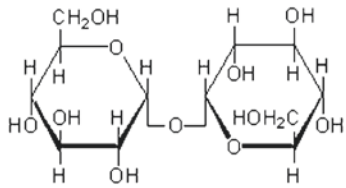
Cellobiose
β-D-glucopyranosyl-(1-4)-α-D-glucopyranose



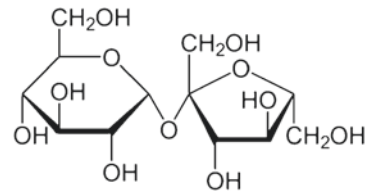
Isomaltose
α-D-glucopyranosyl-(1-6)-β-D-glucopyranose



Gentobiose
β-D-glucopyranosyl-(1-6)-β-D-glucopyranose



Trehalose
α-D-glucopyranosyl-(1-1)-α-D-glucopyranose



Sucrose
β-D-Fructofuranosyl α-D-glucopyranoside

Fig. 4.13 Common disaccharides found in foods

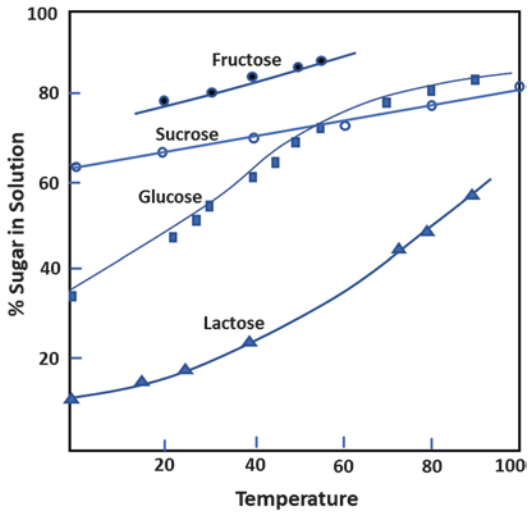


Fig. 4.14 Approximate solubility of some sugars at different temperatures. *Source:* From R.S. Shallenberger and G.G. Birch, *Sugar Chemistry,* 1975, AVI Publishing Co.

Lactose

The sugar in mammalian milk is lactose, which is normally easily digested and converted to energy. Some individuals, however, lack the enzyme lactase, which hydrolyzes lactose and are, therefore, "lactose intolerant." In such individuals the lactose is fermented in the lower gastrointestinal tract causing discomfort and diarrhea. Lactose from cow's milk is less sweet than sucrose and, unlike sucrose, is a reducing sugar. With a few minor exceptions, lactose is the only sugar in the milk of all mammalian species and does not occur elsewhere. Lactose is also a major constituent of the dry matter of cow's milk, as it represents close to 50% of the total solids. The lactose content of cow's milk ranges from 4.4 to 5.2%, with an average of 4.8% expressed as anhydrous lactose. In comparison, the lactose content of human milk is higher, about 7.0%. Lactose is a

disaccharide of D-galactose and D-glucose and is designated as 4-*O*-β-D-galactopyranosyl-D-glucopyranose (Fig. 4.13). It is hydrolyzed by the enzyme β-D-galactosidase (lactase) or by dilute solutions of strong acids. On the other hand, organic acids such as citric acid, which easily hydrolyze sucrose, are unable to hydrolyze lactose. This difference is the basis of the determination of the two sugars in mixtures.

Maltose

Maltose (4-α-D-glucopyranosyl-β-D-glucopyranose) represents an important disaccharide found widely in plants, and is the basic building block of starch and glycogen polysaccharides (Fig. 4.13). When these polysaccharides are hydrolyzed, the primary degradation product is maltose disaccharide. For example, in brewing, starch is hydrolyzed by amylases to maltose (maltose has a characteristic flavor of malt) which is then available for hydrolysis to glucose by glucoamylase, with subsequent conversion to ethanol by yeast. The α-1 → 4 linkage is broken by amylases and maltases. Maltose is also readily broken down in animal gastrointestinal tract and thus provides 4 Kcal energy/g. Maltose is a reducing disaccharide, shows mutarotation, is fermentable, and is easily soluble in water.

Cellobiose

Cellobiose (4-β-D-glucopyranosyl-β-D-glucopyranose) is a reducing disaccharide resulting from the partial hydrolysis of cellulose polysaccharide because it is a building block for cellulose (Fig. 4.13). In cellulose and cellobiose the β-1 → 4 linkage is not hydrolyzed by animal and most microbial enzymes, thus making cellulose more stable than starch in the environment. The cellobiose, its oligomers and cellulose polymers can generally be thought of as non-digestible by animals and provide no caloric value. The polymeric structure is important for plant cell wall structure, and fibers such as cotton.

Other Glucose Disaccharides

Other glucose disaccharides are found at relative low concentrations in nature. Isomaltose is a breakdown product during production of glucose from starch and dextran polysaccharides, and is thus a secondary oligosaccharide; it can also be found in honey. Gentiobiose occurs as a glycoside in amygdalin which is found various stone fruits. Trehalose is found in yeast.

Sugars are important in the texture and appearance of foods, particularly confections, cakes and cookies. The equilibrium between dissolved and crystalline sugars in the cream centers of confections help control the texture. In cookies, sugar can exist in crystalline, glass, and dissolved forms. In the crystalline form most sugars are single anhydrous anomers. When crystallized at temperatures below 50 °C, glucose crystalizes as an α-pyranose monohydrate. D-Lactose crystallizes as α-D-lactose which is soluble in water (up to 20 g/100 mL water), but can be slow to dissolve. In evaporated milk α-D-lactose crystals occasionally cause a gritty defect, whereas, the texture of sweetened condensed milk is dependent on the α-D-lactose crystals.

Sugars in general are very soluble in water and are often formulated as sugar syrups. These highly concentrated syrups are produced by boiling or vacuum evaporation. Syrups of reducing sugars such as corn syrup (mostly glucose) are particularly resistant to crystallization. The worldwide sugar industry takes advantage of the crystallization of sucrose from concentrated solutions to produce high purity crystals. Impure solutions are also resistant to crystallization. Hard candy is produced by boiling an aqueous mixture of sucrose and glucose with flavors and colors to produce a glass which is a super-cooled liquid. In cakes, the glucose syrup functions to retain moistness in the crumb and molding characteristics of fondants.

Disaccharide Sugar Alcohols

In recent years, disaccharide sugar alcohols have also become important food ingredients. The most common disaccharide alcohols include isomalt, maltitol, lactitol, and hydrogenated starch hydrolyzates (HSH). Maltitol is hydrogenated maltose, and its structure is shown in Fig. 4.15. It has the highest sweetness of the disaccharide polyols compared to sugar (Table 4.3) (Heume and Rapaille 1996). It also has a low negative

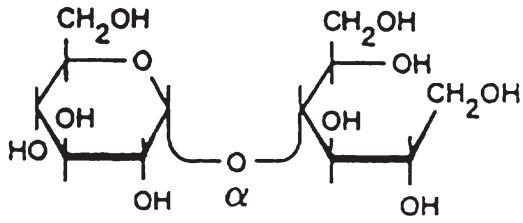


Fig. 4.15 Structure of maltitol

heat of solution and, therefore, gives no cooling effect in contrast to sorbitol and xylitol. Maltitol (Fig. 4.15) exhibits a very high viscosity in solution. Sorbitol and maltitol are derived from starch, whereas lactitol is a disaccharide alcohol, 1 → 4-galactosyl-glucitol, produced by hydrogenation of lactose. It has low sweetness and a lower energy value than other polyols. It has a calorie value of 2 kcal/g and is non-cariogenic (Blankers 1995). It can be used in combination with intense sweeteners like aspartame or acesulfame-K to produce sweetening power similar to sucrose. These combinations provide a milky, sweet taste that allows good perception of other flavors. Isomalt, also known as hydrogenated isomaltulose or hydrogenated palatinose, is manufactured in a two-step process: (1) the enzymatic transglycosylation of the nonreducing sucrose to the reducing sugar isomaltulose; and (2) hydrogenation, which produces isomalt—an equimolar mixture of D-glucopyranosyl-α-(l → l)-D-mannitol and D-glucopyranosyl-α-(l → 6)-D-sorbitol. Isomalt is extremely stable and has a pure, sweet taste. Because it is only half as sweet as sucrose, it can be used as a versatile bulk sweetener (Ziesenitz 1996).

Both monosaccharides and disaccharides contribute flavor and texture in fruits. Vegetables also contribute both sugars and fiber. The sugar in fruit and fruit juices is the major source of calories, and fruits are also good sources of dietary fiber. Cereal based products contribute primarily starch and sugar. Table 4.5 provides examples of sugar, starch, and fiber from a range of plant-based foods. It is important to compare the sugars and starch which provide 4 kcal/g when selecting plant based foods. The dietary fiber which can deliver from 0 to 2 kcal/g provides important bulk in the lower gastrointestinal tract.

Legumes contain several primary oligosaccharides, including raffinose and stachyose, which are based on sucrose (Fig. 4.16). These oligosaccharides are poorly absorbed when ingested, which results in their fermentation in the large intestine. This leads to gas production and flatulence, which present a barrier to wider food use of such legumes. deMan et al. (1975, 1987) analyzed a large number of soybean varieties and found an average content of 1.21% stachyose, 0.38% raffinose, 3.47% sucrose, and very small amounts of melibose. In soy milk, total reducing sugars after inversion amounted to 11.1% calculated on dry basis.

Cow's milk contains traces of oligosaccharides other than lactose. They are made up of two, three, or four units of lactose, glucose, galactose, neuraminic acid, mannose, and acetyl glucosamine. Human milk contains about 1 g/L of these oligosaccharides, which are referred to as the bifidus factor. The oligosaccharides have a beneficial effect on the intestinal flora of infants.

Fructooligosaccharides (FOSs) or "fructans" are also primary oligosaccharides based on sucrose (Fig. 4.17) where an additional one, two, or three fructose units have been added by a β-(2 → l)-glucosidic linkage to the fructose unit of sucrose (Fig. 4.17). The FOSs occur naturally as components of edible plants including banana, tomato, and onion (Spiegel et al. 1994). FOSs are also manufactured commercially by the action of a fungal enzyme from *Aspergillus niger,* β-fructofuranosidase, on sucrose. The three possible FOSs are lF-(l-β-fructofuranosyl)$_{n-1}$ sucrose oligomers with abbreviated and common names as follows: GF_2 (1-kestose), GF_3 (nystose), and GF_4 (1F-β-fructofuranosylnystose). The commercially manufactured product is a mixture of all three FOSs with sucrose, glucose, and fructose. FOSs can also be secondary oligosaccharides if manufactured from the enzymatic breakdown of fructan polysaccharides such as inulin extracted from chicory roots. FOSs are non-digestible by humans. Such prebiotic non-digestible food ingredients beneficially affect the host animal by selectively stimulating the growth of bacteria in the colon which is advantageous to the host (Eggleston and Côté 2003). They do this by serving as selective substrates for probiotic

Table 4.5 Sugars, starches and total fiber in plant based foods (from USDA Nutrient data base)

Food Description	Glucose (g)	Fructose (g)	Sucrose (g)	Total Sugars	Starch	Total Fiber
Apples, raw, red delicious, with skin	2.71	5.9	1.86	10.48	0.05	2.3
Apricots, raw	2.37	0.94	5.87	9.24	–	2
Bananas, raw	4.98	4.85	2.39	12.23	5.38	2.6
Blueberries, raw	4.88	4.97	0.11	9.96	0.03	2.4
Dates, medjool	33.68	31.95	0.53	66.47	–	6.7
Kiwifruit, green, raw	4.11	4.35	0.15	8.99	0	3
Oranges, raw, navels	1.97	2.25	4.28	8.5	0	2.2
Pineapple, raw, all varieties	1.73	2.12	5.99	9.85	0	1.4
Plums, raw	5.07	3.07	1.57	9.92	0	1.4
Raisins, seedless	27.75	29.68	0.45	59.19	2.70	3.7
Raspberries, raw	1.86	2.35	0.2	4.42	0	6.5
Strawberries, raw	1.99	2.44	0.47	4.42	0	6.5
Watermelon, raw	1.58	3.36	1.21	6.2	0	0.4
Asparagus, cooked, boiled, drained	0.42	0.79	0	1.3	0	2
Beans, snap, green, raw	1.51	1.39	0.36	3.26	0.88	2.7
Broccoli, raw	0.49	0.68	0.1	1.7	0	2.6
Carrots, baby, raw	1.04	1	2.72	4.76	0	2.9
Corn, sweet, white, cooked, boiled, drained, without salt	0.7	1.02	6.02	7.73	4.47	2.7
Onions, sweet, raw	2.26	2.02	0.72	5.02	0	0.9
Peas, green, frozen, unprepared	0.08	0.25	4.6	5	4.17	4.5
Peppers, sweet, green, raw	1.16	1.12	0.11	5.93	–	5.5
Potatoes, flesh and skin, raw	0.33	0.27	0.17	0.78	15.44	2.2
Sweet potato, cooked, baked in skin, flesh, without salt	0.57	0.5	2.28	6.48	7.05	3.3
Tomatoes, red, ripe, raw, year round average	1.25	1.37	0	2.63	0	1.2
Beans, kidney, all types, mature seeds, canned	0	–	1.85	1.85	8.9	4.3
Beans, pinto, canned, drained solids	0	–	0.54	0.54	10.21	5.5
Lentils, raw	0	0.27	1.47	2.03	49.9	10.7
Peanuts, all types, dry-roasted, with salt	0	–	4.9	4.9	4.39	8.4
Macaroni, cooked, enriched	0.04	0.03	0.09	0.56	26.01	1.8
Rice, white, long-grain, precooked or instant, enriched, prepared	–	–	0	0	26.33	0.6
Spaghetti, cooked, enriched, with added salt	0.04	0.03	0.09	0.56	26.01	1.8
Cereals, oats, unenriched, cooked with water	0	–	0.25	0.27	11.6	1.7
Cereals ready-to-eat, POST, Shredded Wheat, original big biscuit	0	–	0.55	0.94	65.1	12
Cereals ready-to-eat, RALSTON Corn Flakes	1.48	1.34	4.61	7.84	60.42	2.7

bacteria. Many other non-digestible oligosaccharides are also used as prebiotics, and include oligosaccharides of D-galactose, D-glucose, D-xylose and combinations.

Chemical Reactions of Sugars

Mutarotation

When a crystalline reducing sugar is placed in water, an equilibrium is established between isomers, as is evidenced by a relatively slow change in specific rotation that eventually reaches the final equilibrium value. The working hypothesis for the occurrence of mutarotation has been described by Shallenberger and Birch (1975). It is assumed that five structural isomers are possible for any given reducing sugar (Fig. 4.18), with pyranose and furanose ring structures being generated from a central straight-chain intermediate. Mutarotation is the change in the observed optical rotation when a reducing sugar is dissolved in water and forms different tautomeric forms. In the crystalline form a sugar will have a specific

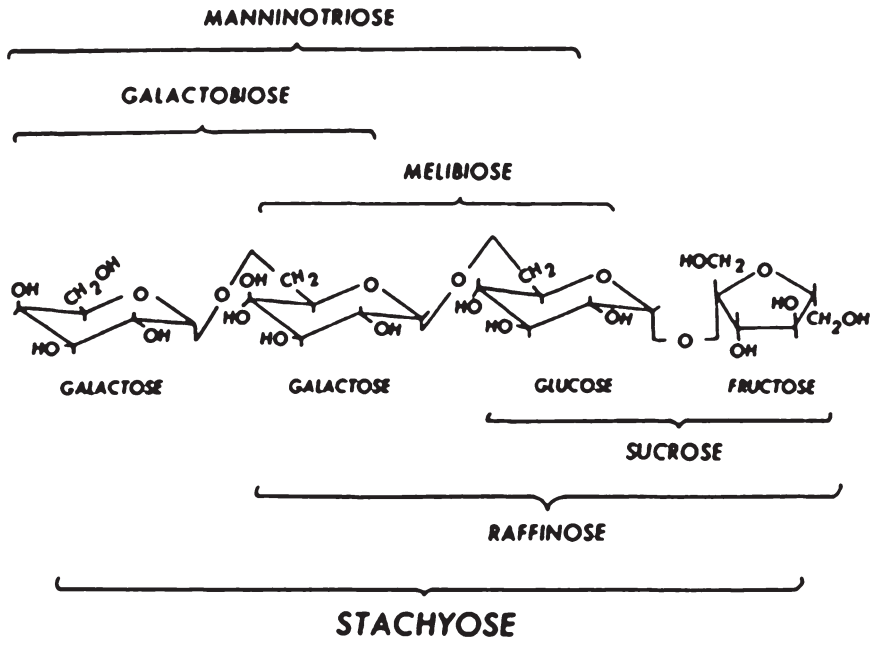


Fig. 4.16 Composition of some major oligosaccharides occurring in foods. *Source:* From R.S. Shallenberger and G.G. Birch, *Sugar Chemistry*, 1975, AVI Publishing Co.

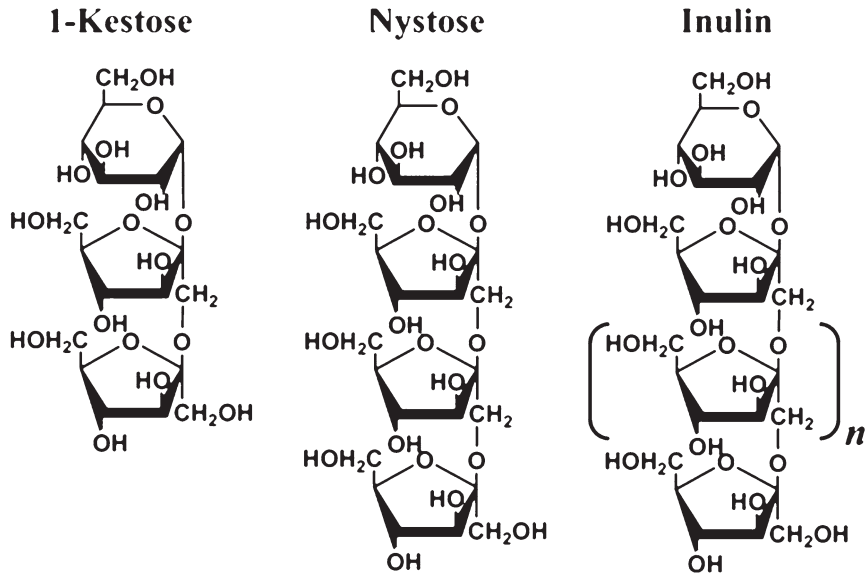


Fig. 4.17 Fructooligosaccharides (FOSs) or Fructan oligosaccharides

form, for example, β-D-glucose either as a pyranose or furanose ring. When the sugar dissolves in water, the ring structure opens and an equilibrium is established among the various isomers of the sugar. Shallenberger and Birch (1975) described the process known as mutarotation. There are five possible isomers possible for a reducing sugar as shown in Fig. 4.18.

The velocity of the reaction is greatly accelerated by acid or base. The rate is at a minimum for pyranose-pyranose interconversions in the pH range 2.5–6.5. Table 4.6 contains the distribution

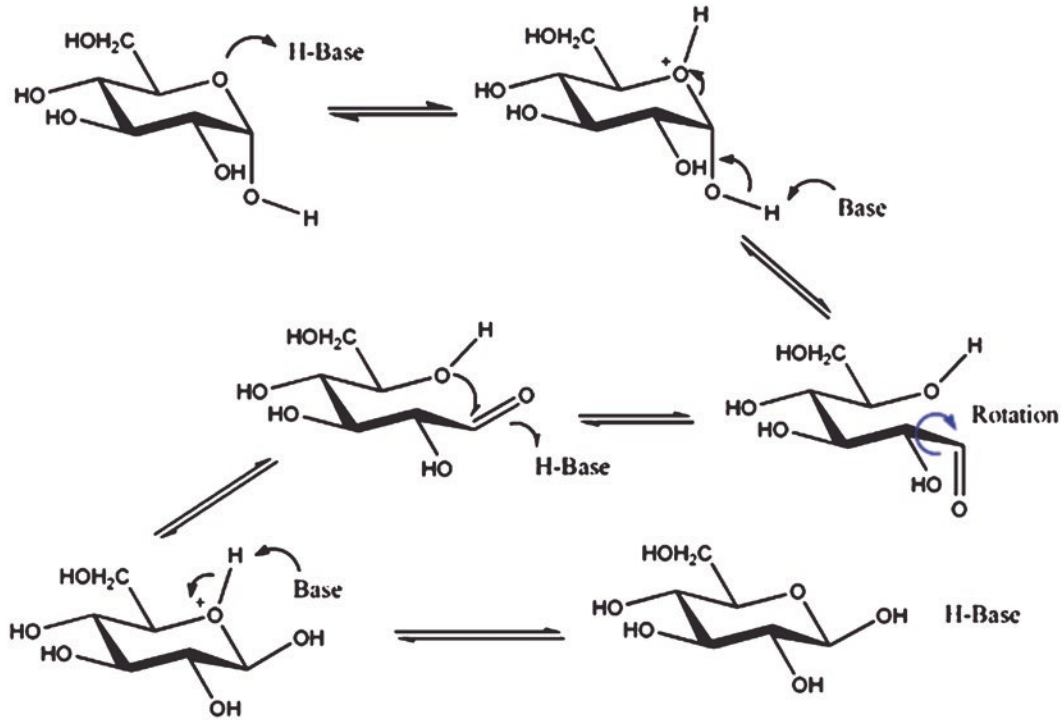


Fig. 4.18 Equilibria involved in mutarotation. *Source:* Wikipedia

Table 4.6 Percentage distribution of isomers of mutarotated sugars at 20 °C.

Sugar	α-Pyranose	β-Pyranose	α-Furanose	β-Furanose
D-Glucose	31.1–37.4	64.0–67.9	–	–
D-Galactose	29.6–35.0	63.9–70.4	1.0	3.1
D-Mannose	64.0–68.9	31.1–36.0	–	–
D-Fructose	4.0	68.4–76.0	–	28.0–31.6

Source: From R.S. Shallenberger and G.G. Birch, *Sugar Chemistry,* AVI Publishing Co.

of mutarotated sugars at room temperature. Both acids and bases accelerate mutarotation rate, with bases being more effective. This was expressed by Hudson (1907) in the following equation:

$$K_{25}^{\circ} = 0.0096 + 0.258\left[H^{+}\right] + 9.750\left[OH^{-}\right]$$

This indicates that the effect of the hydroxyl ion is about 40,000 times greater than that of the hydrogen ion. The rate of mutarotation is also temperature dependent; increases from 1.5 to 3 times occur for every 10 °C rise in temperature.

When sugars are in the open ring form they tend to be more reactive particularly when amines are present and the Maillard browning reaction takes place. This is discussed in Chap. 3. The rate of this reaction is increased in the presence of acid or base. The rate is slowest between pH 2.5 and 6.5. At alkaline pH the hydroxyl ion will increase the rate by as much as 40,000-fold. Sugars in solution are unstable and undergo a number of reactions, which have been comprehensively reviewed by Clarke et al. (1997). In addition to mutarotation, which is the first reaction to occur when a sugar is dissolved, enolization and isomerization, dehydration and fragmentation, anhydride formation and polymerization may all take place. These reactions are outlined in Fig. 4.19, using glucose as an example. Compounds (1) and (2) are α and β forms in equilibrium during mutarotation with the aldehydo form (5). Heating results in dehydration of

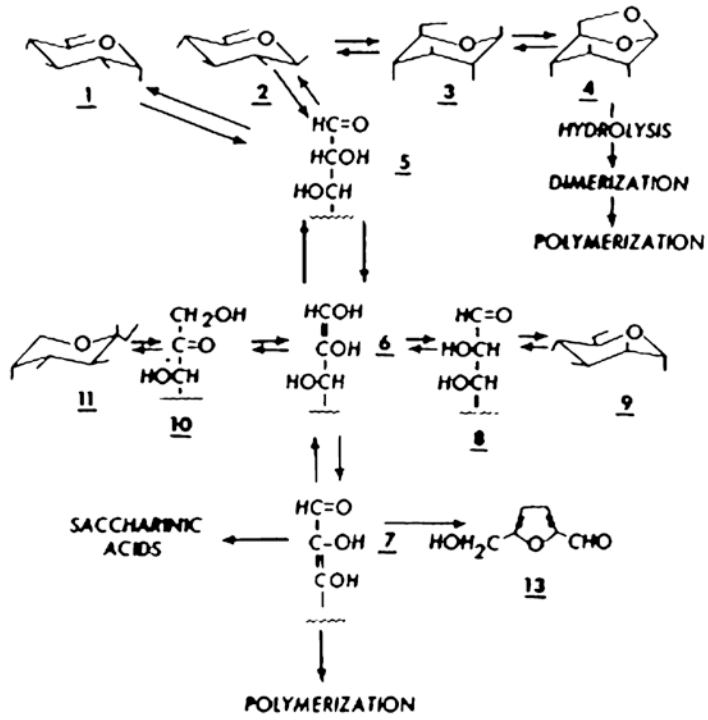


Fig. 4.19 Reactions of reducing sugars in solution. *Source:* From R.S. Shallenberger and G.G. Birch, *Sugar Chemistry*, 1975, AVI Publishing Co.

the *IC* conformation of β-D glucopyranose (3) and formation of levoglucosan (4), followed by the sequence of reactions described under caramelization. Enolization is the formation of an enediol (6). These enediols are unstable and can rearrange in several ways. Since the reactions are reversible, the starting material can be regenerated. Other possibilities include formation of keto-D-fructose (10) and β-D-fructopyranose (11), and aldehydo-D-mannose (8) and α-D-mannopyranose (9). Another possibility is for the double bond to move down the carbon chain to form another enediol (7). This compound can give rise to saccharinic acids (containing one carboxyl group) and to 5-(hydroxy)-methylfurfural (13). All these reactions are greatly influenced by pH. Mutarotation, enolization, and formation of succharic acid (containing two carboxyl groups) are favored by alkaline pH, formation of anhydrides, and furaldehydes by acid pH. It appears from the aforementioned reactions, that holding a glucose solution at alkaline pH is likely to yield a mixture of sugars. When an acid solution of sugar of high concentration is left at ambient temperature, reversion takes place. This is the formation of disaccharides. The predominant linkages in the newly formed disaccharides are α-D-1 → 6, and β-D-1 → 6. A list of reversion disaccharides observed by Thompson (1954) in a 0.082 N hydrochloric acid solution or in D-glucose is shown in Table 4.7.

Caramelization

Caramelization involves the oxidation of sugars, and is used extensively in cooking to produce nutty flavors and brown color. Moreover, caramel food colorants have been a mainstay in the food and beverage industries for decades (Boyd 2016). During the controlled, heat process volatile chemicals are released producing the characteristic caramel flavor. The reaction involves the removal of water and the breakdown of the sugar. The caramelization reaction varies depending on the type of sugar. Sucrose and glucose begin to caramelize around 160 °C (320 °F) whereas fructose caramelizes at a lower temperature of 110 °C (230 °F), because it is more labile to acid degradation. This latter effect can be seen in baked goods made from honey or fructose syrup yield products with a darker color.

Table 4.7 Reversion disaccharides of glucose in 0.082 N HCl

Glucose disaccharides	Reversion (%)
β, β-trehalose (β-D-glucopyranosyl β-D-glucopyranoside)	0.1
β-sophorose (2-O-β-D-glucopyranosyl-β-D-glucopyranose)	0.2
β-maltose (4-O-α-D-glycopyranosyl-β-D-glycopyranose)	0.4
α-cellobiose (4-O-β-D-glucopyranosyl-α-D-glucopyranose)	0.1
β-cellobiose (4-O-β-D-glucopyranosyl-β-D-glucopyranose)	0.3
β-isomaltose (6-O-α-D-glucopyranosyl-β-D-glucopyranose)	4.2
α-gentiobiose (6-O-β-D-glucopyranosyl-α-D-glucopyranose)	0.1
β-gentiobiose (6-O-β-D-glucopyranosyl-β-D-glucopyranose)	3.4

Source: From A. Thompson et al. Acid Reversion Products from D-Glucose, J. Am. Chem. Soc., Vol. 76, pp. 1309–1311, 1954

The formation of the caramel pigments is considered a non-enzymatic browning reaction in the absence of nitrogenous compounds. When sugars are subjected to heat in the absence of water or are heated in concentrated solution, a series of reactions occurs that finally leads to caramel formation. The initial stage is the formation of anhydro sugars. Glucose yields glucosan (1,2-anhydro-α-D-glucose) and levoglucosan (1,6-anhydro-β-D-glucose), which have widely differing specific rotations: +69° and −67°, respectively. These compounds may dimerize to form a number of reversion disaccharides, including gentio-biose and sophorose, which are also formed when glucose is melted (Shallenberger and Birch 1975).

Caramelization of sucrose starts with the melting of the sugar at high temperatures followed by boiling. Sucrose first decomposes into glucose and fructose, which is followed by a condensation step, in which the individual sugars lose water and react with each other. Hundreds of new aromatic compounds are formed having a range of complex flavors.

Melting and caramelization of sucrose have been of significant importance to the food and confection industries for over 150 years (Gelis 1858; Cunningham and Dorée 1917). Geliś (1858) introduced the terms “caramelan”, “caramelen”, and “caramelin”:

Caramelization Products:

$$2C_{12}H_{22}O_{11} = 4H_2O \cdot C_{24}H_{36}O_{18} \textit{Caramelan}$$

$$3C_{12}H_{22}O_{11} = 8H_2O \cdot C_{36}H_{50}O_{25} \textit{Caramelen}$$

Continued heating yields caramelin $C_{125}H_{188}O_{80}$

Caramelan is the first product formed through loss of water from sucrose molecules as a result of dehydration and polymerization reactions. Caramelan is brown and results in a bitter flavor; von Elbe (1936) reported that caramelan was a mix of colorless components and a dark brown humin. The 84% alcohol soluble product results from an approximate 12% loss in mass as water and the formula $C_{12}H_{18}O_9$. Caramelen is darker than caramelan, and is not soluble in 84% ethanol. Carmelen results from a further 15%, loss in water, and the greater degree of polymerization with the formula $C_{36}H_{50}O_{25}$. Caramelin is generated at 22% loss of mass as water. Caramelin is a mix of strongly colored, hot-water-soluble substances more highly polymerized materials with the average formula $C_{96}H_{102}O_{51}$. These amorphous products are complex mixtures that are formed when sucrose is heatied at 180–190 °C (Gelis 1858; Cunningham and Dorée 1917). Further work has shown that a reaction of anhydrofructose and anhydroglucose (glucosan) produced isosaccharosan (anhydrous sucrose)(Pictet and Adrianoff 1924). These studies demonstrated the complexity of the carmelization reaction when sugars are heated. Time and temperature have major effects on the caramel products formed. Several other sugars exhibit similarities in thermal behavior to sucrose melting and cara-

melization (Raemy et al. 1983). Other sugars, such as glucose, fructose, lactose, maltose, and xylose form colored products when the sugars are heated (von Elbe 1936; Raemy et al. 1983; Feather and Harris 1973; Lappalainen et al. 2006). Industrial caramelization processes, as related to manufacturing of sugar-based caramels, differ significantly from spontaneous caramelization of sugars during heating. Caramels are produced from a mix of caramel ingredients, as reviewed by Martin (1955) and Sengar and Sharma (2014).

Caramelization of sucrose requires a temperature of about 200 °C. At 160 °C, sucrose melts and forms glucose and fructose anhydrides (levulosans). At 200 °C, the reaction sequence consists of three distinct stages well separated in time. The first step requires 35 min of heating and involves a weight loss of 4.5%, corresponding to a loss of one molecule of water per molecule of sucrose. This could involve formation of compounds such as isosacchrosan. Pictet and Strieker (1924) showed that the composition of this compound is 1,3′; 2,2′-dianhydro-α-D-glucopyranosyl-β-D-glucopyranosyl-β-D-fructo-furanose (Fig. 4.20). After an additional 55 min of heating, the weight loss amounts to 9%, and the pigment formed is named caramelan. This corresponds approximately to the following equation:

$$2C_{12}H_{22}O_{11} - 4H_2O \cdot C_{24}H_{36}O_{18}$$

The reaction is initiated by the formation of anhydro sugars (Shallenberger and Birch 1975). Glucose is converted to glucosan (1,2,anhydro-α-D glucose and levoglucosan (1,6-anhydro-β-D glucose), which can dimerize to form multiple reversion disaccharides such as sophorose and gentiobiose.

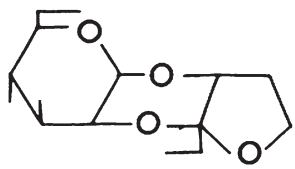


Fig. 4.20 Structure of isosacchrosan. *Source:* From R.S. Shallenberger and G.G. Birch, *Sugar Chemistry,* 1975, AVI Publishing Co.

The pigment caramelan is soluble in water and ethanol and has a bitter taste. Its melting point is 138 °C. A further 55 min of heating leads to the formation of caramelen. This compound corresponds to a weight loss of about 14%, which is about eight molecules of water from three molecules of sucrose, as follows:

Caramelen is soluble in water only and melts at 154 °C. Additional heating results in the formation of a very dark, nearly insoluble pigment of average molecular composition $C_{125}H_{188}O_{80}$. This material is called humin or caramelin. The typical caramel flavor is the result of a number of sugar fragmentation and dehydration products, including diacetyl, acetic acid, formic acid, and two degradation products reported to have typical caramel flavor by Jurch and Tatum (1970), namely, acetylformoin (4-hydroxy-2,3,5-hexane-trione) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone.

Crystallization

An important characteristic of sugars is their ability to form crystals. In the commercial production of table sugar (sucrose), crystallization is an important purification step. Generally, the more pure a solution of a sugar, the easier and faster it will crystallize. Non-reducing oligosaccharides also crystallize relatively easily. The fact that certain reducing sugars crystallize with more difficulty has been ascribed to the presence of anomers and ring isomers in solution, which makes these sugars intrinsically "impure" (Shallenberger and Birch 1975). Mixtures of sugars crystallize less easily than single sugars. In certain foods, crystallization is undesirable, such as the crystallization of lactose in sweetened condensed milk or ice cream.

Crystallization of Sucrose in Commercial Manufacture of Table Sugar

Factors that influence growth of sucrose crystals have been listed by Smythe (1971). They include supersaturation of the solution, temperature, relative velocity of crystal and solution, nature and concentration of impurities, and nature of the crystal surface. Crystal growth of sucrose consists of two steps: (1) the mass transfer of sucrose

molecules to the surface of the crystal, which is a first-order process; and (2) the incorporation of the molecules in the crystal surface, a second-order process. Under typical conditions, overall growth rate is a function of the rate of both processes, with neither being rate-controlling. The effect of impurities are various: (1) Viscosity can increase, thus reducing the rate of mass transfer, (2) impurities can involve adsorption-occlusion on specific surfaces of the crystal, thereby reducing the rate of surface incorporation, (3) inclusion whereby the impurity is incorporated inside the crystal by the physical capture of syrup or mother liquor, and (4) co-crystallization can occur when there is a different solid impurity with a lower free energy (lower solubility) than that of sucrose in the process.

The crystal structure of sucrose has been established by X-ray diffraction and neutron diffraction studies. The packing of sucrose molecules in the crystal lattice is determined mainly by hydrogen bond formation between hydroxyl groups of the fructose moiety. As an example of the type of packing of molecules in a sucrose crystal, a projection of the crystal structure along the *a* axis is shown in Figs. 4.21 and 4.22. The dotted square represents one unit cell. The crystal faces indicated in this figure follow planes between adjacent sucrose molecules in such a way that the furanose and pyranose rings are not intersected.

The processing and refining of sugar (sucrose) represents an excellent example of the seeding crystallization process for the large-scale manufacture of sugars. A seed slurry is prepared with a ball mill whereby powdered sugar (average 50 μm size) is mixed with isopropyl alcohol and ground for 24 h. A 1.5–4.0 μm seed slurry is produced, however, different sizes are produced for different vacuum pan crystallizations depending on the final crystal product. The seed pan is added to the vacuum pan containing the correct supersaturated syrup or mother liquor. The growth of sucrose crystals is a two-step process.

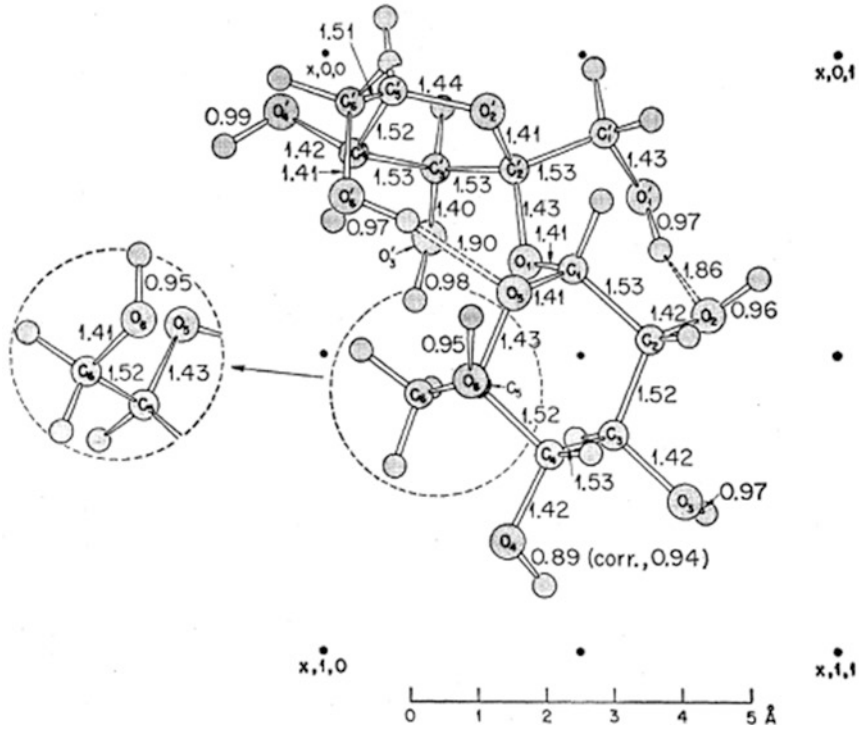
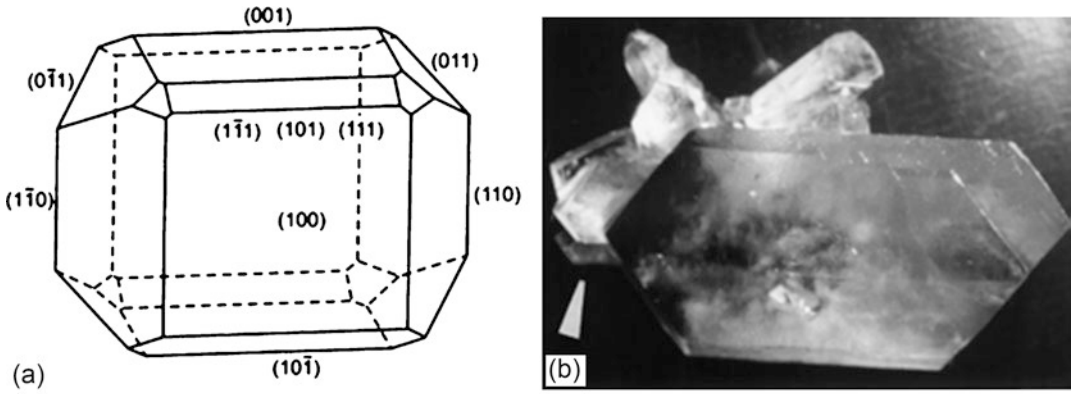


Fig. 4.21 The reference molecule (asymmetric unit) in the sucrose crystal structure viewed in projection along the a axis. The insert shows a clearer view of a portion of the molecule, projected along a line in the plane of a and b, 30° from a. Numbers attached are bond lengths (A)



(a) Drawing of a sucrose crystal grown in slightly supersaturated solution . The b-axis is the polar axis. (b) Intergrowths on M1 110N and neighboring faces in pure solution (arrow: twin with twinning axis [0 0 1]).

Fig. 4.22 Projection of a sucrose crystal along the b axis (Wang et al. 2000)

First the sucrose molecules migrate to the surface of the growing crystal (a first-order reaction) followed by the incorporation of the sucrose molecules into the crystalline matrix (a second order reaction).

Commercially available refined sugar (sucrose) has a very high purity (>99.9%). To obtain sugar, a pure product from both sugarcane and sugar beet, rather complex isolation and purification processes are followed. Essentially there are a series of separations of non-sucrose impurities from sucrose. Sugarcane processing occurs in two stages (Eggleston 2008). Firstly, the juice is extracted from sugarcane and converted into raw sugar (98–99% purity; golden yellow/brown crystals) at factories (mills) near where sugarcane is grown. Secondly, after raw sugar has been transported to the refinery, it is refined using very similar unit processes used in raw sugar manufacture, to the white, refined edible sugar. In comparison to sugarcane, sugar beets are grown in temperate areas and are processed directly into white sugar at nearby factories. A flow chart of the typical unit processes in the manufacture of raw sugar from sugarcane at a factory is outlined in Fig. 4.23.

Juice is extracted from sugarcane by either tandem milling or diffusion. Extracted juice is then purified in the clarification unit process. Clarification processes can vary, but the most common is hot lime clarification (Eggleston et al. 2003). The clarified juice is then concentrated through a series of multiple-effect vacuum evaporators to syrup of ~65% dissolved solids. Syrup from the final evaporator unit is then concentrated, under vacuum at lower temperature than in evaporation to minimize the chemical and thermal degradation of sucrose, and crystallized. The vacuum pans are seeded with finely ground sucrose to allow larger sucrose crystals to form. A mixture of sucrose crystals and mother liquor (massecuites) is produced that is then separated in centrifuges, and the mother liquor is re-concentrated and recrystallized to give two more crops of crystals. The final liquor is the by-product molasses.

Unlike sugarcane factories that are limited to operation during the sugarcane harvest season, raw sugar refineries operate year round. Since the production of refined sugar from raw sugar at the refinery is also a series of separations of non-sugars from sucrose (Fig. 4.24), it consists of many similar processes as in raw sugar manufacture at a factory (Fig. 4.23). There is, however, a greater onus at the refinery to remove color and ash, so after clarification there are decolorization or demineralization processes that can differ from refinery to refinery. The first stage of the refining

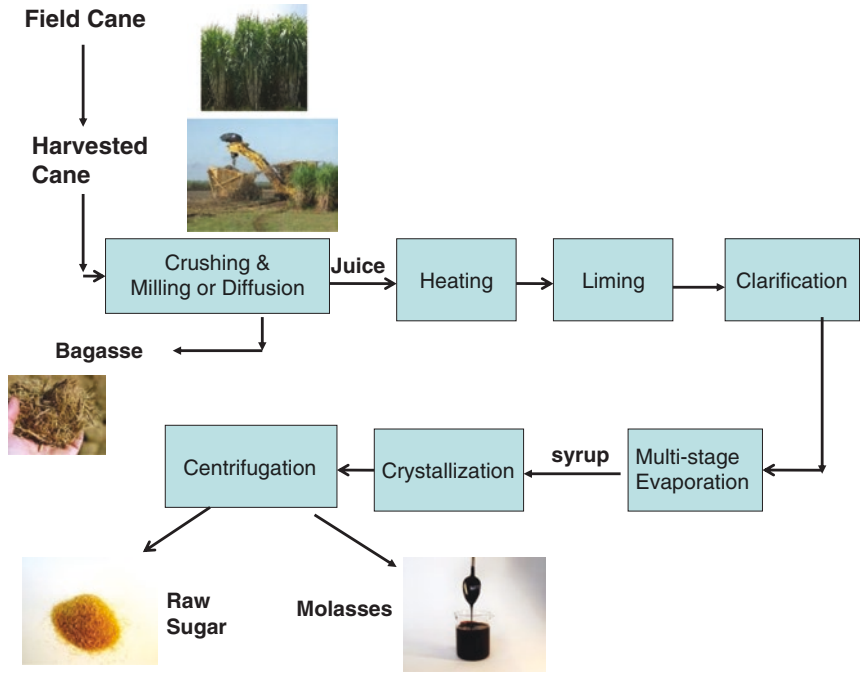


Fig. 4.23 Basic flow chart of the raw sugar manufacturing process in a sugarcane factory

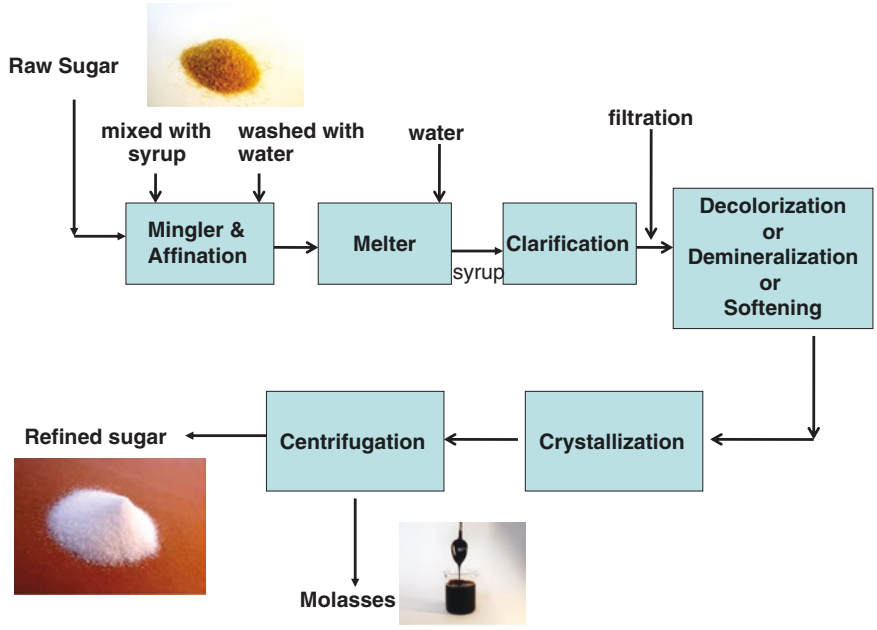


Fig. 4.24 Basic scheme of the white, refined sugar manufacturing process in a sugar refinery

process is named affination, where the raw sugar is mixed with syrup (magma) and then centrifuged and washed with water to remove the molasses layer around the raw sugar crystals. Nowadays, many refiners are trying to eliminate affination because it is high energy and cost intensive. The affined sugar is then melted in water to create a melt liquor of ~68% dissolved solids. The melt

liquor is then clarified by either a phosphatation or carbonation clarification process; both clarification processes remove turbid particles and some, but not all, colorants. The clarified syrup is then decolorized with either ion exchange resins or granular activated carbon (Eggleston 2008). Desalting ion exchange resins can also remove ash or minerals. Multi-stage crystallization is the final purification process at the refinery to produce white, refined sugar sold in solid or liquid form.

Production of refined, white sugar from sugar beets has some similarities to refined cane sugar production, as both are a series of process units aimed at separating and removing impurities from sucrose. However, dissimilarities exist as sugar beet is a tuberous root and sugarcane is a grass (Eggleston 2008). A basic scheme of white, refined sugar manufacture from sugar beets is illustrated in Fig. 4.25. Sugar beets at the factory, are washed, and sliced into "V" shaped cossettes. Cossettes are added to a counter-current diffuser of a different design than sugarcane diffusers. Sucrose and impurities are extracted with hot water at 85 °C in the diffuser. The generated diffuser juice is then heated to maintain an 85 °C temperature, before it is purified with a double-carbonatation clarification process (Fig. 4.25). In some sugar beet factories, sulfur dioxide is added to filtered, clarified juice to minimize color formation during subsequent processing. The resulting clarified "thin" juice is then concentrated to 65% dissolved solids ("thick" juice) across multiple-effect vacuum evaporators, then triple-crystallized and centrifuged to produce white refined sugar. In many sugar beet factories additional purification steps are employed, such as softening, demineralization, or color removal with ion-exchange resins or granular activated carbon.

Crystallization of Lactose

Lactose can occur in two crystalline forms, the α-hydrate and the β-anhydrous forms and can occur in an amorphous or glassy state. The most common form is the α-hydrate ($C_{12}H_{22}O_{11}·H_2O$), which can be obtained by crystallization from a supersaturated solution below 93.5 °C. When crystallization is carried out above 93.5 °C, the crystals formed are of β-anhydrous type. Some

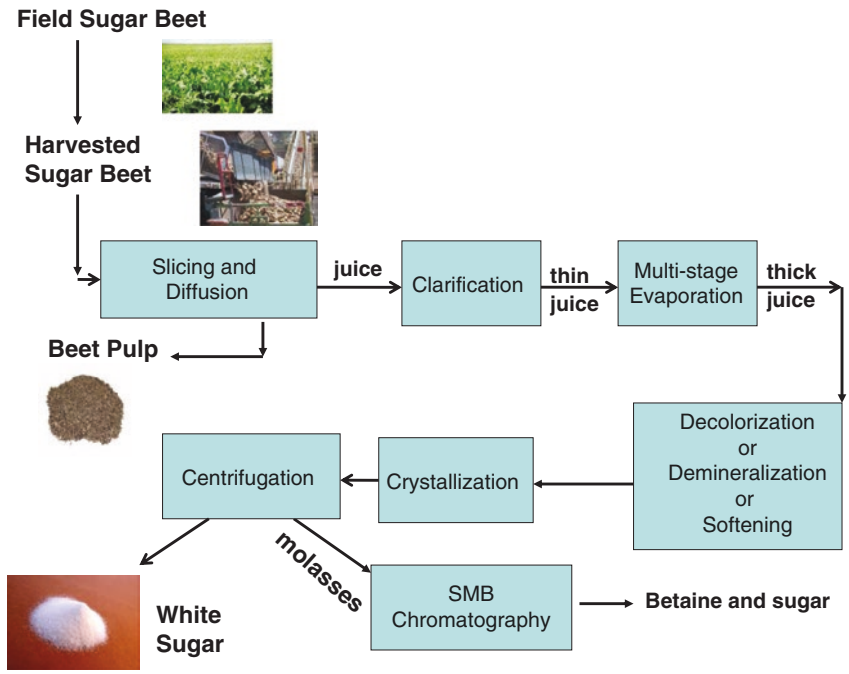


Fig. 4.25 Basic scheme of the white, refined sugar manufacturing process in a sugar beet factory

Table 4.8 Some physical properties of the two common forms of lactose

Property	α-Hydrate	β-Anhydride
Melting point[a]	202 °C (dec.)	252 °C (dec.)
Specific rotation[b] $[\alpha]_D^{20}$	+89.4°	+35°
Solubility (g/100 mL) Water at 20 °C	8	55
Water at 100 °C	70	95
Specific gravity (20 °C)	1.54	1.59
Specific heat	0.299	0.285
Heat of combustion (cal/g)	3761.6	3932.7

Source: From R. Jenness, *Principles of Dairy Chemistry,* 1959, John Wiley and Sons
[a]Values vary with rate of heating, α-hydrate losses H_2O (120 °C)
[b]Values on anhydrous basis, both forms mutarotate to +55.4°

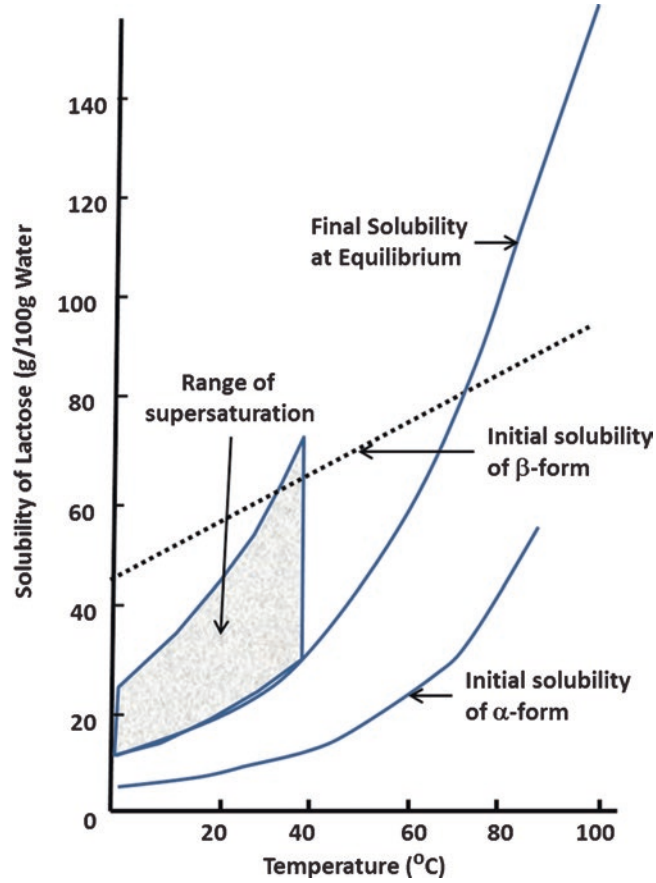


Fig. 4.26 Solubility of lactose in water. *Source:* From E.O. Whittier, Lactose and Its Utilization: A Review, *J. Dairy Sci.,* Vol. 27, p. 505, 1944

properties of these forms have been listed by Jenness (1959) (Table 4.8).

Under normal conditions the α-hydrate form is the stable one, and other solid forms spontaneously change to that form provided sufficient water is present. At equilibrium and room temperature, the β-form is much more soluble and the amount of α-form is small. However, because

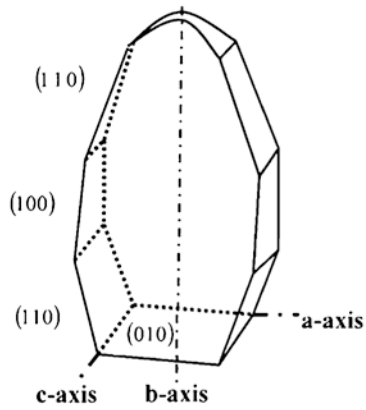
of its lower solubility, the α-hydrate crystallizes out and the equilibrium shifts to convert β-into α-hydrate. The solubility of the two forms and the equilibrium mixture is represented in Fig. 4.26.

The solubility of lactose is less than that of most other sugars, which may present problems in a number of foods containing lactose. When milk is concentrated 3:1, the concentration of lactose approaches its final solubility. When this product is either cooled or when sucrose is added, crystals of α-hydrate may develop. Such lactose crystals are very hard and sharp; when left undisturbed they may develop to a large size, causing a sensation of grittiness or sandiness in the mouth. This same phenomenon limits the amount of milk solids that can be incorporated into ice cream.

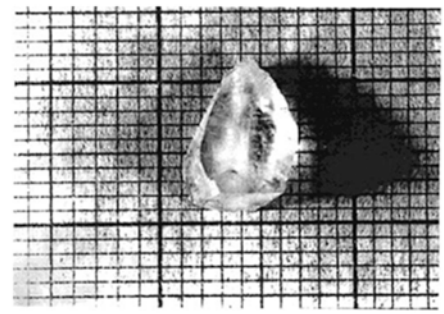
The crystals of α-hydrate lactose usually occur in a prism or tomahawk shape. The latter is the basic shape and all other shapes are derived from it by different relative growth rates of the various faces. The shape of a α-hydrate lactose crystal is shown in Fig. 4.27. The crystal has been characterized by X-ray diffraction, and the following constants for the dimensions of the unit cell and one of the axial angles have been established: $a = 0.798$ nm, $b = 2.168$ nm, $c = 0.4836$ nm, and $\beta = 109°47'$. The crystallographic description of the crystal faces is indicated in Fig. 4.27. These faces grow at different rates; the more a face is oriented toward the β direction, the slower it grows and the (0T0) face does not grow at all.

Amorphous or glassy lactose is formed when lactose-containing solutions are dried quickly. The dry lactose is non-crystalline and contains the same ratio of alpha/beta as the original product. This holds true for spray or roller drying of milk products and also during drying for moisture determination. The glassy lactose is extremely hygroscopic and takes up moisture from the atmosphere. When the moisture content reaches about 8%, the lactose molecules recrystallize and form α-hydrate crystals. As these crystals grow, powdered products may cake and become lumpy.

Both lactose and sucrose have been shown to crystallize in an amorphous form at moisture contents close to the glass transition temperature (Roos and Karel 1991a,b; Roos and Karel 1992). When amorphous lactose is held at constant water content, crystallization releases water to the remaining amorphous material, which depresses the glass transition temperature and accelerates crystallization. These authors have done extensive studies on the glass transition of



Characteristic tomahawk-shaped morphology of R-lactose monohydrate crystallized from aqueous solution.



Typical crystal of R-lactose monohydrate grown by the slow cooling of a saturated solution

Fig. 4.27 Crystallographic representation of a tomahawk crystal of α-lactose *Monohydrate a*nd photo of R-lactose crystal (Raghavan et al. 2000)

amorphous carbohydrate solutions (Roos 1993; Roos and Karel 1991d).

Seeding is a commonly used procedure to prevent the slow crystallization of lactose and the resulting sandiness in some dairy products. Finely ground lactose crystals are introduced into the concentrated product, and these provide numerous crystal nuclei. Many small crystals are formed rapidly; therefore, there is no opportunity for crystals to slowly grow in the supersaturated solution until they would become noticeable in the mouth.

Compounds Related to Sugars

Sugars can be appended with amino groups in a class of molecules named amino sugars. Amino sugars form the backbone of chitin in insects, crustaceans, mollusks, and some mushrooms. In chitin the amino group of glucosamine is acetylated and exists in polymeric form (Fig. 4.28). Glucosamine is frequently bound to proteins as part of their glycoprotein structures, i.e., ovomucin in egg whites.

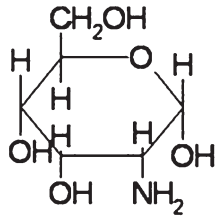


Fig. 4.28 Structure of glucosamine

In glucosamine one of the hydroxyl groups is replaced with an amino group. With chitosan the amino group is acetylated through addition of an acetate group (Fig. 4.29). Chitosan is also frequently partially hydrolyzed to remove the acetate group resulting in the formation of chitosan. Chitin is structural in insects and] crustaceans. Chitosan has interesting properties existing as a polyamine including absorption and anti-microbial activity.

Galacturonic acid is a sugar where the C6 carbon is oxidized to a carboxylic acid (Fig. 4.30). D-galactose can also be oxidized at the C1 position (D-galactonic acid) and at both the C1 and C6 positions (*meso*-galactaric acid (mucic acid). Galacturonic is one of the main monomers in pectin. In pectin the carboxylic acid is generally acetylated.

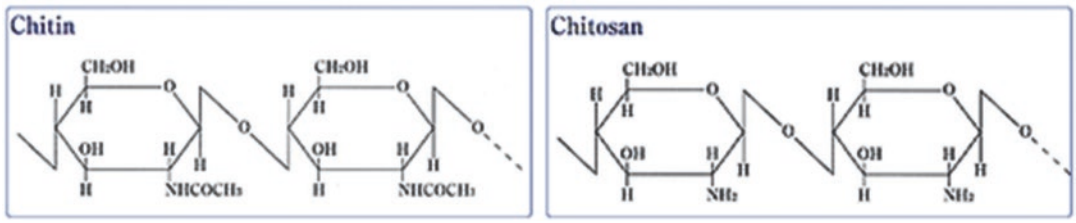
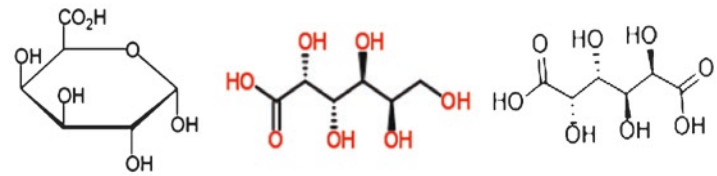


Fig. 4.29 Repeat unit structures of chitin and chitosan polysaccharides

Fig. 4.30 Different structures of galacturonic acids



Polysaccharides

Polysaccharides, like oligosaccharides, consist of monosaccharaides bound together by glycosidic bonds. Less than 10 monosaccharaides are considered oligosaccharides and greater than 10 are polysaccharides. The number of monosaccharides in the polymer is referred to as the degree of polymerization (DP). There are only a few polysaccharides with DPs less than 100, most are in the 200–300 DP range. Starches and cellulose can be much larger. Cellulose can have DPs from 7000 to 15,000. It has been estimated that over 90% of the carbohydrate mass in the world is in the form of polysaccharides.

Homoglycans are polymers like starch and cellulose where all of the monosaccharide constituents are the same, in these examples D-glucopyranosyl units. Homoglycans includes polymers that are branched like amylopectin. Polymers where two types of monosaccharides are included are called di-heteropolymers. And those with three different monomers are tri-heteropolymers, etc.

Starch

The major carbohydrate polysaccharide in plant tubers and seed endosperm is starch (Buléon et al. 1998; Blazek et al. 2011). Starch is a hompolymer of D-glucose and is a storage carbohydrate in plants. It occurs as small granules with the size range and appearance characteristic to each botanical plant species. The granules can be shown by ordinary and polarized light microscopy and by X-ray diffraction to have a highly ordered crystalline structure. Morphologies are shown in Fig. 4.31 by confocal laser microscopy.

Each granule contains several million amylopectin molecules packed with a much larger number of smaller amylose molecules. While corn is the largest single source of commercial starch, other commonly used sources are wheat, rice, potato, and tapioca (Fig. 4.31). The amylose polymer is composed of glucose units in a linear polymer while amylopectin is a highly branched polymer (Fig. 4.32). Both starch polymers are composed of α-D-glucose units in the 4C1 conformation. In amylose these are linked -(1 → 4)-, with the ring oxygen atoms all on the same side,

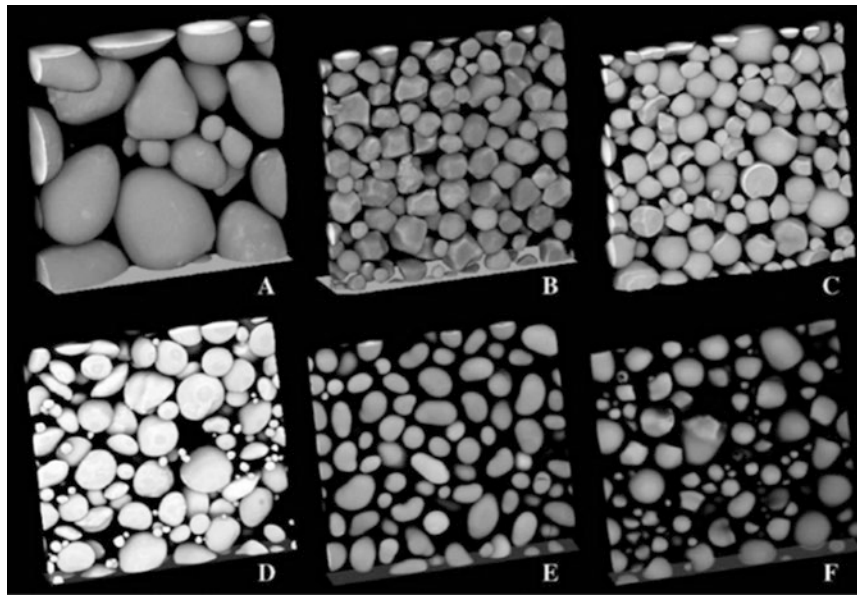


Fig. 4.31 3-D Images of the starch granules from (**a**) potato, (**b**) corn, (**c**) tapioca, (**d**) wheat, (**e**) mung bean, (**f**) Sweet Potato: Coloring Rhodamine, Objective 63w, Electronic Zoom 1, Image size 160 × 160 × 36 μ. van de Velde F, van Riel J, Tromp RH. 2002. Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). J Sci Food Agric. 82, 13, 1528–1536

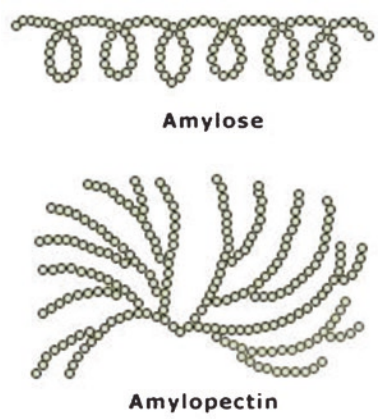


Fig. 4.32 The helical organization of amylase and the branched structure of amylopectin

Table 4.9 The color produced by reaction of iodine with amyloses of different chain length

Chain length	No. of helix turns	Color produced
12	2	None
12–15	2	Brown
20–30	3–5	Red
35–40	6–7	Purple
<45	9	Blue

whereas in amylopectin about one residue in every 20 is also linked -(1 → 6)- forming branch-points. The degree of branching and amylose to amylopectin ratios are highly variable depending on the botanical source of the starch. For example, amylomaizes contain over 50% amylose whereas 'waxy' maize has almost none (~3%) (Li and Yeh 2001; Singh et al. 2003). The number of glucose units may range in various starches from a few hundred to several thousand units. In the most common starches, such as corn, rice, and potato, the linear fraction is the minor component and represents about 17–30% of the total. Some varieties of pea and corn starch may have as much as 75% amylose. The characteristic blue color of starch produced with iodine relates exclusively to the linear, amylase fraction. The polymer chain takes the form of a helix, which forms inclusion complexes with iodine molecules. The inclusions of iodine are due to an induced dipole effect and consequent resonance along the helix. Each turn of the helix is made up of six glucose units and encloses one molecule of iodine. The length of the chain determines the color produced (Table 4.9). Many industrial starch methods are based on this amylose-iodine reaction, although some starch enzymatic methods are also available. Such iodometric methods, measure total starch in food ingredients or food products, but do not provide information on how much of the starch is soluble or insoluble (granular). Just recently, however, a new method has been reported by Cole et al. (2016), that is based on microwave-assisted sonication, which is capable of measuring total, insoluble and soluble starch in food products.

Amylose and amylopectin molecules differ greatly in structure and function. Fig. 4.33 illustrates the linear linkages of amylose that result in a helical structure, as well as the branching of amylopectin. Amylose has a lower molecular weight with a relatively extended shape of a helical rod. Amylopectin is an extremely large molecule but tends to be tightly packed. The presence of amylose tends to reduce the crystallinity of the amylopectin and influence the ease of water penetration into the granules. Although the α-(1 → 4) links are capable of relatively free rotation around the (ϕ) phi and (ψ) psi torsions, hydrogen bonding between the O3′ and O2 oxygen atoms of sequential residues tends to encourage a helical conformation. These helical structures are relatively stiff and result in hydrophobic surfaces.

Amylopectin can be isolated from 'waxy' maize starch whereas amylose (without amylopectin) is best isolated after specifically hydrolyzing the amylopectin with pullulanase (Vorwerg et al. 2002). Genetic modification of starch crops has enabled the control of amylose and amylopectin in crops which improves the ability to control functionality of the starches (Gidley et al. 2010).

Starch granules are partially crystalline; native starches contain between 15 and 45% crystalline material (Oates 1997). The crystallinity can be demonstrated by X-ray diffraction techniques. Two polymorphic forms, A and B polymorphs, have been described. There is also an intermediate C form. Crystallinity results from intertwining of amylopectin chains with a linear component of over ten glucose units to form a double helix (Fig. 4.34).

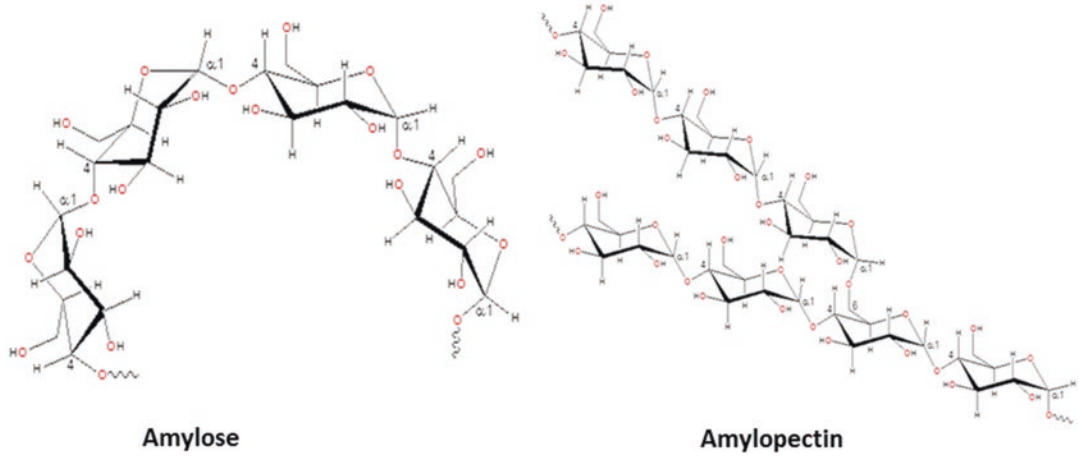


Fig. 4.33 Illustration of the molecular arrangement of amylose and amylopectin starch polysaccharides. Amylose structure shows the helical nature of the glucose polymers and amylopectin illustrates the branching

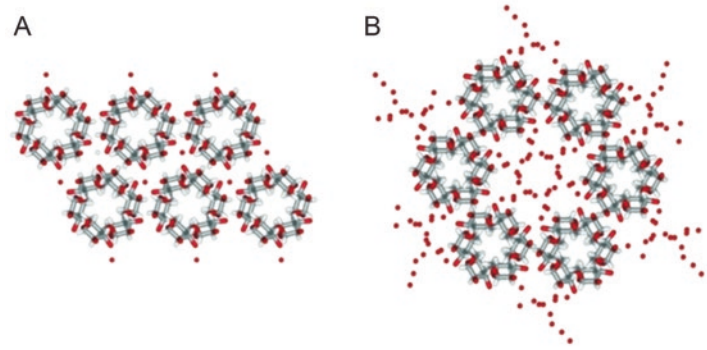


Fig. 4.34 Hexagonal packing of A-type (**a**) and B-type (**b**) starch crystalline polymorphs. (**a**) The dense structure of the A-type only allows few structured water molecules (*red dots*). (**b**) The more open B-type makes space for more structured water molecules (Damager et al. 2010)

Amylose molecules are composed of mostly unbranched chains with 500–20,000 α-(1 → 4)-D-glucose units. Occasionally amylose will have a few α-1 → 6 branches and phosphate groups bound to –OH groups of some glucose units may be found (Hoover 2001). Amylose can form an extended shape (hydrodynamic radius 7–22 nm; Parker and Ring 2001). More typically amylose is found as a left-handed single helix or with parallel left-handed double helical junction zones (Imbert et al. 1988). Single helical amylose has hydrogen-bonding between the O_2 and O_6 atoms on outside surface of the helix with only the ring oxygen internal to the helix.

Amylopectin is formed by non-random α-1 → 6 branching of the amylose-type α-(1 → 4)-D-glucose structure. Typically amylopectin molecules contain about a million residues. Approximately 5% of the residues represent branch points. There are usually slightly more 'outer' unbranched chains (A-chains) than 'inner' branched chains (called B-chains). There is only one chain (C-chain) containing the single reducing group (Fig. 4.35). The A-chains contain between 13 and 23 residues. There are two main fractions of long and short internal B-chains with the longer chains (greater than about 23–35 residues) connecting between clusters and the shorter chains similar in length to the terminal A-chains.

Each amylopectin molecule contains up to two million glucose residues in a compact structure with a hydrodynamic radius of 21–75 nm (Bertoft et al. 2008) (compare with waxy maize amylopectin >300 nm (Juna et al. 2011). The amylopectin molecules are oriented radially in the starch granule and as the radius increases the

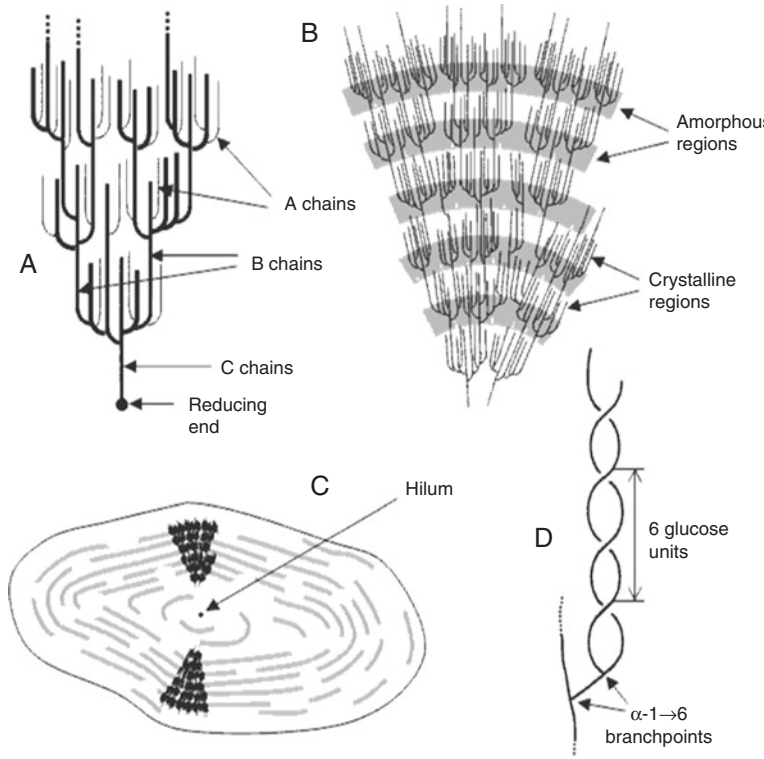


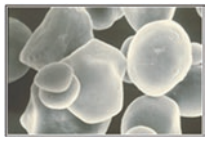
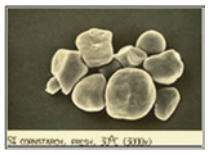
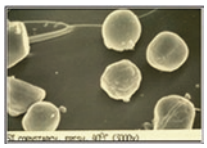
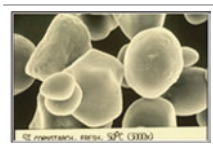
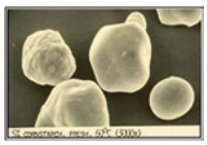
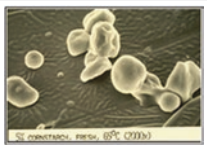
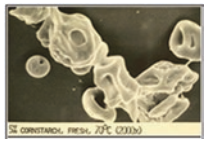
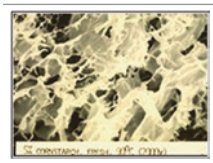
Fig. 4.35
Representations of key elements in starch structure: Type A Amylopectin double-helical chains can either form the more open hydrated. Type B hexagonal crystallites or the denser Type A crystallites. Type C with staggered monoclinic packing, dependent on the plant source of the granules (Parker and Ring 2001). Type A, with unbroken chain lengths of about 23–29 glucose units is found in most cereals

number of branches increases filling the space. The increased branching results in the formation of concentric regions of alternating amorphous and crystalline structure. In Fig. 4.35, A illustrates the essential features of amylopectin. B—the organization of the amorphous and crystalline regions (or domains) of the structure generating the concentric layers that contribute to the "growth rings "that are visible by light microscopy. C—the orientation of the amylopectin molecules in a cross section of an idealized entire granule. D—the likely double helix structure taken up by neighboring chains and giving rise to the extensive degree of crystallinity in granule. It is postulated that the crystalline structure consists of parallel left-handed helices with six residues per turn. An alternative arrangement of interconnecting clusters has been described for some amylopectins (Bertoft 2004).

Type B, with slightly longer unbroken chain lengths of about 30–44 glucose units is found in banana, some tubers such as potato and high amylose cereal starches. Type C structure, which is a combination of types A and B, can be found in peas and beans (Fig. 4.35). Starch granule architecture has been recently comprehensively described (Tang et al. 2006).

Starch has many important functions in foods. It is used for water binding and as a thickener, an emulsion stabilizer and gelling agent. An excellent review of starch functionality in foods can be found in Copeland et al. (2009). Starch is a major component in many foods and ingredients such as wheat flour, where it is 80% of the flour. Starch, therefore, inherently delivers function to the final product. Refined starches are also added to foods to provide functionality such as gelation or thickening. In plant tissue, such as cereals, starch is found with radial, tight packing to form dehydrated granules which contain about one water molecule per glucose. The shape and size of the granules in the ingredients are specific to the botanical source (maize 2–30 μm; wheat 1–45 μm; potato 5–100 μm (Jobling 2004). The swelling function is determined by the shape and size of the granules. Starch granules are generally either larger and lenticular (lens-like, A-starch) with large swelling power or smaller and spheri-

Table 4.10 The progressive heating of starch leading to gelatinization

	Raw starch that has not had moisture added does not undergo gelatinization. By definition, gelatinization is a phenomena which takes place in the presence of heat and moisture. The dry raw starch, if heated, would undergo dextrinization. This certainly would affect the starch paste viscosity and starch gel strength. The paste viscosity would be decreased and gel strength decreased
	If a "limited amount" of moisture is added to the raw starch you may get partial gelatinization. This condition exists in baked products
	Cornstarch at a 5% level in 95% water would have a slight change occur if heat is initiated. Water might be slightly ADSORBED onto the surface of the granule. Actually, in the research from which these images came, I found that I got a difference in paste viscosity and ultimate op as measured by viscosity if I allowed cornstarch to sit in water at room temperature. This led me to believe that there is some initiation adsorption upon the granule at room temperature (27C)
	If this 5% dispersion of cornstarch was heated to 40C I would expect more water would be ADSORBED onto the surface of the granule, the hydrogen bonding between the starch polymers within the granule might begin to be loosened slightly. In some types of starches water might even begin to be ABSORBED into the granule
	If this 5% dispersion of cornstarch was heated to 50C I would expect more water would be ADSORBED onto the surface of the granule, the hydrogen bonding between the starch polymers within the granule would begin to be loosened. This would allow the water to penetrate into the granule becoming ABSORBED by the granule. Additionally, some of the amylose may begin to work itself off the granule surface, thus, opening the structure even more
	If this 5% dispersion of cornstarch was heated to 60–65C I would expect more water would be ADSORBED onto the surface of the granule, the hydrogen bonding between the starch polymers within the granule would loosen. This would allow the water to penetrate into the granule becoming ABSORBED by the granule. Additionally, some of the amylose would work itself off the granule surface, thus, opening the structure even more. This in turn would allow even more of the water to become ABSORBED and more amylose to work itself out into a colloidal dispersion outside of the granule. The long amylose polymer is a colloid in characteristics
	This is intermediate between 60 and 70C. The precise changes are affected by rate of heating, condition of the starch and other factors
	If this 5% dispersion of cornstarch was heated to 70–90C I would expect more water would be ADSORBED onto the surface of the granule, the hydrogen bonding between the starch polymers within the granule would loosen. This would allow the water to penetrate into the granule becoming **ABSORBED** by the granule. Additionally, the amylose would work itself off the granule surface, thus, opening the structure even more. This in turn would allow even more of the water to become **ABSORBED** and more amylose to work itself out into a colloidal dispersion outside of the granule. The long amylose polymer is a colloid in characteristics
	At some point between 60 and 95C we would likely have gelatinization occur. This might be measured by loss of birefringence, increased viscosity, translucency, increased susceptibility to enzyme action, X-ray diffraction or some other chemical or physical means. At this point, the starch granule is swollen as much as possible. It is a starch sol until you remove it from the heat and begin to allow the amylose and some amylopectin to recrystallize, i.e. realign
	In some instances, when heated to 90C the starch granule could reach optimum gelatinization and be a nice swollen granule sack. In other cases, this may allow the sack to "implode" and loose their contents as there is not enough structure and hydrogen bonding to hold the polymers together. It is interesting that overcooking, as with overstirring, will decrease the starch paste colloidal sol viscosity

cal (B-starch) with less swelling power (Ao et al. 2007). Granules contain regions of amylopectin which contain both crystalline (~30%) and amorphous areas. As starch granules become hydrated they swell, lose crystallinity and leach amylose out of the granule. High amylose starches exhibit lower swelling and lower gel strength than lower amylose containing starches (Table 4.10).

Amylose exhibits the important function of acting as a hydrocolloid. Its extended conformation is the cause of the high viscosity of water-soluble starch over a broad temperature range. The extended helical chains possess a relatively hydrophobic inner surface that does not hold water effectively. The hydrophobic core of the amylose helix serves as a binding site for hydrophobic molecules such as lipids and aroma compounds. Amylose also forms useful gels and films. When heated amylose is cooled the chains form an association and crystallization (retrogradation) on cooling and storage. When starch gels are cooled slowly some starches form a precipitate of crystalline like material. This phenomenon is referred to as retrodegradation. It is essentially a precipitation of linear amylose molecules. Typically starches with smaller amylose polymers (400 glucose units) as in corn starch are more prone to retrogradation than starch from potato where amylose polymers are longer (2000 glucose units). Freezing accelerates the retrogradation process. After a freeze thaw cycle starch gels frequently become spongy and with slight pressure the water will begin to separate from the gel. Bread staling is a practical example of retrodegradation. After baking and initial cooling the amylose is already partially retrograded. Retrogradation is most readily observed in the staling of baked products where the texture becomes hard. It can be reversed by reheating the product. The retrogradation decreases storage stability resulting in shrinkage and the release of water (syneresis). Increasing amylose concentration decreases gel stickiness but increases gel firmness. Retrogradation is influenced by lipid content, amylose/amylopectin ratio, chain length of amylose and amylopectin, and solid concentration (Chung and Liu 2009). Amylopectin interferes with the interaction between amylose chains (retrogradation) and its solution can lead to an initial loss in viscosity and followed by a more slimy consistency. Mixing with κ-carrageenan, alginate, xanthan gum and low molecular weight sugars can also reduce retrogradation. At high concentrations, starch gels are both pseudoplastic and thixotropic with greater storage stability. Their water binding ability (high but relatively weak) can provide body and texture to foodstuffs, making it useable as a fat replacement.

In the native undamaged state starch granules are insoluble in water, however, they will reversibly imbibe small amounts of water and swell slightly. When heated in water the order and crystallization within the granule is disrupted in a process referred to as gelatinization. The irreversible loss of order in the granule is characterized by a loss in birefringence, granule swelling and loss of crystallinity. Gelatinization occurs over a temperature range (Table 4.10) with the larger starch granules gelatinizing first. Throughout the process there is leaching of amylose. Measurement of gelatinization and temperature ranges are dependent on the method used for measurement and the ratio of water to starch. Several early stages of gelatinization can be observed under a hot stage polarizing light microscope. The loss of birefringence (disappearance of the maltese cross) is a useful means of measuring gelatinization. The initial loss of birefringence (initiation) is the first stage, the mid-point, and the complete loss of birefringence are recorded. The complete loss of birefringence is then defined as complete gelatinization, thus defining the gelatinization temperature range.

When heated in excess water after gelatinization is complete, the starch granules continue to swell. Throughout this process the leaching of amylose continues. Eventually the granules will be completely disrupted. This disruption is accentuated by applying shear force as one would observe during extrusion. This disruption results in the formation of starch paste (pasting) which is a solubilization of the amylose and amylopectin in a continuous phase. One can also observe granule fragments or ghosts of starch granules in the mixture. Complete disruption is rare but there

is more disruption under shear stress as in extrusion. When cooled the starch paste forms a firm rigid gel.

Starch gelatinization is an endothermic process and the temperatures and enthalpies of gelatinization are frequently measured by differential scanning calorimetry (DSC). In the gelatinization process water acts as a plasticizer for the starch polymers. When starch granules are heated in sufficient water (60%) the plasticized amorphous regions in the starch granule undergo a transition from the glassy to the rubbery state. This specific transition temperature is the glass transition temperature (Tg). This is similar to the definition of a glass which is a solid capable of supporting its own weight against flow, while a rubber is an undercooled liquid that can exhibit viscous flow.

In typical food processing conditions there is sufficient heat and water for the starch granules to swell beyond the point where the process can be reversed. Water enters the spaces between chains, disrupts interchain bonding, and establishes hydration layers in the granule separating the macromolecules. This lubrication or plasticizing effect causes the starch polymers to be more separated and enables them to slip or be considered partially dissolved. Ultimately the starch will swell to several times the original size.

The properties of cooked starches exhibit a range of characteristics that can be used to further classify starches (Table 4.11). Cereal starches (corn, wheat and rice) form viscous short bodied pastes and result in opaque gels on cooling. Potato and tapioca starches form clear, weak gels on cooling. Waxy starches form heavy bodied stringy clear pastes upon cooling and relatively poor gel formation. High amylose corn starch requires very high temperatures for gelatinization resulting in short bodied firm opaque gels on cooling. Starches fill a wide range of roles in food production; mainly they are used to absorb water and produce viscous fluids, pastes and gels for control of texture in foods. The extent of gelatinization of starch in baked goods has a major impact on product properties including texture, storage stability and rate of digestion. In high fat low moisture cookies and pie crusts as much as 90% of the starch in the product is not gelatinized. On the other extreme in white bread and angel food cake 96% of the starch is gelatinized and in many cases deformed.

Resistant Starch

Resistant starch (RS) is starch that is not digested in the normal gastric process (Englyst et al. 1987; Sajilata et al. 2010) and as a result falls in the category of dietary fiber. RS is not rapidly digested like ordinary starch, and imparts the biological benefits of fermentable dietary fiber. RS is defined as the fraction of starch, which escapes digestion in the small intestine, and may be fermented by the micribiome in the large intestine (Englyst et al. 1992). Various factors contribute to starch's resistance to digestion, thus there are four categories of RS, each with similar resis-

Table 4.11 A summary of the size and some physical characteristics of commonly used food starches

	Corn Starch	Waxy Maize	High-Amylose Corn	Potato	Tapioca	Wheat	Rice
Granule size (μm)	2–30	2–30	2–24	5–100	4–35	2–55	<1–9
% Amylose	28	<2	50–70	21	17	28	0–21
Gelatinization/Pasting°C	62/80	63/72	66/170[a]	58-65	52-65	52-85	65–75
Relative viscosity	Medium	Medium	Very Low	Very High	High	Low	low
		High					
Paste Rheology	Short	Long	Short	Long	Long	Long	Short
Paste clarity	Opaque	Cloudy	Opaque	Clear	Clear	Opaque	Opaque
Retrodegredation	High	Low	High	Low	Low	High	High

When choosing a starch for a particular application these characteristics should be considered
Source: From BeMiller JN et al. (2008). Carbohydrates in *Fennema's Food Chemistry, Fourth Edition* S. Damodaran, KL. Parkin, OR. Fennema (eds) p 84–151. Taylor and Francis
[a]No increase in viscosity was observed up to 100 °C. Pasting does not occur until temperatures approach 170 °C

Table 4.12 Types of resistant starch

Type of RS	Description	Food sources
RS_1	Physically protected	Whole or partially milled grains and seeds, legumes
RS_2	Ungelatinized resistant granules with type B crystallinity, slowly hydrolyzed by α-amylase	Raw potatoes, green bananas, some legumes, high amylose corn starch
RS_3	Retrograded starch	Cooked and cooled potatoes, bread, corn flakes, foods with repeated moist heat treatment and cooling
RS_4	Chemically modified starches by crosslinking or acylation	Foods where modified starches are added, i.e. bread, cakes

tance properties but very different origins. The four categories of RS are listed in Table 4.12.

RS_1 is the physically protected form of starch found in green bananas, grains, and pulses. It is physically inaccessible because it is not hydrated and attack by enzymes is prevented by the tissue structures. RS_2 is raw starch granules, where the starch is tightly packed in a radial pattern and is relatively dehydrated. This dense structure limits the accessibility of digestive enzymes and accounts for the resistant nature. RS_2 is typically found in raw potato, banana, and high-amylose starch cereals. RS_3 is retrograded starch. The formation of RS_3 occurs when the starch granule is completely hydrated, the amylose leaches out of the granules into the solution as a random coil polymer. When the solution cools the amylose polymer chains begin to reassociate as double helices which are stabilized by hydrogen bonds in a gel (Wu and Sarko 1978). A typical example of RS_3 formation occurs when bread or rolls become stale. The stiff texture of stale products is a result of the retrogradation process. This effect can be partially reversed by reheating the product. RS_4 includes structures of modified starches obtained by chemical treatments like distarch phosphate ester. Starch with structure intermediate between the more crystalline resistant starch (for example, RS_3 in staled bread) and more amorphous rapidly digestible starch (for example, in boiled potato) is slowly digestible starch (Lehmann and Robin 2007). Slowly digestible starch results in slower release of glucose causing smaller postprandial blood glucose peaks and is therefore useful in the diabetic diet.

RS is classified as a component of fiber on the basis of the recent definitions of dietary fiber given by AACC (2000) and NAS (2002). Portions of RS consist of low-molecular-weight dextrins, however, the bulk consists of polymers, of which retrograded amylose often forms the major fraction (Ranhotra et al. 1991a). RS has an impact similar to soluble dietary fiber in the colonic health by increasing crypt cell production rate, or decreasing the colonic epithelial atrophy in comparison with no-fiber diets. RS influences tumorigenesis, and reduces serum cholesterol and triglycerides. One of the modes of action for RS is that it is fermented to short chain fatty acids by the microbiome. The short chain fatty acids are rapidly utilized by the colonic epithelium.

Hydrolysis of Starch

Hydrolyzed starches have many applications in foods ranging from limited degrees of hydrolysis for texture modifiers and foam stabilization, to greater degrees hydrolysis for use as sweeteners and fermentation substrates. Most carbohydrate polymers, including starch, are readily hydrolyzed by hot acids or enzymes. Greater hydrolysis of the starch results in the formation of dextrins. The dextrins tend to result in less viscous preparations and can be used at higher levels in foods as fillers. They can also be used as coatings for panning and carriers of spray dried flavors and colors. Some dextrins retain long linear chains of amylose fragments which result in the formation of strong gels. More extensive hydrolysis of starch with acid or enzymes results in the formation of maltodextrins. Maltodextrins are categorized by their chain length which is expressed as dextrose equivalents. The degree of hydrolysis is expressed as dextrose equivalent (DE), defined as the amount of reducing sugars present as dextrose and calculated as a percentage of the total dry matter. Table 4.13 lists corn syrups with various DE. The Dextrose Equivalent

Table 4.13 Composition of representative corn syrups

Type of conversion	Dextrose *equivalent*	Saccharides (%)							
		Mono-	Di-	Tri-	Tetra-	Penta	Hexa-	Hepta-	Higher
Acid	30	10.4	9.3	8.6	8.2	7.2	6.0	5.2	45.1
Acid	42	18.5	13.9	11.6	9.9	8.4	6.6	5.7	25.4
Acid-enzyme	43	5.5	46.2	12.3	3.2	1.8	1.5	–	29.5[a]
Acid	54	29.7	17.8	13.2	9.6	7.3	5.3	4.3	12.8
Acid	60	36.2	19.5	13.2	8.7	6.3	4.4	3.2	8.5
Acid-enzyme	63	38.8	28.1	13.7	4.1	4.5	2.6	–	8.2[a]
Acid-enzyme	71	43.7	36.7	3.7	3.2	0.8	4.3	–	7.6[a]

Source: From J.D. Commerford, Corn Sweetener Industry, in *Symposium: Sweeteners,* I.E. Inglett, ed., 1974, AVI Publishing Co.
[a]Includes heptasaccharides

(DE) is related to the average degree of polymerization (DP) in the maltodextrin by the equation:

$$DE = 100/DP.$$

The DE can also be considered as the percentage of reducing power that would come from pure glucose. The DE, therefore, is inversely related to average molecular weight of remaining polymers in the material.

When starches are further hydrolyzed to DE values of 20–60 they are referred to as corn syrup solids. These syrups are hygroscopic and rapidly dissolve in water. Syrups with a DE of 42 are extensively used in food products (Table 4.13). The syrups have a high osmolality which inhibits growth of most microorganisms yet are highly resistant to crystallization. An example is pancake syrup which is colored with caramel and added flavor, but consists primarily of DE 42 syrup. The acid conversion process has a practical limit of 55 DE; above this value, dark color and bitter taste become prominent. There is a fairly constant relationship between the composition of acid-converted corn syrup and its DE. The composition of syrups made by acid-enzyme or dual-enzyme processes cannot be as easily predicted from DE.

Maltodextrins are defined as hydrolyzates with measurable DE value of less than 20. Low DE maltodextrins have the highest molecular weight and are non-hygroscopic, whereas, high DE maltodextrins have the lowest molecular weight can become very hygroscopic. Maltodextrins are excellent bulk fillers for foods and contribute little or no sweetness. Further hydrolysis results it mixtures of malto-oligosaccharides, maltose and glucose. Maltodextrins (DE < 20) have compositions that reflect the physico-chemical characteristic of the starch used, particularly the amylose/amylopectin ratio of the starch. A maltodextrin with DE 12 shows retrogradation in solution, producing cloudiness. In comparison, a maltodextrin from waxy corn at the same DE does not show retrogradation because of the higher level of α-1 → 6 branches. As the DE decreases, the differences become more pronounced. A variety of maltodextrins with different functional properties, such as gel formation, can be obtained by using different starch raw materials. Maltodextrins of varying molecular weights are plasticized by water and decrease the glass transition temperature. Maltodextrins can retard the crystallization of amorphous sucrose and, at high concentrations, totally inhibit sucrose crystallization (Roos and Karel 1991c). Maltodextrins with low DE and with little or no remaining polysaccharide can be produced by using two enzymes. Alpha-amylase randomly hydrolyzes 1 → 4 linkages to reduce the viscosity of the suspension. Pullulanase is specific for 1 → 6 linkages and acts as a debranching enzyme. The application of these two enzymes makes it possible to produce maltodextrins in high yield (Kennedy 1985). The action of

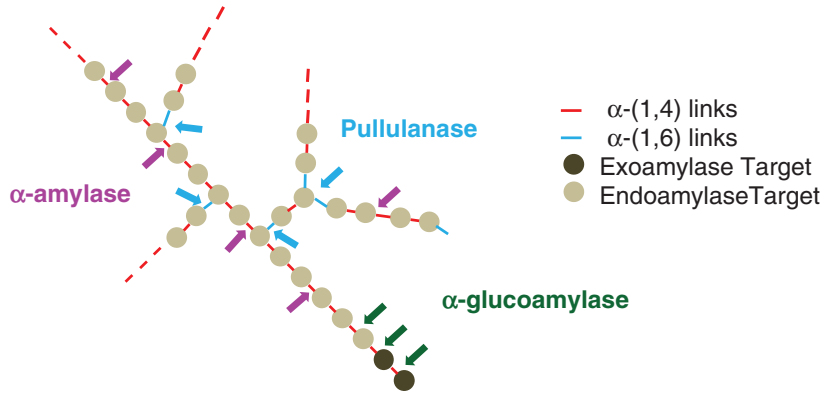


Fig. 4.36 Schematic representation of the action of starch-degrading enzymes. β-Amylase can also hydrolyze disaccharide maltose units from the non-reducing end of amylose and amylopectin

starch degrading enzymes is discussed in more detail below.

Starch Degrading Enzymes

There are various starch degrading enzymes, with different actions, that are described below and illustrated in Fig. 4.36.

α-Amylase

The enzymatic hydrolysis of starch by α-amylase is important for both processing and *in vivo* digestion. α-Amylase is an endoamylase that cleaves both amylose and amylopectin producing oligosaccharides. The products are the result α-amylase hydrolyzing the 1 → 4 linkages of the starch. It does not attack double helix regions or starch helical sections that are complexed with lipids. The resulting hydrolysis products from amylopectin remain branched via 1 → 6 linkages. The bacterial amylases used in processing starch are similar to the amylases in animal digestive tracts. This is why starches with large helical regions are resistant to digestion.

Glucoamylase

Glucoamylase is an exo-amylase that is used in combination with α-amylase to produce D-glucose syrups and crystalline D-glucose. Glucoamylase requires the starch to be gelatinized and sequentially hydrolyzes glucose units of the non-reducing ends amylose and amylopectin. It has the ability to hydrolyze both 1 → 4 and 1 → 6 linkages. Glucoamylase can completely hydrolyze starch to glucose, however combing it with α-amylase makes the process more efficient by generating more non-reducing ends for hydrolysis.

β-Amylase

β-Amylase hydrolyzes disaccharide maltose units from the non-reducing end of amylose and amylopectin. It does not cleave 1 → 6 linkages. Therefore, when amylopectin is the substrate, in addition to maltose it yields pruned or unhydrolyzed fragments which are named limit dextrins.

Pullulanase and Isoamylase

Pullulanase and isoamylase are "debranching" enzymes that specifically hydrolyze 1 → 6 bonds in amylopectin. This produces shorter linear 1 → 4 chains.

High Fructose Corn Syrup (HFCS)

High fructose corn syrup HFCS) is a major ingredient in the food and beverage industry. HFCS was first marketed in the 1970s and is less expensive than granulated sugar (sucrose). The major use of HFCS has been in beverages and soft-drinks which, at its peak, accounted for 75–80% use (Eggleston et al. 2017). The use of HFCS, however, peaked in 1999 due to consumer concern rather than price or technical use. In particular, consumers are concerned about a possible link between HFCS and metabolic diseases like obesity and diabetes. The flow chart for the commercial production of HFCS is illustrated in Fig. 4.37. The starting material for HFCS production is corn starch. This is slurried in water, mixed with a thermally stable α-amylase and rapidly heated, where the starch is rapidly gelatinized and hydrolyzed by the α-amylase. After cooling to 55–60 °C, the liquefied starch is further hydrolyzed with glucoamylase. The hydrolyzed mixture is then clarified, concentrated, passed through activated carbon and ion exchange beds. Seed crystals are added and D-glucose or D-glucose hydrate crystals are produced. To produce HFCS the solution of D-glucose is passed through a column containing immobilized glucose isomerase (glucofructoisomerase). Glucose isomerase catalyzes the isomerization of D-glucose to D-Fructose through a trans-enediol intermediate, and it was this reaction that opened the way for HFCS production (Dziezak 1987). The equilibrium of the mixture is approximately 58% glucose and 42% fructose. Fructose concentrations are further increased by passing the equilibrium mixture over a cation-exchange column which retains fructose. The fructose is then eluted and can be used to produce the 55% fructose containing sweetener that is typically used in beverages.

Starch Hydrolyzates: Corn Sweeteners

As previously stated, starch can be hydrolyzed by acid or enzymes or by a combination of acid and enzyme treatments. When acid is used, the corn is sprayed with hydrochloric acid and heat applied. This can be accomplished by direct spraying with hydrochloric acid or by moistening the starch and applying hydrogen chloride gas. After heat treatment the hydrochloric acid is neutralized and the desired starch product is washed with water and dried. In lightly treated starches the granules remain intact but the starches cook more quickly and result in thinner and more clear (less turbid) and less viscous solutions. These starches are used for coatings, film formation and pan coating of nuts and candies. Corn starch is an excellent starting material to produce strong, fast setting gels in products like jelly beans and processed cheese loafs. A larger variety of products are obtained from starch hydrolysis by using various starches such as corn, wheat, potato, and cassava (tapioca) starch. Glucose syrups, known in the United States as corn syrups, are hydrolysis products of starch with varying amounts of glucose monomer, dimer, oligosaccharides, and polysaccharides. Depending on the method of hydrolysis used, different compositions with a broad range of functional properties can be obtained.

The initial step in starch hydrolysis by enzymes is also illustrated in Fig. 4.37, and involves the use of a heat-stable endo-α-amylase. This enzyme randomly attacks α-1 → 4 glycosidic bonds resulting in rapid decrease in viscosity. These enzymes can be used at temperatures as high as 105 °C. This reaction produces maltodextrins (Fig. 4.37). The next step is saccharification by using a series of enzymes that hydrolyze either the α-1 → 4 linkages of amylose or the α-1 → 6 linkages of the branched amylopectin. The action of the various starch-degrading enzymes is shown in Fig. 4.37 (Olsen 1995). In addition to products containing high levels of glucose (95–97%), sweeteners with DE of 40–45 (maltose), 50–55 (high maltose), and 55–70 (high conversion syrup) can be produced. High dextrose syrups can be obtained by saccharification with amyloglucosidase. At the beginning of the reaction dextrose formation is rapid but gradually slows down. This decelleration is caused by formation of branched dextrins by enzyme catalyzed transglycosylation reactions, and because at high

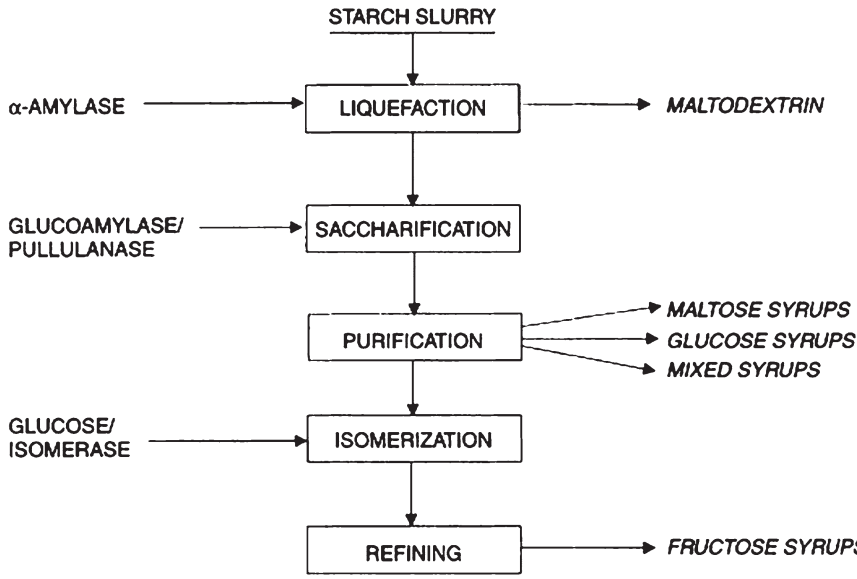


Fig. 4.37 Major steps in enzymatic starch conversion to high fructose corn syrups. *Source:* Reprinted from H.S. Olsen, Enzymic Production0 of Glucose Syrups, in *Handbook of Starch Hydrolysis Products and Their Derivatives,* M.W. Kearsley and S.Z. Dziedzic, eds., p. 30, © 1995, Aspen Publishers, Inc.

dextrose levels the repolymerization of dextrose into isomaltose occurs.

Modified Starches

Chemical modification and physical modifications of starch are frequently used to impart desired functionality in foods. There is a wide array of modified starches available from different starches, as well as various types and extents of modification. Modification of starches results in pastes that more effectively withstand food processing conditions, including shear, heat and acidic conditions, compared to native starches. Modifications can be made to reduce the heat required for gelatinization, increased or decreased paste viscosity, gel clarity, gel strength, reduced syneresis, improved film formation and improved heat stability. Generally chemical modification of starch occurs at low levels (degree of substitution) on the hydroxyl group of the starch. These degrees of substitution (DS) only range from 0.002 to 0.2% but still, however, dramatically alter the functional attributes of the starch.

The properties of starches can be modified by chemical treatments that result in products suitable for specific purposes in the food industry (Whistler and Paschall 1967). Starches are used in food products to produce viscosity, promote gel formation, and provide cohesiveness in cooked starches. When a slurry of starch granules is heated, the granules swell and absorb a large amount of water; this happens at the gelatinization temperature (see Table 4.10), and the viscosity increases to a maximum. The swollen granules then start to collapse and break up, and viscosity decreases. Starch can also be modified by acid treatment, enzyme treatment, cross-bonding, substitution, oxidation, or heat (Table 4.14). Acid treatment results in thin boiling starch. The granule structure is weakened or completely destroyed as the acid penetrates into the intermicellar areas, where a small number of bonds are hydrolyzed. When this type of starch is gelatinized, a solution or paste of low viscosity is obtained. A similar result may be obtained by enzyme treatment. The thin boiling starches yield low-viscosity pastes but retain the ability to form gels on cooling. Acid-converted waxy starches with low amylose levels, produce stable gels that remain clear and fluid when cooled. Acid-

Table 4.14 Primary reactions in starch modification

Physical	Gelatinization	Solubility	Functionality
Heat/Moisture	Above gelatinization temperature but low	Increased paste stability, higher gelatinization temperature	Increases resistant starch
	(27%) moisture		
Annealing	40–60% moisture	Higher gelatinization temperature, slower hydration	Increases resistant starch
	Below gelatinization temperature		
Pre-gelatinization	100 °C excess water	Cold water dispersible	Instant foods, rapid hydration foods
	Drum Drying		
	Extrusion		
Dextrinization	Dry roast acidified Starch	High solubility, higher reducing sugars	Coatings, films, fat replacers in dairy and baked products
Modification			
Partial Acid	HCL, H_2SO_4	Lower molecular weight	Confections, batters and coatings
Hydrolysis	H_3PO_4		
Enzymatic Hydrolysis	Below gelatinization temperature with amylase	Lower molecular weight	Confections, batters and coatings
Alkaline Treatment	NaOH, KOH		
Oxidation	Peracetic acid	Low viscosity, clear gels	Batters, coatings binders and film forming
	Hydrogen Peroxide		
	Sodium hypochlorite		
Derivatization			
Etherification	Propylene oxide	Improved clarity, less retrogradation. Freeze/thaw stability	Gravies, dips sauces, puddings, pie filling
Esterifcation	Acetic Anyhdride	Lower gelatinization temperature, clear pastes	Refrigerated foods, emulsion stabilizers, encapsulation
	Tripolyphosphat	Low retrogradation	
	1-Octonyl-succinic anhydride		
	Acetic + Adipic anhydrides		
	Sodium ortho-phosphate		
	Sodium Tripoly phosphate		
	Phospo-oxy chloride		
Crosslinking	Sodium ortho-phosphate	High stability granules, high gelatinization temperature, Resists shear	Increase viscosity in soups, sauces and dairy products
	Sodium Tripoly phosphate		
	Phospho-oxychloride		

converted starches with higher amylose levels are more likely to form opaque gels on cooling. The acid conversion is carried out on aqueous granular starch slurries with hydrochloric or sulfuric acid at temperatures of 40–60 °C. The action of acid incorporates a preferential hydrolysis of linkages in the non-crystalline areas of the granules. The granules are weakened and no longer swell; they take up large amounts of water and produce pastes of low fluidity.

Cross-bonding of starch involves the formation of chemical bonds between different areas in the starch granule. This makes the granules more resistant to rupture and degradation on swelling and provides a firmer texture. The number of cross-bonds required to modify the starch granule is low; a large change in viscosity can be obtained by as few as 1 cross-bond per 100,000 glucose units. Increasing the number of cross-bonds to 1 per 10,000 units results in a product that does not swell on cooking. There are two methods to cross-link starch. The first, which gives a product known as distarch adipate, involves treating an aqueous slurry of starch with a mixture of adipic and acetic anhydrides under mildly alkaline conditions. After the reaction the starch is neutralized, washed, and dried. The second method, which produces distarch phosphate, involves treating a starch slurry with phosphorous oxychloride or sodium trimetaphosphate under alkaline conditions. Since the extent of cross-linking is low, the amount of reaction product in the modified starch is low. Free and combined adipate in cross-linked starch is below 0.09%. In distarch phosphate, the free and combined phosphate, expressed as phosphorus, is below 0.04% when made from cereal starch other than wheat, 0.11% if made from wheat starch, and 0.14% if made from potato starch (Wurzburg 1995).

Substitution of starch is achieved by reacting some of the hydroxyl groups in the starch molecules with monofunctional reagents that introduce different substituents. The action of the substituents lowers the ability of the modified starch to associate and form gels. This is achieved by preventing the linear portions of the starch molecules to form crystalline regions. The different types of substituted starch include starch acetates, starch monophosphates, starch sodium octenyl succinate, and hydroxypropyl starch ether. These substitution reactions can be performed on unmodified starch or in combination with other treatments such as acid hydrolysis or cross-linking.

Acetylation of starch is undertaken on suspensions of granular starch with acetic anhydride or vinyl acetate. No more than 2.5% of acetyl groups on a dry starch basis are introduced, which equates to a degree of substitution of about 0.1%. Acetyl substitution reduces the ability of starch to produce gels on cooling and also increases the clarity of the cooled sol.

Starch phosphates are monophosphate esters, i.e., only one hydroxyl group is substituted in contrast to the two hydroxyl groups involved in production of cross-bonded starch. They are pro-

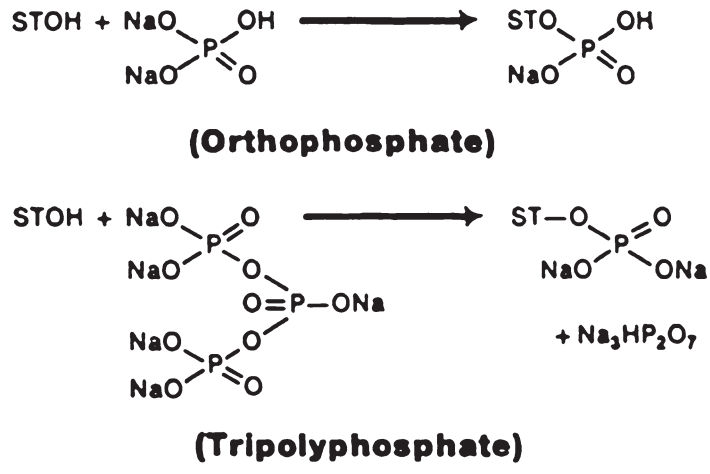


Fig. 4.38 Phosphorylation of starch with sodium ortho- or triphosphate

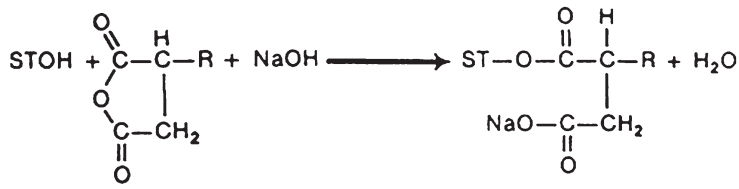
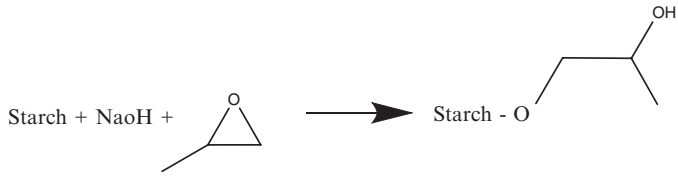


Fig. 4.39 Reaction of starch with octenyl succinic anhydride



Starch plus propylene oxide under alkaline conditions produces hydroxyproplyl starch

Fig. 4.40 Hydroxypropylation of starch

duced by mixing an aqueous solution of ortho-, pyro, or tripolyphosphate with granular starch; drying the mixture; and subjecting this to dry heat at 120–170 °C. The level of phosphorus introduced into the starch does not exceed 0.4%. The introduction of phosphate groups as shown in Fig. 4.38 gives the product an anionic charge (Wurzburg 1995). Starch monophosphates give dispersions with higher viscosity, better clarity, and better stability than the unmodified starch. They also have higher stability at low temperatures and during freezing.

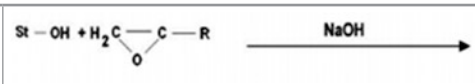
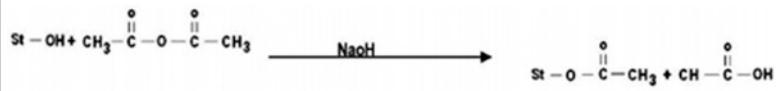
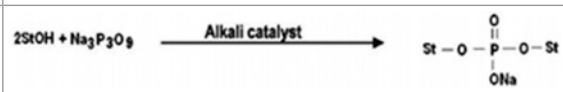
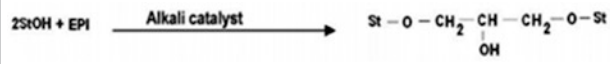
Starch sodium octenyl succinate is a lightly substituted half ester produced by reacting an aqueous starch slurry with octenyl succinic anhydride as shown in Fig. 4.39. The level of introduction of substituent groups is limited to 1 for about 50 anhydroglucose units. The treatment may be combined with other methods of conversion. The introduction of the hydrophilic carboxyl group and the lipophilic octenyl group makes this product amphiphilic and gives it the functionality of an emulsifier (Wurzburg 1995).

Hydroxypropylated starch is prepared by reacting an aqueous starch suspension with propylenol oxide under alkaline conditions at temperatures from 38 to 52 °C. The reaction (Fig. 4.40) is often combined with the introduction of distarch cross-links (Wurzburg 1995).

Oxidized starch is prepared by treating starch with the strong oxidant of sodium hypochlorite. Although this starch is sometimes described as chlorinated starch, no chlorine is introduced into the molecule. The reaction is undertaken by combining a starch slurry with a solution of sodium hypochlorite. Under alkaline conditions carboxyl groups are formed that modify linear portions of the molecule so that association and retrogradation are minimized. In addition to the formation of carboxyl groups, a variety of other oxidative reactions may occur including the formation of aldehydic and ketone groups. Oxidation increases the hydrophilic character of starch and lessens the tendency toward gel formation.

Dextrinization or pyroconversion of starch occurs through the action of heat on dry, powdered starch. Usually the heat treatment is undertaken with hydrochloric or phosphoric acid at levels of 0.15% and 0.17%, respectively. After addition of the acid, the starch is dried and heated in a cooker at temperatures ranging from 100 to 200 °C. Two types of reaction occur, hydrolysis and transglucosidation. At low degree of conversion, hydrolysis is the main reaction and the resulting product is known as white dextrin.

Table 4.15 Some common starch modification reactions

Modification type	
Etherification Hydroxyalkyl starch (with alkylene oxide)	
Esterification Starch acetate (with vinyl acetate)	
Starch acetate (with acetic anhydride)	
Starch phosphate (with orthophosphates)	
Carboxymethyl starch (with mono chloro acetic acid)	
Cross-linking	
With $PoCI_3$	
With STMP	
With EPI	

St starch, *$POCl_3$* phosphorus oxychloride, *STMP* sodium tri-meta phosphate

Table 4.16 Properties and applications of modified starches

Process	Function/property	Application
Acid conversion	Viscosity lowering	Gum candies, formulated liquid foods
Oxidation	Stabilization; adhesion gelling clarification	Formulated foods, batters, gum confectionery
Dextrins	Binding; coating; encapsulation; high solubility	Confectionery, baking (gloss), flavorings, spices, oils, fish pastes
Cross-linking	Thickening; stabilization; suspension; texturizing	Pie fillings, breads, frozen bakery products, puddings, infant foods, soups, gravies, salad dressings
Esterification	Stabilization; thickening; clarification; when combined with cross-linking, alkali sensitive	Candies, emulsions, products gelatinized at lower temperatures
Etherification	Stabilization; low-temperature storage	Soups, puddings, frozen foods
Dual modification	Combinations of properties	Bakery, soups and sauces, salad dressings, frozen foods

Source: Reprinted with permission from O.B. Wurzburg, Modified Starches, in *Food Polysaccharides and Their Applications,* A.M. Stephen ed., p. 93, 1995. By courtesy of Marcel Dekker, Inc.

Transglucosidation involves initial hydrolysis of α 1 → 4 glucosidic bonds and recombination with free hydroxyl groups at other locations. In this manner new randomly branched structures or dextrins are formed; this reaction happens in the more highly converted products known as yellow dextrins. The dextrins have film-forming properties and are used for coating and as binders.

The chemical reactions and changes in structure of chemically modified starch have been

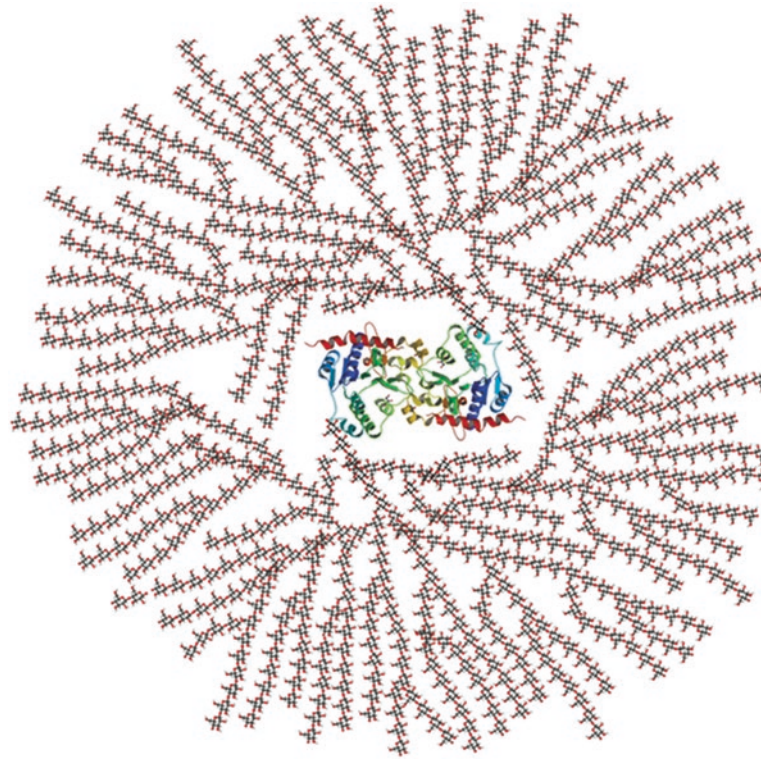


Fig. 4.41 Schematic two-dimensional view of glycogen. A core protein of glycogenin is surrounded by branches of glucose unites. The entire globular granule may contain around 30,000 glucose units. Source: W.D. McArdle, F. I. Katch; V. L. Katch, In *Exercise Physiology: Energy, Nutrition, and Human Performance (2006). Lippincott Williams & Wilkins*

comprehensively described by Singh et al. (2003). Table 4.15 summarizes the primary starch modification reactions. These modifications result in many useful food applications for chemically modified starch. Table 4.16 summarizes some common applications of chemically modified starch.

The properties and applications of modified starches are summarized in Table 4.10 (Wurzburg 1995). The application of modified starches as functional food ingredients has been described by Luallen (1985).

Glycogen

Humans, animals, and fungi produce glycogen in muscle as an energy reserve. Glycogen is a highly branched polysaccharide of glucose. Figure 4.41 illustrates the highly branched structure of glycogen.

Glucoses are linked together linearly by α-(1 → 4) glycosidic bonds, and branches are linked to the chains with α-1 → 6 glycosidic bonds. The outermost branches of the chains are 6–7 glucose units long. Glycogen synthesis is initiated by autoglucoslylation of a protein, glycogenin-1 (Fig. 4.41) (Lomako et al. 2004).

In humans, glycogen, hydrated with three or four parts of water, is produced and stored primarily in the cells of the liver and muscles. Muscle glycogen is converted into glucose by muscle cells, and liver glycogen coverts to glucose for use throughout the body. Glycogen is found in the form of granules in the cytosol/cytoplasm in many cell types, and plays an important role in the glucose cycle. Glycogen is similar to starch in is readily rehydrolyzed to produce glucose for the muscle tissue on demand.

In the liver, when a meal containing carbohydrates or protein is eaten and digested, blood glucose levels rise, and the pancreas secretes insulin. Insulin acts on blood glucose in the liver to stimulate the action of several enzymes including glycogen synthase. Glucose are added to the chains of glycogen as long as both insulin and glucose

remain plentiful. When it is needed for energy, glycogen is broken down by glycogen phosphorylase and converted again to glucose (Manners 1991). In comparison, muscle cell glycogen serves as an immediate reserve source of available glucose for muscle cells only.

Glycemic Index

The glycemic index (GI) is a number associated with a particular type of food that indicates the food's effect on a person's blood glucose (also called blood sugar) level). A value of 100 represents the standard, which equivalent to pure glucose. The GI represents the rise in a person's blood sugar level 2 h after consumption of the food, and is useful to understand who the body breaks down available carbohydrates in foods, i.e., starch. The glycemic effect of foods depends on a few factors: type of starch or carbohydrate, physical entrapment of the carbohydrates within the food, and fat and protein contents of the foods (Anon 2017). The GI is typically applied in the context and quantity of the food and the amount of carbohydrate in the food that is actually consumed. A related measure, the glycemic load (GL), factors this by multiplying the glycemic index of the food by the carbohydrate content of the serving. Watermelon, for example, had a high GI but a low GL for the quantity usually consumed. Glycemic index Charts often give only one value per food, but variations occur due to, for example, variety, ripeness, cooking methods, processing, and the length of food storage (Anon 2017).

Cellulose

Cellulose is one of the most widely distributed compounds in nature generally existing as homologus polymers accompanied by other polysaccharides and lignins. Cellulose can have DP as high as 10,000 which would translate to a molecular weight of approximately 1,620,000 Da. It provides structural support to most plants and includes the major component in wood. Cotton cellulose is used for fabric and can be used in some food applications. Cellulose is a polymer of β-D-glucose joined with β-1 → 4 linkages. In the native state, cellulose occurs as extended ribbons of twofold chain geometry stabilized by hydrogen bonding of each successive residue to its nearest neighbors. The great strength, fibrous character, insolubility and inert characteristics of cellulose, integral to its skeletal function in plant cell walls are due to the ordered packing of the cellulose chains (Rees 1977) The hydrogen bonds result in a high degree of crystallinity and dense structure contributing to areas that do not absorb water effectively and exhibit a high degree of enzyme resistance. When foods are dried the amorphous regions of cellulose become increasing crystalline in nature and result in toughness of dried products.

In food products, the crystalline area of the cellulose absorbs water poorly, thus it is highly resistant to enzymatic attack. The amorphous regions absorb water and swell although they do not dissolve and they remain essentially indigestible. Higher degrees of crystallinity provide greater tensile strength and increased elastic modulus. An example of this is that when foods like carrots are dehydrated, crystallinity increases and the foods become tougher. Heating cellulose causes modest reductions in hydrogen bonding which results in some swelling of the cellulose.

Cellulose and modified celluloses are used in a wide range of foods to provide important physical characteristics including bulk replacements of digestible carbohydrates in low calorie foods. Unmodified cellulose and its derivatives can be used to provide bulk, imbibe oils or flavors, or serve as carriers to improve the flow characteristics of intense sweeteners or flavors. The attributes of cellulose are based on its physical characteristics including limited interaction with water, its insolubility in water, specific rheological characteristics it brings to the product and the resulting texture of the product. For example, finely ground or microcrystalline cellulose provides bulk to low calorie foods. Chemically modified celluloses provide emulsification, modification of texture, emulsification, foam stabilization, water binding and ice crystal formation. With modified cellulose the functions are determined by the chain length of the cellulose poly-

Table 4.17 Applications of commercial food cellulose products

Cellulose/modified cellulose	Food applications
Hydroxypropyl cellulose	Whipped toppings, mousses, extruded foods
Hydroxypropylmethyl cellulose	Whipped toppings, mousses, baked goods, bakery fillings, icings, fried foods, sauces, dressings, frozen desserts, reduced fat foods
Methyl cellulose	Sauces, soups, breads, tortillas, fried foods, reduced fat foods, foams
Microcrystalline cellulose	Dressings, sauces, baked goods, beverages, whipped toppings, reduced fat foods
Powdered cellulose	Breads, cookies, pastries, pasta, imitation cheese, cereals, canned meats
Sodium carboxymethyl cellulose	Frozen desserts, baked goods, dressings, sauces, beverages, animal foods, reduced calorie foods, extruded foods

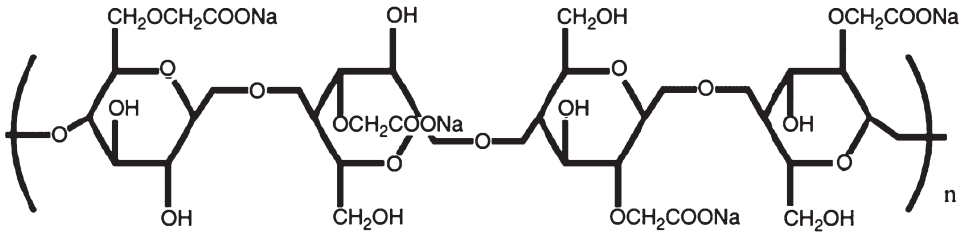


Fig. 4.42 Carboxymethyl modified cellulose repeating units

mer, the physical characteristics of the particular cellulose (amount of amorphous or crystalline region), the chemical modification, the degree of modification and the interactions with other ingredients in the food matrix.

The addition of carboxyl groups increases the hydrophilic nature of the cellulose, where the addition of hydrocarbons such as methyl or ethyl groups makes the cellulose more hydrophobic. The chain length of the cellulose polymer influences the change in viscosity with longer chains resulting in greater increases in viscosity. When producing films or coatings shorter chain length is generally preferred. Table 4.17 summarizes the applications of some commonly used modified celluloses.

Carboxymethyl modification of cellulose is one of the most common modifications used to produce cellulose gum (Fig. 4.42). The characteristics of the gum are influenced by chain length and degree of substitution. Longer chains result in higher viscosity. Higher substitution increases water holding capacity. Addition of water soluble solvents such as glycerine and ethanol increase the viscosity of the gum. Increasing the degree of substitution increases the tolerance to dilution of water by ethanol substantially. Carboxymethylcellulose solutions can remain clear at up to 50% ethanol in the solution. When the degree of substitution is lower the gum is less soluble but binds more water.

Microcrystalline Cellulose

Microcrystalline cellulose is a type of purified and partially depolymerized cellulose (DP is typically less than 400) that is white, odorless, tasteless, and occurs as a crystalline powder made up of porous particles. MCC is synthesized from α–cellulose. It can be synthesized by different processes including reactive extrusion, enzyme mediated reactions, steam explosion, and acid hydrolysis. The role of these reactions is to destroy the amorphous regions of cellulose, leaving only the crystalline domains. MCC has become a very valuable additive in the food, pharmaceutical, cosmetic, and other industries. In the food industry, MCC can be used as an important base in functional foods to: (1) maintain emulsification and foam stability, (2) maintain high temperature stability, (3) improve liquid stability, and (4) act as a nutritional supplement and thickener.

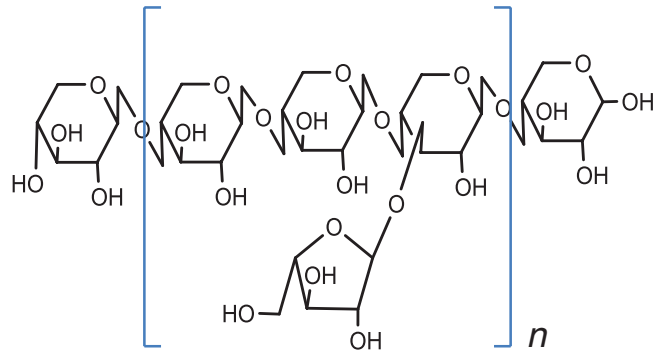


Fig. 4.43 Wheat flour pentosan repeating unit of L-arabinofuranosyl linked by β1 → 4 glycosidic bonds to D-xylopyranose backbone

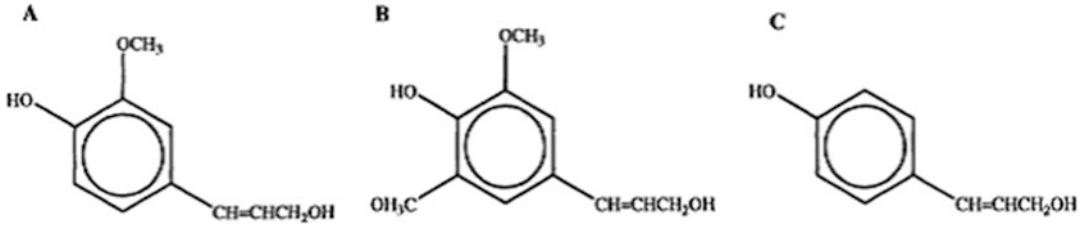


Fig. 4.44 Monomeric components of lignin: (**a**) *trans*-coniferyl alcohol, (**b**) *trans*-sinapyl alcohol, (**c**) *trans*-p-coumaryl alcohol

Pentosans/Hemicelluloses

Pentosans and hemicelluloses are groups of non –starch and non-cellulosic polysaccharides which are widely distributed in plants. Hemicelluloses are water insoluble polysaccharides and pentosans are water soluble non-strachy polysaccharides (D'Appolonia et al. 1971). Hemicelluloses are structural plant polysaccharides that are mostly composed of non-glucose containing sugars. They are classified based on the carbohydrate sugars in the polymer. Sugars in hemicelluloses can be xylose, arabinose, mannose or galactose. The hemicelluloses generally contain polymers of two to four different sugar units. Cereals are common sources of hemi-celluloses and pentosans. Arabinose and xylose are the primary pentoses found in pentosans and hemicellulose. Hexose units include glucose, galactose, rhamnose, glucuronic acid and 4-)-methyl-D-glucuronic acid.

In wheat flour the pentosans are arabinoxylans as shown in Fig. 4.43. The hemicelluloses in wheat contain 59% L-arabinose, 38.5% D-xylose, and 9% D-glucuronic acid. It is a highly branched with arabinose on the side chains and an acidic polysaccharide core with seven to eight xylopyranose units and a one D-glucuronic acid attached by a 1 → 2 linkage at the branch point.

The water-soluble pentosans are highly branched, highly viscous, and gel forming. Because of these properties, it is thought that the pentosans may contribute to the structure of bread dough. Hoseny (1984) has described the functional properties of pentosans in baked foods. One of the more significant properties is due to the water-soluble pentosans, which form very viscous aqueous solutions. These solutions are subject to oxidative gelation with certain oxidizing agents. The cross-linking of protein and polysaccharide chains creates high molecular weight compounds that increase the viscosity and thereby change the rheological properties of dough. Wheat flour contains 2–3% arabinoxylans. These water soluble pentosans are viscous and gel forming. Wheat flour contains 2–3% of water soluble and insoluble pentosans. The soluble portion represents about 0.5–0.8% of the dry weight of the flour. It is estimated that the pentosans absorb about one third of the water in a normal dough. Treatment of doughs with pentosanase hydrolyzes the pentosans to oligomers and monomers of arabinose and xylose thereby drastically

reducing the viscosity of the dough and reducing bake time for cookies and crackers. Pentosanase treatment is also important in reducing the viscosity of low/no fat doughs.

Lignin

Although lignin is not a polysaccharide, it is included in this chapter because it is a component of dietary fiber and an important constituent of plant tissues. Lignin is present in mature plant cells and provides mechanical support, conducts solutes, and provides resistance to microbial degradation (Dreher 1987). Lignin is always associated in the cell wall with cellulose and hemicelluloses, both in close physical contact but also joined by covalent bonds. Lignins are defined as polymeric natural products resulting from enzyme-initiated dehydrogenative polymerization of three primary precursors: *trans*-coniferyl, *trans*-sinapyl, and *trans*-p-coumaryl alcohol (Fig. 4.44). Lignin occurs in plant cell walls as well as in wood, with the latter having higher molecular weights. Lignin obtained from different sources differs in the relative amounts of the three constituents as well as in molecular weight. The polymeric units have molecular weights between 1000 and 4000 Da. The polymeric units contain numerous hydroxylic and ether functions, which provide opportunities for internal hydrogen bonds. These properties lend a good deal of rigidity to lignin molecules. One of the problems in the study of lignin composition is that separation from the cell wall causes rupturing of lignin-polysaccharide bonds and a reduction in molecular weight so that isolated lignin is never the same as the *in situ* lignin (Sarkanen and Ldwig 1971).

Cyclodextrins

When starch is treated with a glycosyltransferase enzyme (CGTase), (E.C. 2.4.1.19; CGTase) cyclic polymers are formed that contain six, seven, or eight glucose units. These are known as α-, β-, and γ-cyclodextrins, respectively. The structure of β-cyclodextrin is shown in Fig. 4.45. These ring structures have a hollow cavity that is relatively hydrophobic in nature because hydrogen atoms and glycosidic oxygen atoms are directed to the interior. The outer surfaces of the ring are

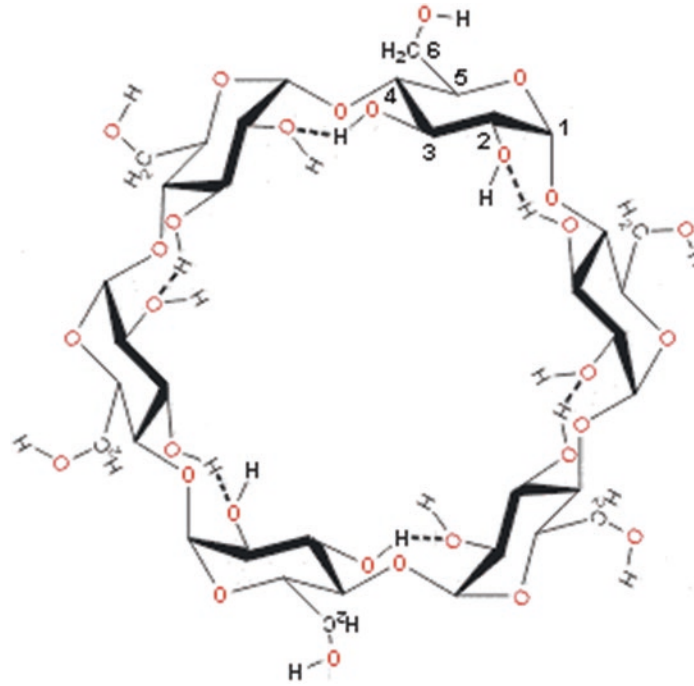


Fig. 4.45 Molecular structure of cyclodextrin showing linkages

Table 4.18 Properties of the main cyclodextrins used in food products

Cyclodextrin	Mass	Outer diameter (nm)	Cavity diameter (nm)		Cavity volume (mL/g)	Solubility, g/kg H_2O	Hydrate H_2O	
			Inner rim	Outer rim			Cavity	External
α, $(glucose)_6$	972	1.52	0.45	0.53	0.10	129.5	2.0	4.4
β, $(glucose)_7$	1134	1.66	0.60	0.65	0.14	18.4	6.0	3.6
γ, $(glucose)_8$	1296	1.77	0.75	0.85	0.20	249.2	8.8	5.4

Source: From Sabadini 2006; http://www1.lsbu.ac.uk/water/cyclodextrin.html#r915

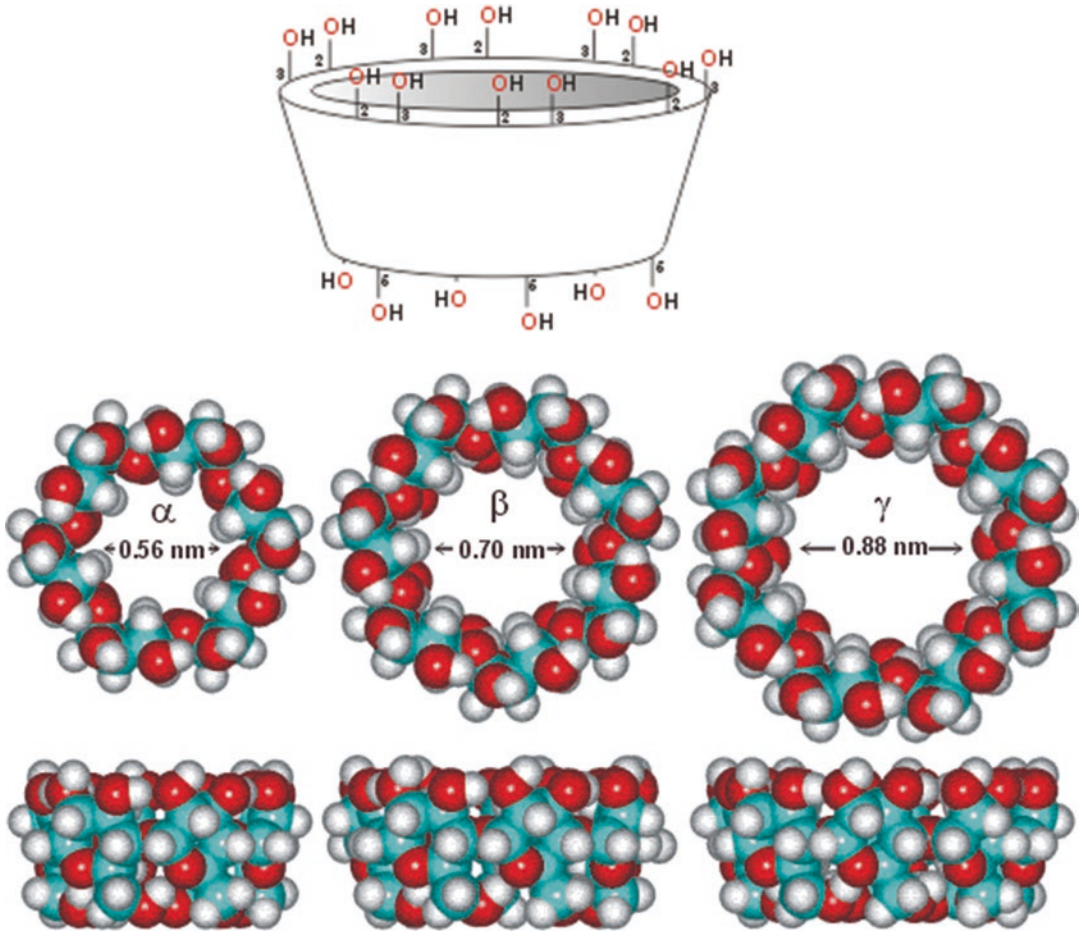


Fig. 4.46 Structures of α, β and γ cylodextrins

hydrophilic because polar hydroxyl groups are located on the outer edges. The hydrophobic nature of the cavity allows molecules of suitable size to be complexed by hydrophobic interaction. These stable complexes may alter the physical and chemical properties of the guest molecule. For example, vitamin molecules could be complexed by cyclodextrin to prevent degradation. Other possible applications have been described by Pszczola (1988). A disadvantage of this method is that the complexes may become insoluble. This can be overcome by derivatization of the cyclodextrin, for instance, by selective méthylation of the C2 and C3 hydroxyl groups (Szejtli 1984).

Cyclodextrins have many applications in the food industry (Astray et al. 2009). The three most

widely used cyclodextrins contain six, seven, or eight D-glucopyranonsyl residues (α-, β-, and γ-cyclodextrin respectively) linked in a ring by α-1 → 4 glycosidic bonds. The glucose residues have the $^4C_1$(chair) conformation. All three cyclodextrins have similar structures differing only in number of glucose residues (Table 4.18). Their shape is like a bottomless bowl. The molecule is stiffened by hydrogen bonding between the 3-OH and 2-OH groups around the outer rim.

The different chain lengths of the polymers result in different dimensions of the cavities. The cavities have different diameters dependent on the number of glucose units (empty diameters between anomeric oxygen atoms illustrated in Fig. 4.46. The side rim depth (shown below in the diagrams) is the same for all three (at about 0.8 nm).

Cyclodextrin rings have a wider rim with the 2- and 3-OH groups and the narrower rim with 6-OH group on its flexible appendage. The hydrophilic groups are on the outside of cyclodextrin and the inner surface is hydrophobic lined with the ether-like anomeric oxygen atoms and the C3-H and C5-H hydrogen atoms. The hydrophobic cavity contains about 3 (α-DC), 7 (β-DC) or 9 (γ-DC) poorly held and easily displaceable water molecules. The hydrophilic cyclodextrin molecules may bind non-polar suitably-sized aliphatic and aromatic compounds such as aroma compounds and lipophilic drugs in the hydrophobic cavity. They may bind in 1:1, 2:1 and 1:2 ratios dependent on the molecules involved (for example, two molecules of γ-cyclodextrin bind well to single C_{60}-fullerene molecules (Buvári-Barcza et al. 2001). Such binding also allows cyclodextrins to be used to increase the water solubility of normally hydrophobic compounds or minimize undesirable properties such as odor or taste in certain food additives. Cyclodextrin complexes are now widely used in the pharmaceutical, food and cosmetic and toiletry fields (Hedges 1998).

The cyclodextrins, by themselves, are natural, non-toxic additives. The hydroxyl groups may be

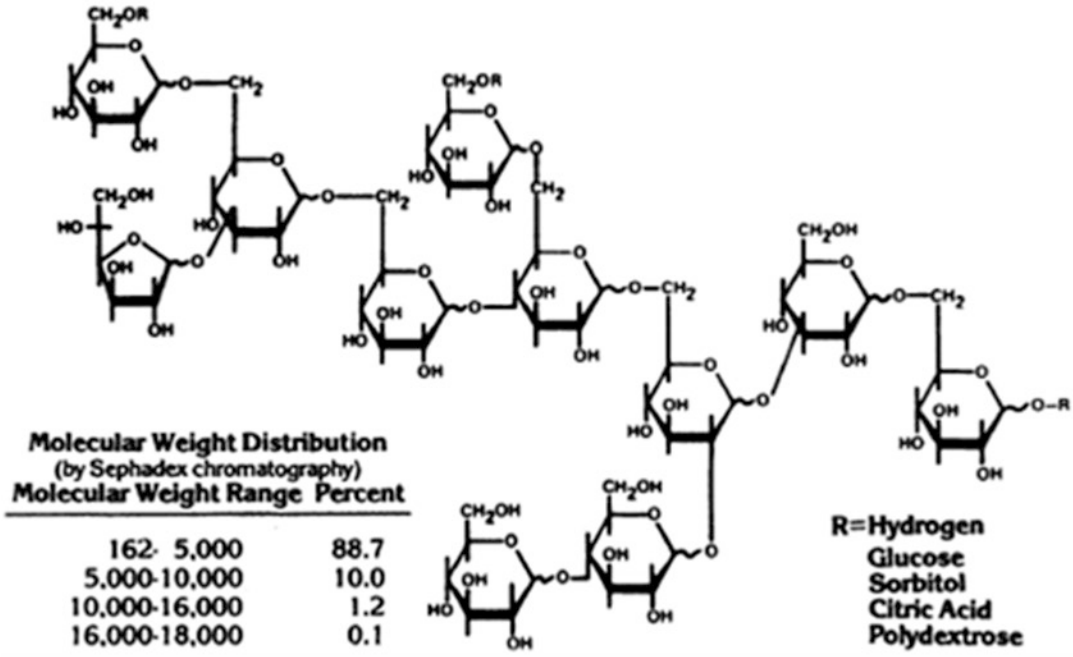


Fig. 4.47 Hypothetical structure of polydextrose repeating unit

derivatized to modify the specificity, physical and chemical properties of the cyclodextrins. The 6-OH groups are most easily derivatized.

Polydextrose

Polydextrose is synthetically produced low calorie polymer. It is produced by a random transglycosylation polymerization of glucose with minor amounts of sorbitol and citric acid. Polydextrose is a randomly bonded condensation polymer of glucose. It is synthesized in the presence of minor amounts of sorbitol and citric acid. The polymer contains all possible types of linkages between glucose monomers, resulting in a highly branched complex structure (Fig. 4.47). Because of the material's unusual structure, it is not readily broken down in the human intestinal tract and therefore supplies only 1 calorie per g. It is described as a *bulking agent* and can be used in low-calorie diets. It provides no sweetness. When polydextrose use is combined with artificial sweeteners, a reduction in calories of 50% or more can be achieved (Smiles 1982).

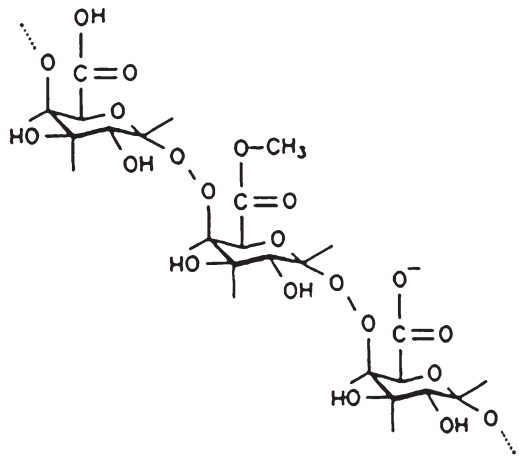


Fig. 4.48 A repeating segment of galacturonic acid units in pectin. *Source:* Reprinted with permission from D.G. Oakenfull, The Chemistry of High Methoyxl Pectins, in *The Chemistry and Technology of Pectin*, R.H. Walter, ed., p. 87, © 1991, Academic Press

Pectins

The middle lamellae of plant cell walls contain pectic materials that serve to cement the cellulosic network and help control the movement of water. These pectic materials can be attached to cellulose fibers and/or xyloglucans through glycosidic bonds. When heated under acidic conditions the pectin substances are hydrolyzed to form pectin and as the reaction continues they become soluble pectin. As fruit ripens similar hydrolysis takes place. Pectin is primarily composed of repeating units of α-1 → 4-galacturonic acid. The structure is a homologous polymer of 1 → 2-α-D-galactopyranosyluronic acid (Fig. 4.48). Pectins can also contain α-D-galactouronan which is a heteogenious section formed by repeating units of 1 → 2-α-L-rhamnosyl-(1-L)α-D-galactosyluronic acid. The main blocks in pectin are branched galacturonan chains that are periodically interrupted with rhamnose units which causes bending in the chain. The rhamnose units can carry many sidechain units. The branched units alternate with unbranched blocks. The rhamnose in the branched blocks form galactan and arabinan blocks or arabinogalactan side chains form through a 1 → 4 linkage with the rhamnose. The galacturonic acid is partially esterified with methyl groups, and the degree of esterification varies among plant species. Pectins are all similar but various species exhibit differences in chain length, degree of esterification and in some cases other sugars will be on the side chains. The number of units between branch points ranges from 8 to 20 residues.

The most important factors in determining properties are chain length and degree of methylation. Pectins range from 9 to 12% methyl esters, although the theoretical maximum would be 16%, no pectin has been reported with that extent of methyoxylation. Treatment of pectin with alkali results in hydrolysis of the methyl esters. Complete hydrolysis of the esters results in forming pectic acid which is completely insoluble.

In the presence of calcium ions, sugar and acid pectin forms gels. These gels are three dimensional networks of pectins that bind large amounts

of water. The pectins for this purpose should be branched and form interchain associations through hydrophobic, hydrogen or ionic bonds. The ability to form these gels is controlled by the composition of the side chains, degree of polymerization, composition of the side chains and degree of methylation.

Two types of pectins are commonly used in foods. They are referred to as High methoxy- (HM) and Low methoxy- (LM) based on degree of methylation. HM pectin has 50–80% of the acid sites esterified with methyl esters and the LM has 25–50% esterification. The HM forms acid gels and the LM forms gels with Calcium ions acting as bridges between exposed carboxylic acid groups. For HM gels to form stable gels they require the pH to be below 3.6 and the sugar content to be at least 55% by weight. The HM gels are formed as a result of hydrogen and hydrophobic bonding that are stabilized as a result of the high sugar concentration which acts as a dehydrating agent in the system.

Commercially pectins are graded based on the quantity of sugar required to gel one part pectin to an acceptable firmness. Common conditions for establishing pectin grades are pH 3.2 to 3.5, sugar 65 to 70% and pectin from 0.2 to 1.5%. Commercial pectin grades range from 100 to 500. The degree of methylation in Rapid Set pectin is over 70%. The pectin gels form at a pH 3.0 to 3.4. The gel strength is determined by chain length: the longer the chain the firmer the gel. Degree of methylation does not have a significant effect on gel strength. Slow set pectin has a degree of methylation of 50–70% in a pH range from 2.8 to 3.2. These pectins form gels at lower temperatures than the rapid set. LM pectins form gels with Calcium ions and are not influenced significantly by sugar or pH. Gel strength is dependent on degree of methylation and molecular weight.

The gel formation of pectin (homogalacturonan) with calcium ions is one of the most common uses of pectin in foods. The gel is formed when calcium ions interact with the free carboxylic acid groups on the galacturonan polymer. These pectin gels are used in jams, jellies, confections, desserts, and dairy products such as yogurt. The degree gelatinization and the strength of the pectin gel is determined by the ratio of free carboxylic acids to methyl esters. Furthermore, the occurrence of more free carboxylic acid groups results in stronger gels.

Table 4.19 Pectin levels in selected fruits and vegetables by two studies

Product	Campbell and Palmer 1978		Zilversmit et al. 1979
	Range	Average	
Apples	0.71–0.84	0.78	0.78
Apricots	0.71–1.32	1.02	1.00
Bananas	0.59–1.28	0.94	0.94
Beans	0.27–1.11	0.69	0.70
Blackberries	0.68–1.19	0.94	0.94
Carrots	1.17–2.92	2.04	2.00
Cherries	0.24–0.54	0.39	0.39
Dewberries	0.51–1.00	0.76	nl[a]
Grapes	0.09–0.28	0.19	0.19
Grapefruit	3.30–4.50	3.90	3.90
Lemons	2.80–2.99	2.90	2.90
Loganberries	0.59	0.59	0.59
Oranges	2.34–2.38	2.36	2.36
Raspberries	0.97	0.97	0.97
Squash	1.00–2.00	1.50	nl
Sweet Potatoes	0.78	0.78	0.78

nl[a] not listed

The pectin content of fruits and vegetables range widely as shown in Table 4.19. In juice manufacture pectin can present problems, because it holds water and reduces the amount of juice that can be extracted. This is frequently resolved by adding pectinases to hydrolyze the pectins in the juice extract.

Pectin is one of the most versatile stabilizers and gelling agents in food applications. Traditionally, pectin was primarily used in the production of jams and fruit jellies in low as well as high sugar products. It helps develop the desired texture, helps keep fruit distributed in the product and prevents water migration to the surface. Some typical applications are listed below:

- Bakery fillings and toppings
  - Fruit preparations for dairy applications
- Fruit applications
  - Jams, jellies, and desserts
- Dairy application

Table 4.20 Structure function properties and applications of polysaccharides

Polysaccharide source	Molecular structure	Function	Applications
Agar Red seaweeds (*Gelidium)*	Sulfated polymers of (1 → 3)-β-D-Galactose and (1 → 4)-3,6-anhydro-α-L-Galactose alternating; pyruvate and methyl side chains	Gelation	Confections, dairy, meat products
		In vivo –Non-digestible	
Carboxymethyl cellulose Modified cotton cellulose	HO_2C-CH_2—group ether linked to O-6 of the linear (1 → 4)-β-D-glucose polymer	Water retention, stabilizer thickener	Ice cream, syrups, meat products, cakes
		In vivo—	
Carageenans	Sulfated polymers of (1 → 3)-β-D-Galactose and (1 → 4)-3,6-anhydro-α-D-Galactose alternating; pyruvate and methyl side chains	Gelation, thickener Stabilizer	Ice cream, desserts, instant pudding, dressing, meat products
Red Seaweed (*Gracilaria, Gigartina, Eucheuma*		*In vivo* –Non-digestible	
Guar Gum/Guar seeds	Linear β-D-(1 → 4)-manan changes appended with α-D-galactose side chains	Water retention, stabilizer, viscosity, retards ice crystal growth	Ice Cream, milk products, desserts, bread, cakes, icings, sauces
Locust Bean Gum/ Locust bean		*In vivo*—bulking agent ~2 kcal/g	
Gum Arabic Stems of *Acacia senegal*	L-Arabino-(1 → 3)and (1 → 6)-β-D-galactan, branched with L-rhamnose and D-galacturopnic acid	Emulsifier, encapsulation, thickener	Confections, dairy, meat products, beverages
		Reduces surface tension, increases fizzing in carbonated beverages	
		In vivo—nearly 100% digestible	
Pectins Citrus, apple etc.	Partially esterified with methyl groups or acetylated Linear and branched (1 → 4)-*a*-D-galacturonic acid;	Gelation, stabilizer, thickener	Jams, Jellies, Preserves, dairy, confections, beverages
		In vivo –	
Xanthan gum	Cellulosic backbone with D-mannose and D-galacturonic acid side chains; Mannose is pyruvylated and acetylated	Thickener, stabilizer	Dairy, dressings, beverages
Xanthomonas campestris		*In vivo*—Non-digestible	

  - Acidified milk and protein drinks
  - Yoghurts (thickening)
- Confectionery
  - Fruit jellies
  - Neutral jellies
- Beverages
- Nutritional and Health Products
- Pharmaceutical and Medical Applications

Gums

This large group of polysaccharides and their derivatives is characterized by its ability to give highly viscous solutions at low concentrations. Gums are widely used in the food industry as gelling, stabilizing, and suspending agents. Compounds in this group come from different sources and may include naturally occurring compounds as well as their derivatives, such as exudate gums, seaweed gums, seed gums, microbial gums, and starch and cellulose derivatives. Table 4.20 lists the source and molecular structure of many of the gums as well as other polysaccharides (Stephen 1995). These polysaccharides are extensively used in the food industry as stabilizers, thickeners, emulsifiers, and gel formers (Stephen 1995).

Gums have hydrophilic molecules, which can combine with water to form viscous solutions or gels. The nature of the molecules influences the properties of the various gums. Linear polysac-

charide molecules occupy more space and are more viscous than highly branched molecules of the same molecular weight. The branched compounds form gels more easily and are more stable because extensive interaction along the chains is not possible. The linear neutral polysaccharides readily form coherent films when dry, and they are good coating agents. Solutions are not tacky. Solutions of branched polysaccharides are tacky because of extensive entangling of the side chains and because the dried solutions do not form films readily. The dried material can be more easily redissolved than can the dried linear compounds.

Neutral polysaccharides are only slightly affected by change in pH, and salts at low concentrations also have little effect. High salt concentration may result in removal of the bound water and precipitation of the polysaccharide. Some polysaccharides have long straight chains with many short branches. Such compounds, for example, locust bean gum and guar gum, combine many properties of the linear and the branched polysaccharides. Some gums have molecules containing many carboxyl groups along the chains; examples are pectin and alginate. These molecules are precipitated below pH 3 when free carboxyl groups are formed. At higher pH values, alkali metal salts of these compounds are highly ionized, and the charges keep the molecules in extended form and extensively hydrated. This results in stable solutions. Divalent cat-ions such as calcium may form salt bridges between neighboring molecules, resulting in gel formation and—if much calcium is present—precipitation. Examples of polysaccharides with strong acid groups are furcellaran and carrageenan. Both are seaweed extractives with sulfuric acid ester groups. Because the ionization of sulfuric acid groups is not reduced much at low pH, such gums are stable in solutions of low pH values.

Gums can be chemically modified by introduction of small amounts of neutral or ionic substituent groups. Substitution or derivatization to a degree of substitution (DS) of 0.01 to 0.04 is often sufficient to completely alter the properties of a gum. The effect of derivatization is much less dramatic with charged molecules than with neutral ones.

Introduction of neutral substituents along the chains of linear polysaccharides results in increased viscosity and solution stability. Some of the commonly introduced groups are methyl, ethyl, and hydroxymethyl. Acid groups can be carboxyl, introduced by oxidation, or sulfate and phosphate groups. Introduction of strongly ionized acid groups may make the polysaccharides mucilaginous.

Gum Arabic

Gum arabic is a dried exudate from acacia trees. It is a neutral or slightly acidic salt of a complex polysaccharide containing calcium, magnesium,

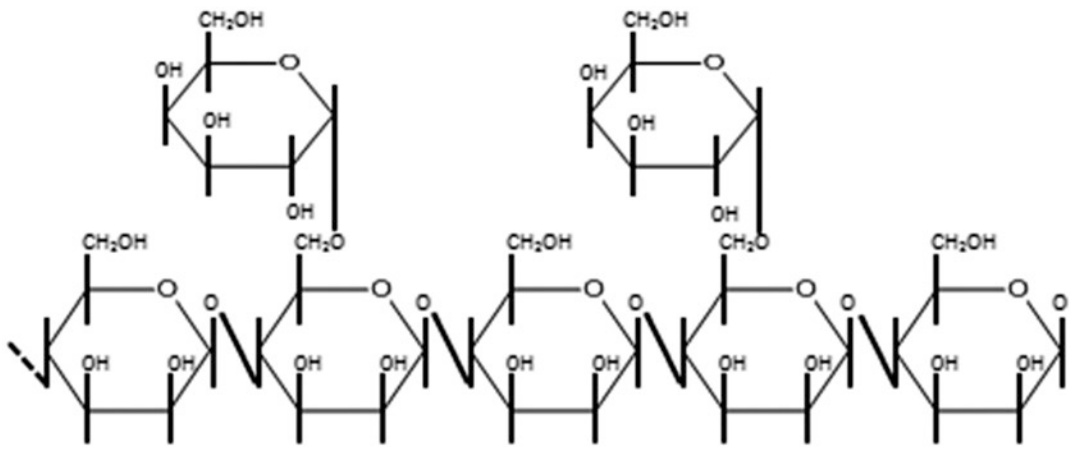


Fig. 4.49 Galactomannan structure found in Guar and locust bean

and potassium anions. The molecule exists in a stiff coil with many side chains and a molecular weight of about 300,000 Da. The molecule is made up of four sugars, L-arabinose, L-rhamnose, D-galactose, and D-glucuronic acid. It is one of the few gums that require high concentration to give increased viscosity and is used as crystallization inhibitor and emulsifier. Gum arabic forms coacervates with gelatin and many other proteins.

Guar and Locust Bean

Guar and locust bean gums are composed of galactomannan polymers, which give solutions of high viscosity and low polymer concentration, but each has its own characteristics. The galactomannan polymers are composed of α (1 → 4)-β-linked D-mannan polymer which is appended with single units of (1 → 6)-α-linked galactopyranosyl side chains as shown on Fig. 4.49. Because of the extent of branching the galactomannans are resistant to crystallization.

Locust bean gum is obtained from the carob bean (*Ceratonia Siliqua*), which is cultivated exclusively around the Mediterranean. The commercial gum contains 88% of D-galacto-D-mannoglycan, 4% of pentoglycan, 6% protein, 1% cellulose, and 1% ash. The molecular weight is about 310,000 Da, and the molecule is a linear chain of D-mannopyranosyl units linked 1 → 4. Every fourth or fifth D-mannopyranosyl unit is substituted on carbon 6 with a D-galactopyranosyl residue. Locust bean gum is known to form tough, pliable films.

Guar gum is obtained from the seed of the guar plant (*Cyamopsis tetragonolubus*) and is more soluble than locust bean gum. It is a straight chain of D-galacto-D-mannogly-can with many single galactose branches. The D-mannopyranose units are joined by β-1 → 4 bonds, and the single D-galactopyranose units are attached by α-1 → 6 linkages. The branches occur at every second mannose unit. The compound has a molecular weight of 220,000 Da, forms viscous solutions at low concentration, and hydrates rapidly in either hot or cold water. At concentrations of 2–3%, gels are formed. Guar gum shows no incompatibility with proteins or other polysaccharides. Guar gum forms tough, pliable films.

Marked synergistic interactions of galactomannans with other specific polysaccharides can occur (Dea et al. 1977). This interaction is sensitive to the mannan/galactose (MG) ratio and thus the type of galactomannan. For example, locust bean gum can form mixed gels with the ordered helical structures of agars, carageenans, and xanthan at polymer concentrations as low as 1 mg/mL (Dea and Morrison 1975).

Agar

Agar is extracted from red algae of the class *Rhodophyceae*. It is soluble in boiling water but is insoluble in cold water. The gels are heat-resistant, and agar is widely used as an emulsifying, gelling, and stabilizing agent in foods. The gel formation properties of agar are unique. At elevated temperatures agarose exists in a "random coil" disordered form, but on cooling forms unique turbid, brittle gels at concentrations as low as 0.04% w/w (Dea and Morrison 1975), which exhibit a marked degree of thermal hysteresis (i.e., gelation takes place at temperatures far

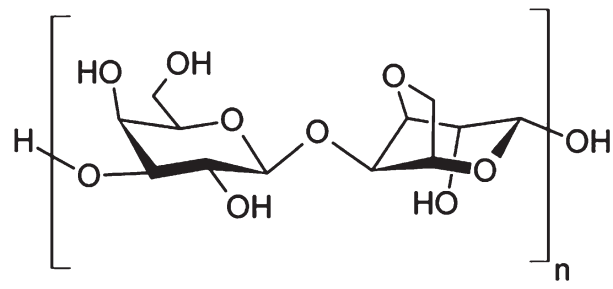


Fig. 4.50 Structure of agarose

below the gel-melting temperature). Molecular weight determinations have given varying results. Osmotic pressure measurements indicate values from 5000 to 30,000 Da; other methods, as high as 110,000. Agar is a mixture of at least two polysaccharides (Glicksman 1969): agarose, a neutral polysaccharide with little or no ester sulfate groups, and agaropectin with 5–10% sulfate groups. The ratio of the two polymers can vary widely. Agarose consists of a linear chain of agarobiose disaccharide units. The structure, as shown in Fig. 4.50, indicates alternating 1 → 4 linked, 3,6-anhydro-L-galactose units and α1 → 3 linked D-galactose units. Agaropectin is a sulfated molecule composed of agarose and ester sulfate, D-glucuronic acid, and small amounts of pyruvic acid. In neutral solutions, agar is compatible with proteins and other polysaccharides. At pH 3, mixing of warm agar and gelatin dispersions cause flocculation. Some gums, such as alginate and starch, decrease the strength of agar gels. Locust bean gum can improve rupture strain of agar gels several times.

Alginates

Alginate gums are salts of alginic acid and they are obtained from the giant kelp *Macrocystis pyrifera* or large, brown algae. Alginic acid is a mixed polymer of anhydro 1 → 4-β-D-mannuronic acid and L-guluronic acid (Fig. 4.51). The most common form is sodium alginate. A property of alginates which is of major importance both for their biological function as structural polysaccharides or food application is the formation of strong, rigid gels on the addition of divalent cations, usually Ca^{2+} (Smidsrod 1974). This functionality also underpins their thickening, suspending, emulsifying, stabilizing, gel-forming, and film-forming properties in foods, and their solubility in both hot and cold water. When no divalent cations are present, solutions have long flow properties. Increasing amounts of calcium ions increase viscosity and result in short flow properties.

Carrageenan

Extracted from Irish moss (*Chondrus crispus*), a red seaweed, carrageenan consists of salts or sulfate esters with a ratio of sulfate to hexose units

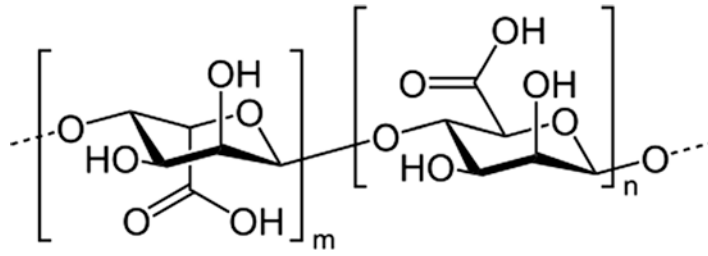


Fig. 4.51 Structure of alginic acid

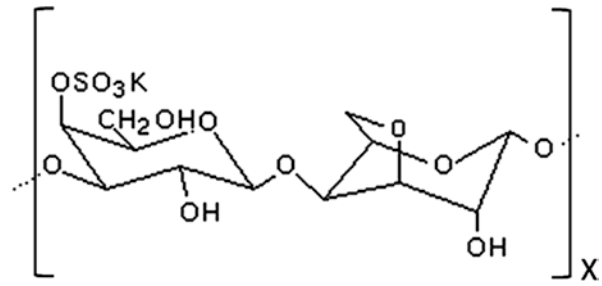


Fig. 4.52 Idealized structure of κ-carrageenan

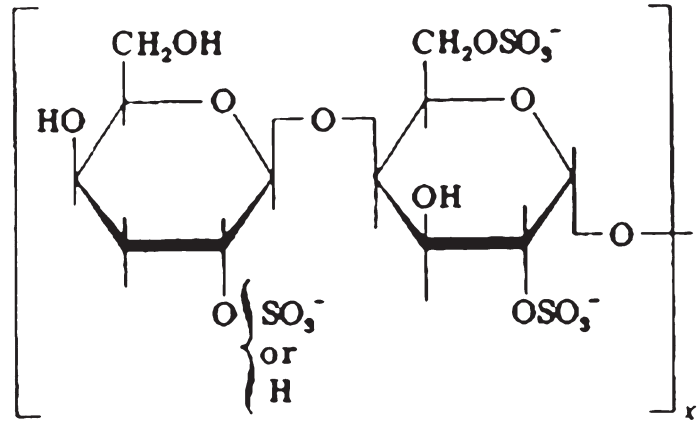


Fig. 4.53 Idealized structure of λ-carrageenan

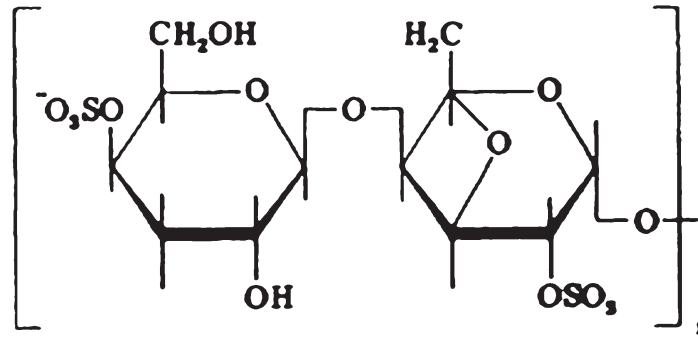


Fig. 4.54 Idealized structure of ι–carrageenan

of close to unity. Three fractions of carrageenan have been isolated, named κ-, λ-, and ι-carrageenan. The idealized structure of κ-carrageenan (Fig. 4.52) is made up of 1 → 3 linked galactose-4-sulfate units and 1 → 4 linked 3,6-anhydro-D-galactose units. Actually, up to 20–25% of the 3,6-anhydro-D-galactose units can be sulfated at carbon 2 and some of the anhydro-D-galactose may occur as galactose-6-sulfate. The 6-sulfate group can be removed by heating with lime to yield anhydro residues, and this treatment results in greatly increased gel strength. The major portion of λ-carrageenan consists of 1 → 3 linked galactose 2-sulfate and 1 → 4 linked galactose 2,6-disulfate (Fig. 4.53); about 30% of the 1,3 galactose units are not sulfated. The 6-sulfate group can be removed with lime treatment but does not result in gel formation. Iota-carrageenan consists mainly of 1 → 3 linked galactose 4-sulfate and 1 → 4 linked 3,6-anhydro-D-galactose 2-sulfate (Fig. 4.54). A certain amount of 6-sulfate groups present can be changed to 3,6-anhydro groups by alkali treatment. The comparative properties of the three types of carrageenan have been listed by Glicksman (1969). Molecular weights of carrageenan vary from 100,000 to 800,000 Da. Carrageenan can form thermally reversible gels whose strengths and gelation temperatures are dependent on the cations potassium and ammonium. The mechanism has been visualized as a zipper arrangement between aligned sections of linear polymer sulfates, with the potassium ions locked between alternating sulfate residues. Other monovalent cations, such as sodium, are not effective, probably because of larger ionic diameter. At low concentrations, carrageenan can alter the degree of agglomeration of caseinate particles in milk. It is a highly effective suspending agent and is used to suspend cacao particles

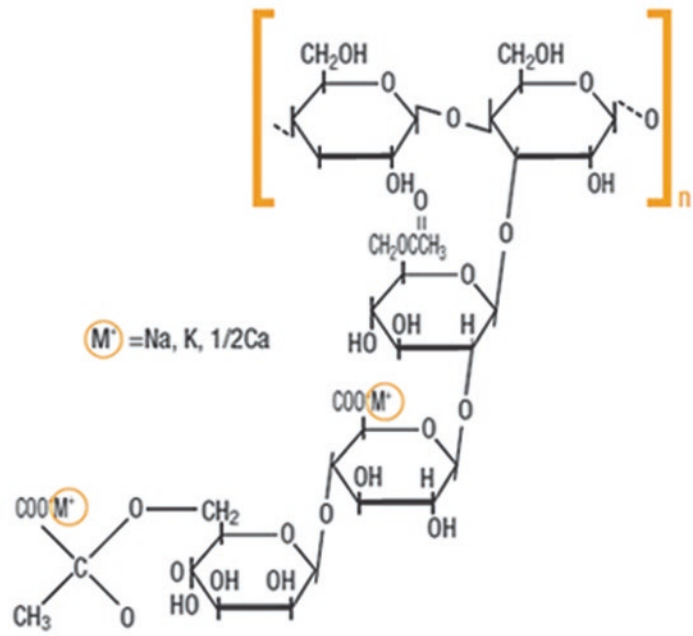


Fig. 4.55 Chemical composition of the repeating unit of xanthan

in chocolate milk at concentrations as low as 0.03%. Carrageenan is often used in combination with starch. The two compounds form complexes that have useful properties in foods. The complexes permit a lowering of the starch content by as much as 50% (Descamps et al. 1986). An example of mixed gels combining carrageenan and whey protein has been described by Mleko et al. (1997). Optimal gelation occurred at pH 6 to 7. The shear stress value of whey protein isolate at 3% concentration was significantly enhanced by the presence of 0.5% κ-carrageenan.

Xanthan

Xanthan, is the extracellular polysaccharide from the plant pathogen *Xanthomas campestris*, is one of the most commercially important water-soluble polymers. It was discovered by USDA-ARS scientist Allene Rosalind Jeanes, and brought into commercial production by Kelco in the early 1960s (Whistler and BeMiller 1973). It has a pentasaccharide repeating unit (Jansson et al. 1975; Melton et al. 1976) in which charged trisaccharide sidechains, which solubilize the polymer, occur on alternate glucose residues of a cellulose-like backbone, giving rise to a highly branched molecule (Fig. 4.55). In solution, xanthan has an ordered conformation (helix) (Morris et al. 1977). The technological use of xanthan as a commercial polysaccharide centers on its unusual solution properties such as its large viscosity increment in the presence of low molecular weight salts (Whitcomb et al. 1977) and having the ability to flow freely with a tenuous, gel-like structure at rest, which is capable of holding particles in suspension over long periods of storage time, for example, in salad dressings.

Dietary Fiber

Originally, the fiber content of food was known as crude fiber and defined as the residue remaining after acid and alkaline extraction of a defatted sample. During the 1970s, the physiological effect of dietary fiber began to attract attention (Ink and Hurt 1987) and the need for better methods for the determination of fiber became apparent. Dietary fiber can be defined as a complex group of plant substances that are resistant to mammalian digestive enzymes. Because the definition is based on physiological properties rather than common chemical properties, the analysis of dietary fiber is not simple. Included in the defi-

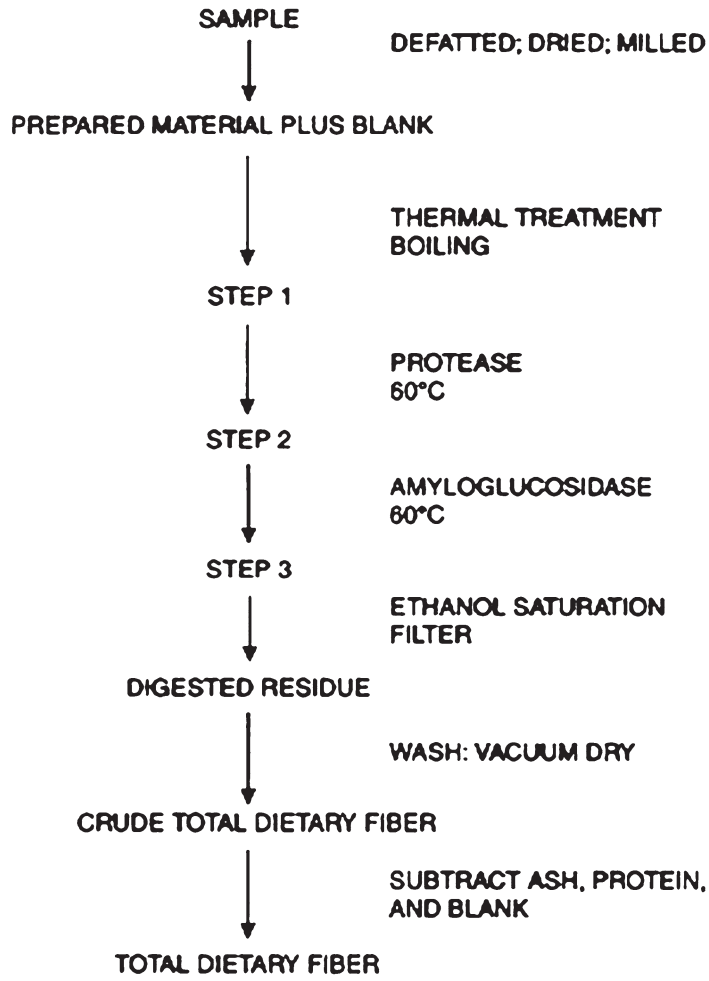


Fig. 4.56 Association of Official Analytical Chemists (AOAC) method for the determination of total dietary fiber. *Source:* Reprinted with permission from AOAC Collaborative Study, *Total Dietary Fiber Method,* © 1984, Association of Official Analytical Chemists

nition of dietary fiber are cellulose, hemicellulose, lignin, cell wall components such as cutin, minerals, and soluble polysaccharides such as pectin. A method for determining total dietary fiber (TDF) that is based on enzymatic digestion has been accepted by the Association of Official Analytical Chemists (AOAC 1984) and is recognized for labeling food products. To determine the calorie content of a food, the TDF can be subtracted from the total carbohydrate content. The AOAC method for total dietary fiber is outlined in Fig. 4.56.

Earlier literature refers to crude fiber, which consists of part of the cellulose and lignin only. This method is now obsolete. The dietary fiber content of foods is usually from 2 to 16 times greater than the crude fiber content. Examples of the difference between the two measurements are given in Table 4.20. One of the first alternative methods was the acid detergent fiber (ADF) method developed by van Soest (1963). In

Table 4.21 Differences between crude fiber (CF) and total dietary fiber (TDF) in some plant materials g/100 g

Plant material	CF	TDF	Ratio
Cellulose	72.5	94.0	1:1.3
Pea hulls	36.3	51.8	1:1.4
Corn bran	19.0	88.6	1:4.7
Distiller's dried grains	10.9	45.9	1:4.2
White wheat bran	8.7	36.4	1:4.2
Citrus pulp	14.4	24.8	1:1.7

Source: Reprinted with permission from M.L. Dreher, *Handbook of dietary Fiber: An Applied Approach,* p. 58 1987. By courtesy of Marcel Dekker, Inc.

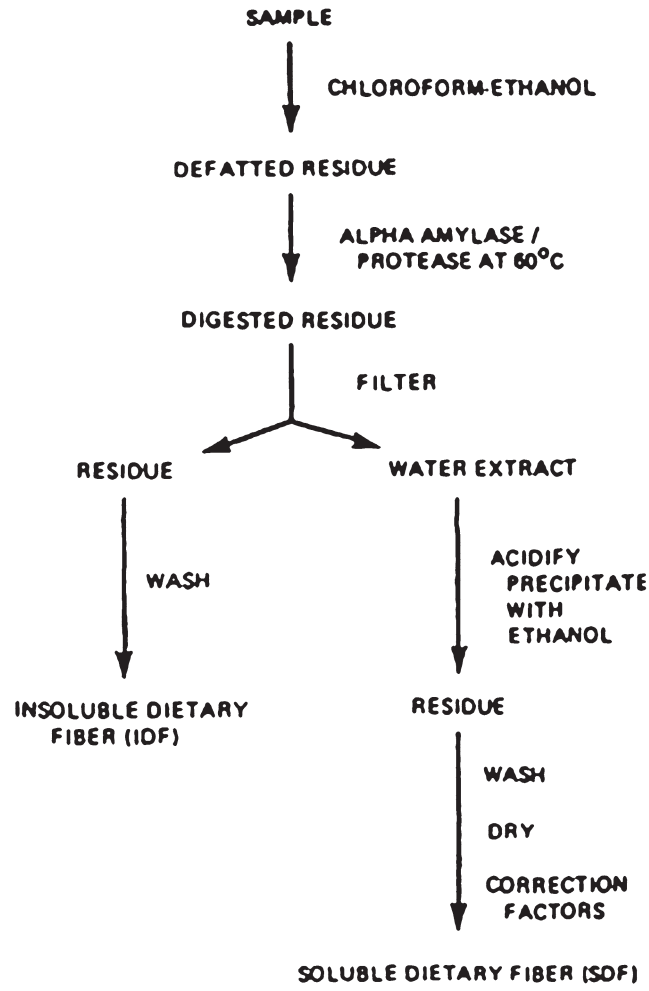


Fig. 4.57 Furda method for the determination of insoluble and soluble dietary fiber. *Source:* Reprinted with permission from I. Furda, Simultaneous Analysis of Soluble and Insoluble Dietary Fiber, in *The Analysis of Dietary Fiber in Food*, W.P.I. James and O. Theander, eds., 1981. By courtesy of Marcel Dekker, Inc.

this procedure, hemi-cellulose is completely extracted, and the residue contains lignin and cellulose. Thereafter, neutral dietary fiber (NDF) methods were developed. These methods measure cellulose, hemicellulose, lignin, cutin, minerals, and protein, but do not account for soluble polysaccharides such as pectins. One of these methods, enzyme-modified neutral detergent fiber (ENDF), has been approved for the determination of insoluble dietary fiber. Chemical methods of determining TDF are known as Southgate type methods (Southgate 1981). This procedure measures cellulose, lignin, and soluble and insoluble non-cellulose polysaccharides (NCP) in terms of hexose, pentose, and uronic acid units. Examples of the determination of TDF by the Southgate method are listed in Table 4.21. Finally, enzymatic gravimetric methods were developed and adopted to determine TDF in food. In these methods the defatted sample is treated with enzymes to degrade proteins and starch. Starch removal is an essential step in these procedures. The various steps evolved in the AOAC method for the determination of total dietary fiber are shown in Fig. 4.56, which shows there is no separation of soluble and insoluble

Table 4.22 Total dietary fiber (TDF) and components as determined by the southgate method (g/100 g dry weight)

	Noncellulose polysaccharides					
Fiber source	Hexose	Pentose	Uronic acid	Cellulose	Lignin	TDF
Wheat bran	6.9	20.9	1.5	7.6	2.9	39.8
Rye biscuit	7.9	8.0	0.5	2.5	0.9	19.8
Dried apple	1.3	1.8	2.7	3.2	1.0	10.0
Citrus pectin	7.6	7.0	77.3	1.6	–	93.5
Potato powder	11.8	1.3	0.8	3.6	–	17.6
Soya flour	3.3	3.8	1.6	2.1	0.3	11.1

Source: Reprinted with permission from M.L. Dreher, *Handbook of Dietary Fiber: An Applied Approach,* p. 66, 1987. By courtesy of Marcel Dekker, Inc.

Table 4.23 Composition of American Association of Cereal Chemists (AACC)–certified food-grade wheat bran

Component	%
Acid detergent fiber	11.9
Neutral detergent fiber	40.2
Total dietary fiber	42.4
Protein	14.3
Lipid	5.2
Ash	5.1
Moisture	10.4
Lignin	3.2
Pectin	3.0
Cutin	0.0
Total starch	17.4
Total sugar	7.0
Pentosan	22.1
Phytic acid	3.4

Source: Reprinted with permission from M.L. Dreher, *Handbook of Dietary Fiber: An Applied Approach*, p. 82, 1987. By courtesy of Marcel Dekker, Inc.

Table 4.24 Overview of dietary fiber methods

Method	Portion removed during analysis	Fiber components determined by method
Crude fiber (CF)	80% lignin, 85% hemicellulose, and 20–60% cellulose	Remainder of the lignin, hemicellulose, and cellulose
Acid detergent fiber (ADF)	Solubilizes cellular components (starch, sugars, fat, nitrogen compounds, and some minerals) plus hemicellulose	Cell wall components, except hemicellulose, as one unit
Neutral detergent fiber (NDF) or enzyme-modified NDF	Solubilizes cellular components: soluble fiber	Cell wall components as one unit
NDF-ADF	–	Hemicellulose
72% sulfuric acid (Klason lignin)	Cellulose	Lignin, insoluble nitrogen compounds, cutin, silica
Permanganate oxidation	Lignin	Lignin (loss in weight)
Southgate-type methods (unavailable carbohydrate)	Solubilizes cellular components; hydrolysis starch	Individual chemical components (including soluble and insoluble polysaccharides) = total dietary fiber (TDF)
Enzymatic methods	Solubilizes cellular components; hydrolysis starch and protein	TDF (indigestible residue); isolation soluble and insoluble fractions
Fractionation methods	–	Isolation and determination of individual components

Source: Reprinted with permission from M.L. Dreher, *Handbook of Dietary Fiber: An Applied Approach*, p. 106, 1987. By courtesy of Marcel Dekker, Inc.

fiber. The method developed by Furda (1981) and shown in Fig. 4.57 provides for separation of soluble and insoluble fiber. The TDF content of some foods as determined by the AOAC method are listed in Table 4.22. The American Association of Cereal Chemists (AACC) makes available a certified standard for reference purposes. The composition of the AACC wheat bran standard is listed in Table 4.23 and illustrates the complexity of what is now known as dietary fiber. An overview of different methods for fiber determinations is presented in Table 4.24 (Dreher 1987).

One of the beneficial effects of dietary fiber is its bulking capacity, and the water-holding capacity of the gums plays an important role in this effect.

Dietary fiber, as now defined, includes the following three major fractions:

1. *Structural polysaccharides*—associated with the plant cell wall, including cellulose, hemicellulose, and some pectins
2. *Structural nonpolysaccharides*—mainly lignins
3. *Nonstructural polysaccharides*—including the gums and mucilages (Schneeman 1986).

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Minerals

5

John W. Finley and John M. deMan

Introduction

In addition to the major components, all foods contain varying amounts of minerals. The mineral material may be present as inorganic or organic salts or may be combined with organic material, as the phosphorus is combined with phosphoproteins and metals are combined with enzymes. More than 60 elements may be present in foods. It is customary to divide the minerals into two groups, the major salt components and the trace elements. The major salt components include potassium, sodium, calcium, magnesium, chloride, sulfate, phosphate, and bicarbonate. Trace elements are all others and are usually present in amounts below 50 parts per million (ppm). Dietary elements in the die can be divided into four basic categories as found in Table 5.1.

A total of 90 naturally occurring elements are found in the earth's crust. Approximately 25 elements are essential for life and are found in living cells. Because most of our diet is derived from living material these essential elements are found in foods in our diet. Foods can also contain nonessential nutrients and toxic elements which often come from contamination during production, harvesting, processing and preparation. The essential elements for humans can be seen on the naturally occurring portion of the periodic table shown in Fig. 5.1.

Mineral elements are present in many forms n foods including chemical compounds, complexes and ionic forms (van Dokkum et al. 1989). The diversity of chemical properties among mineral elements and the vast number of chemical changes that can occur during processing and storage, results in a very large number of mineral species in foods. Many of the mineral species that occur in foods are unstable making it difficult to isolate and characterize specific mineral species in food.

All biological systems contain water and most nutrients are delivered and metabolized in an aqueous environment. Most of the availability and reactivates of minerals are dependent on solubility in water. Generally, the minerals must be in ionic or complexed forms because pure elemental minerals are not absorbed by animals. The forms of the elements depend on the specific chemical properties of the element. For example, the highly water soluble elements Na^+, K^+, Cl^-, and F^-, exist in ionic forms in food. Most other minerals are found as complexes, chelates, or oxygen containing anions.

Much of the chemistry of minerals in food and nutrition can be described in terms of acid/base chemistry. The Bronsted theory of acid base chemistry states:

A Bronsted acid is any substance capable of donating a proton.

A Bronsted base is any substance capable of accepting a proton.

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_5

Table 5.1 Elements in human diets

Essential human elements > 50 *mg/day*	*Essential trace elements* < 50 *mg/day*
Na, K, Ca, Mg, Cl, P	Fe, Cu, I, Co, Mn, Zn, Cr, Ni, Si, F, Mo, Se
Nonnutritive nontoxic elements	*Toxic elements*
Al, B, and Sn	Hg, Pb, As, Cd, and Sb

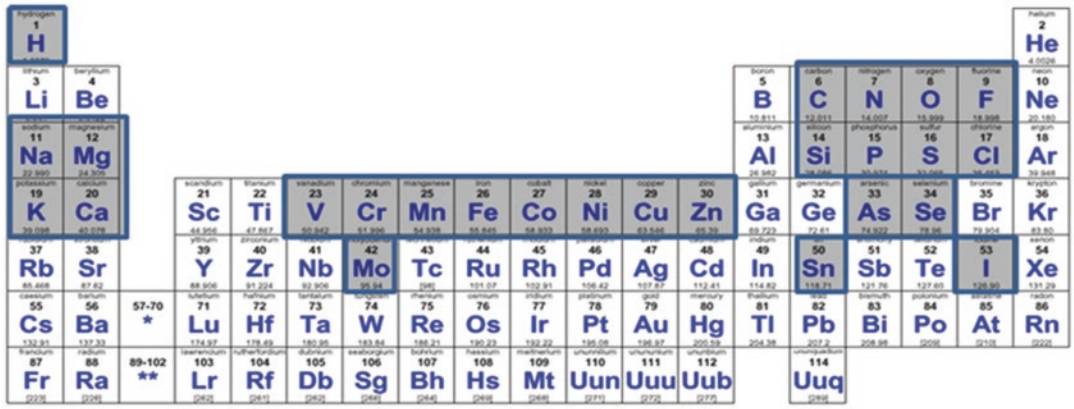

Fig. 5.1 Periodic table of naturally occurring elements. Shaded atoms are of nutritional significance

Foods contain many naturally occurring acids or bases naturally occurring in foods or as and phosphoric or hydrochloric are common inorganic acids in foods. Phosphoric acid is a common ingredient in foods and represents a tri basic acid because it contains three available protons for donation.

$$H_3PO_4 \rightleftarrows H_2PO_4^- + H^+ \qquad pK_1 = 2.22$$
$$H_2PO_4^- \rightleftarrows HPO_4^{2-} + H^+ \qquad pK_2 = 7.10$$
$$HPO_4^{2-} \rightleftarrows PO_4^{3-} + H^+ \qquad pK_1 = 12.40$$

The multiple pK values of phosphoric acid make it useful as a buffering agent and for pH control in many foods and beverages.

The Lewis Theory of acids and bases is based on electrons rather than protons (Shriver et al. 1994):

A Lewis acid is an electron pair acceptor.
A Lewis base is an electron pair donor.
The conventional expression of a Lewis acid base reaction is:

$$A+ : B \rightarrow A - B$$

In the Lewis Theory A must possess unpaired electrons and B must have unpaired electrons. The interaction or bonding occurs when the orbitals of A and B interact to form new molecular orbitals. The stability of the new complex depends on the reduction in energy that occurs when the new molecule is formed. The product of Lewis A and B molecules combining is generally referred to as a complex. Understanding these interactions is key because metal cations in foods are classified as Lewis acid which are bound to Lewis bases. The products from the reaction to metal cations and various molecules in food products range from metal hydrates to metal containing components such as hemoglobin and chlorophyll and metalo-enzymes. The number of Lewis base molecules that can bind to a metal ion is independent of the charge on the metal. The number of potential binding molecules is referred to as the coordination number which can range from 1 to 12, with 6 being the most common number. For example when the Fe^{+3} ion is in water it binds six water molecules forming an octahedron structure as illustrated in Fig. 5.2.

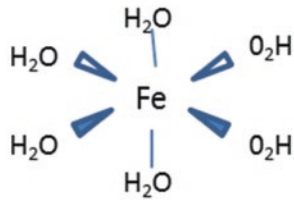

Fig. 5.2 Ferric ion in acidic conditions, coordinated with six water molecules in an octahedral arrangement

The electron donating species are referred to as ligands. A ligand must contain an oxygen donating atom generally they are Oxygen, Nitrogen or Sulfur atoms in an organic molecule. The ligands are classified by the number of bonds they can share with a metal ion. The stability of the complexes is expressed as stability constant or formation constant, shown below the metal ligand complex can be represented was M for metal ion, L_n for the number of ligands and K_n is the stability or formation constant (Shriver et al. 1994).

$$M + L_n \rightleftarrows ML_n \quad K_n = \frac{ML_n}{[M][L_n]}$$

The stability constants or Log K for some typical metal complexes are listed in Table 5.2.

Many food components can form ligands for metal ions, including water, carbohydrates, proteins, phospholipids and organic acids. Figure 5.3 illustrates the mon-dentate formation with water and homogeneous and heterogonous dentate with oxalate and glycine respectively.

The stabilities pf Lewis acid and base complexes are proportional to the driving force of the reaction. The reaction is described quantitatively by the Gibbs free energy equation:

$$\Delta G = \Delta H - T\Delta S.$$

where ΔG is the free energy in the reaction system, ΔH is the enthalpy change, T is the absolute temperature and ΔS is the entropy change. When ΔG is negative reactions will occur spontaneously, and become more favorable as ΔG becomes more negative. For a spontaneous reaction to occur enthalpy must decrease or entropy must increase. A decrease in enthalpy represents a decrease in the electronic energy of the system. An increase in entropy means that there is an increase in the randomness of the system.

A chelate is a complex that forms when a multi-dentate ligand forms two or more bonds with a metal ion. This results in a ring structure which includes the metal ion. For this to occur the chelating ligand must contain at least two functional groups capable of donating electrons. These ligands must be spatially close enough so that when the metal is included a ring can form. Chelates are more thermodynamically stable than other simple complexes.

Kratzer and Vohara described critical factors for the formation of chelates:

1. Five and six membered saturated rings tend to be more stable than smaller rings
2. The greater the number of rings in a chelate the greater the stability
3. Stroger Lewis bases tend to form stronger chelates
4. Charged ligands are more stable than uncharged ligands
5. Relative strengths of ligand bonds:
   Oxygen as donor: $H_2O > ROH > R_2O$
   Nitrogen as donor: $H_3N > RN_2 > R_3N$
   Sulfur as donor: $R_2S > RSH > H_2S$
6. Enhanced resonance in the chelate increases stability
7. Large bulky ligands are less stable chelators

Chelates are effected by many factors in complex systems like foods. The concept of the Gibb's free energy can be a useful predictor of stability. This can be seen in the comparison of Cu^{2+} complexing with ammonia or ethylene diamine (Shriver et al. 1994).

$$Cu\left(H_2O\right)_6^{2+} + NH_3 \rightarrow \left[Cu\left(H_2O\right)_4\left(NH_3\right)_2\right]^{2+} + H_2O$$
$$\left(\Delta H = -46\,kJ\,mol^{-1};\ \Delta S = -8.4\,JK^{-1}\,mol^{-1}\ \text{and}\ \log K = 7.7\right)$$

$$Cu\left(H_2O\right)_6^{2+} + NH_2CH_2CH_2NH_2 \rightarrow \left[Cu\left(H_2O\right)_4\left(NH_2CH_2CH_2NH_2\right)\right]^{2+} + 2H_2O$$
$$\left(\Delta H = -kJ\,mol^{-1};;DS = +23\,JK^{-1}\,mol^{-1};;\log K = 10.1\right)$$

Table 5.2 Major mineral content of some foods

Food	Na	K	Ca	Fe[a]	P
	mg/100 g food				
Whole milk	371	1330	912	0.47	776
Butter (unsalted)	11	24	24	0.02	24
Cheddar cheese	653	76	710	0.14	455
Monterey cheese	781	81	705	0.72	444
Yogurt skim milk	77	255	199	0.09	157
Egg whole	142	138	56	1.75	99
Egg white	166	163	7	0.08	15
Beef carcass	59	267	8	1.83	154
Pork carcass	42	253	19	0.69	155
Chicken meat only raw	77	229	12	0.89	173
Turkey whole meat raw	118	235	11	0.86	190
Fish Cod raw	54	413	16	0.38	203
Fish Tuna Yellowfin	45	441	4	0.77	278
Corn flour yellow whole	5	315	7	2.38	272
Wheat whole grain	2	363	34	3.6	357
Asparagras	2	202	24	2.16	70
Broccoli raw flowers	27	325	48	0.88	66
Carrots, baby raw	78	237	32	0.89	28
Kale, raw	38	491	150	1.47	92
Onions, raw	4	146	23	0.21	29
Peas raw	5	244	25	1.47	108
Potatoes fresh raw	6	425	12	0.81	425
Black beans	5	1483	123	5.02	352
Kidney beans, raw	12	1359	83	6.69	406
Apple Fuji raw	1	109	7	0.11	13
Orange, Raw Florida	0	169	43	0.09	12
Peach raw	0	190	9	0.32	31
Digiorno pepperoni pizza	663	268	209	0.9	268
McDonalds Double Cheeseburger	668	216	178	2.24	166
KFC Original Thigh with breading	781	237	64	0.88	230

[a]Iron discussed below as a trace element

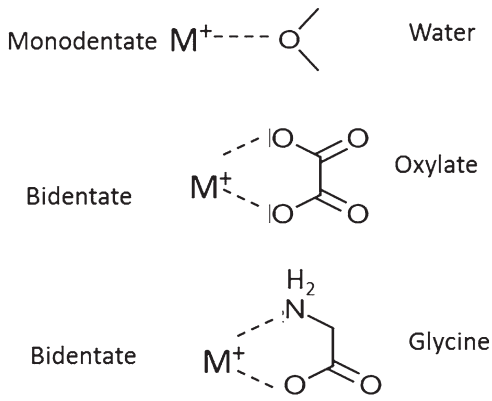

Fig. 5.3 Examples of metal ions coordinated with different ligands

Both complexes have a single Cu^{+2} and two Nitrogens, yet the ethylenediamine complex is much more stable than the ammonia complex with the Log formation constant for the ammonia complex is 7.5 compared to 10.1 for the ethylenediamine complex. Both entropy and enthalpy contribute to the difference, however entropy change is the major factor. Ammonia forms a monodentate ligand whereas ethylenediamine forms a bidentate ligand bond with copper. The entropy change is a result of the change in the number of independent molecules in solution. The first reaction the number of independent molecules is the same on both sides of the

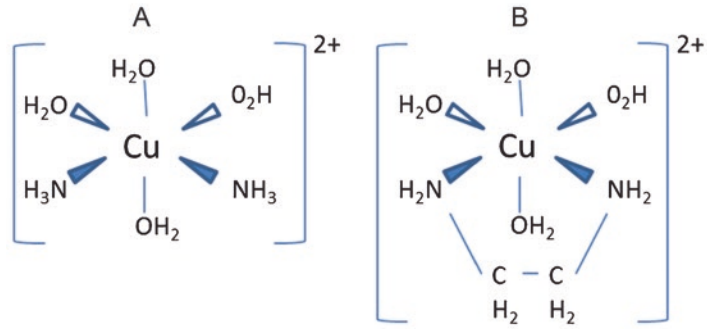

Fig. 5.4 Cupric ion Cu^{2+} complexed with ammonia (**a**) and chelated with etylenediamine (**b**)

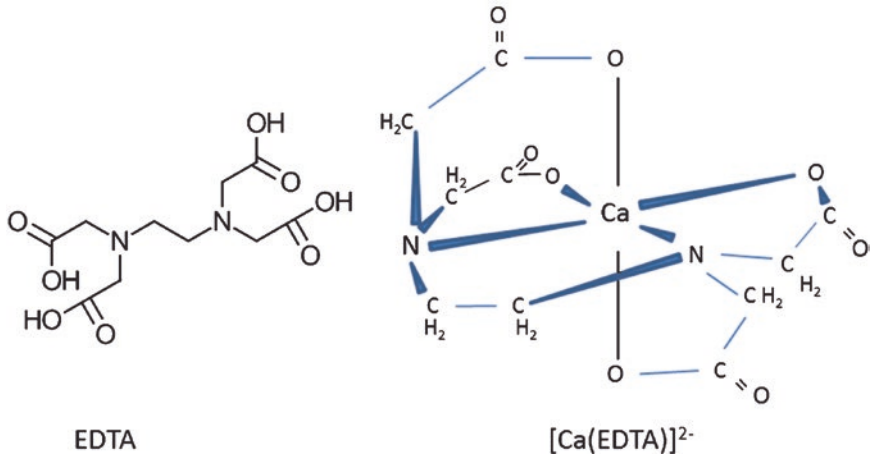

Fig. 5.5 EDTA and calcium EDTA chelate

equation, thus the entropy change is small. The chelation reaction results in a net increase in the number of independent molecules in solution, increasing the entropy (Figs. 5.4 and 5.5).

Ethylenediamine tetraacetic acid (EDTA) is an even stronger chelator. EDTA is a hexadentate chelator displacing six water molecules from a metal.

$$\mathrm{Ca}\left(\mathrm{H_2O}\right)_6^{2+} + \mathrm{EDTA}^{4-} \rightarrow \mathrm{Ca}\left(\mathrm{EDTA}\right)^{2-} + 6\mathrm{H_2O}\,\Delta\mathrm{S} = +118\,\mathrm{J\,K^{-1}\,mol^{-1}}$$

The EDTA chelate contains five membered rings, which enhance stability. EDTA is widely used as a chelater in foods and other biological systems. ss in food sequester metal ions such as copper and ion preventing their action as proxidants.

The chelate's stability depends on the nature of the metal ion and is related to the electronegative chelate normally decreases with decreasing pH. In a chelate the donor atoms can be N, O, P, S, and Cl; some common donor groups are $–NH_2$, =C=O, =NH, –COOH, and –OH–O–$PO(OH)_2$. Many metal ions, especially the transition metals, can serve as acceptors to form chelates with these donor groups. Formation of chelates can involve ring systems with four, five, or six members. Some examples of four- five- and six-membered ring structures are given in Fig. 5.6. Other examples of food components that can be considered metal chelates are hemoglobin and myoglobin, vitamin B_{12}, and calcium caseinate (Pfeilsticker 1970). It has also been proposed that the gelation

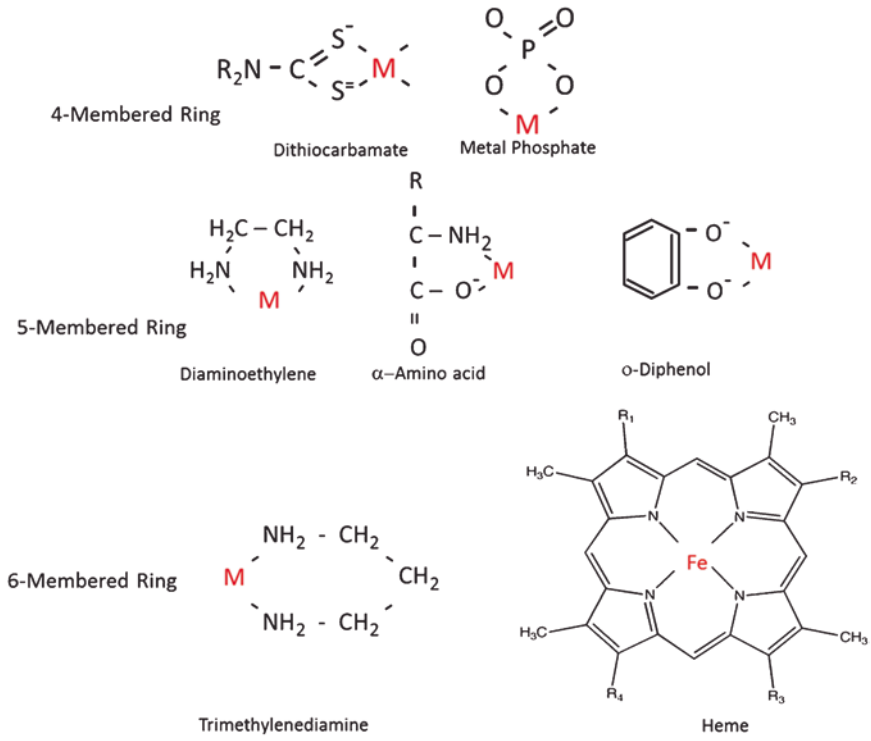

Fig. 5.6 Examples of metal chelates. With four, five, and six membered rings

of certain polysaccharides, such as alginates and pectates, with metal ions occurs through chelation involving both hydroxyl and carboxyl groups (Schweiger 1966). A requirement for the formation of chelates by these polysaccharides is that the OH groups be present in vicinal pairs.

The minerals in foods are usually determined by ashing or incineration. This destroys the organic compounds and leaves the minerals behind. However, determined in this way, the ash does not include the nitrogen contained in proteins and is in several other respects different from the real mineral content. Organic anions disappear during incineration, and metals are changed to their oxides. Carbonates in ash may be the result of decomposition of organic material. The phosphorus and sulfur of proteins and the phosphorus of lipids are also part of ash. Some of the trace elements and some salts may be lost by volatilization during the ashing. Sodium chloride will be lost from the ash if the incineration temperature is over 600 °C. Clearly, when we compare data on mineral composition of foods, we must pay great attention to the methods of analysis used.

Some elements appear in plant and animal products at relatively constant levels, but in a number of cases an abundance of a certain element in the environment may result in a greatly increased level of that mineral in plant or animal products. Enrichment of elements in a biological chain may occur; note, for instance, the high mercury levels reported in some large predatory fish species such as swordfish and tuna.

Interactions with Other Food Components

The behavior of minerals is often influenced by the presence of other food constituents. The recent interest in the beneficial effect of dietary fiber has led to studies of the role fiber plays in the absorption of minerals. It has been shown (Toma and Curtis 1986) that mineral absorption is decreased by fiber. A study of the behavior of

iron, zinc, and calcium showed that interactions occur with phytate, which is present in fiber. Phytates can form insoluble complexes with iron and zinc and may interfere with the absorption of calcium by causing formation of fiber-bound calcium in the intestines.

Iron bioavailability may be increased in the presence of meat (Politz and Clydesdale 1988). This is the so-called meat factor. The exact mechanism of this effect is not known, but it has been suggested that amino acids or polypeptides that result from digestion are able to chelate nonheme iron. These complexes would facilitate the absorption of iron. In nitrite-cured meats some factors promote iron bioavailability (the meat factor), particularly heme iron and ascorbic acid or erythorbic acid. Negative factors may include nitrite and nitrosated heme (Lee and Greger 1983).

Major Minerals

Foods provide most of the minerals important for good health. Table 5.2 contains some of mineral content of some foods illustrating the range of composition of the macro-minerals in foods.

Sodium

The human body contains approximately 1.4 g/kg Sodium. It is present mostly as an extracellular component maintaining the osmotic pressure of extracellular fluid. The U.S. Food and Drug Administration recommends that individuals consume no more than 2300 mg of sodium per day, and that certain groups limit intake to 1500 mg/day. The "Dietary Guidelines for Americans 2010" addresses sodium intake in detail. The average daily sodium intake for Americans is 3400 mg/day, an excessive amount that raises blood pressure and poses health risks in some individuals. It is recommended that Americans limit daily sodium consumption to 2300 mg.

Salt provided one of the earliest means of food preservation and remains as a successful preservative in salted meat products. The emergence of refrigeration and other methods of food preservation, has reduced the requirement for salt as a preservative (He et al. 2007). Salt also provides flavors that we rapidly become accustomed to and many people develop strong preferences for high salt levels, especially in processed foods. The tastes and flavors associated with historical salt use have come to be expected, and the relatively low cost of enhancing the palatability of processed foods has become a key rationale for the use of salt in food (Van der Veer 1985). Sodium continues to play a significant role in reducing the growth of pathogens and organisms that spoil products and reduce their shelf life. High Sodium levels also result enhanced functional roles, such as improving texture. In addition to sodium chloride other sodium-containing compounds are also used for increasing the safety and shelf life of foods or creating physical properties.

Salt is effective as a preservative because it reduces the water activity of foods. Salt's ability to decrease water activity is due to the ability of sodium and chloride ions to associate with water molecules (Fennema 1996; Potter and Hotchkiss 1995). The addition of salt to foods can also cause microbial cells to loose water from osmotic shock, thereby causing cell death or retarded growth (Davidson et al. 2001). Because solutes in aqueous solutions reduce oxygen solubility, interfere with cellular enzymes, or force cells to expend energy to remove sodium ions from the cell, salt can serve to reduce the rate of microbial growth (Shelef et al. 2005).

Potassium

The concentration of Potassium in the human body is approximately 2 g/kg, making it the most predominant cation in intracellular fluid. Potassium is localized mostly within cells where it regulates intracellular osmotic pressure. Potassium is frequently used as a replacement for Sodium in salt substitutes. Generally the population consumes too much Sodium and too little potassium.

Recommended dietary allowance guidelines for Potassium vary depending on age. Infants from 0 to 6 months old should receive 400 mg daily, and those from 7 to 12 months old need 700 mg. The RDA for children from 1 to 3 years old is 3000 mg each day, those from 4 to 8 years old warrant 3800 mg and those from 9 to 13 years old need 4500 mg. Children older than 13 and adults should get 4700 mg/day, except for lactating women, who require 5100 mg.

Low potassium levels, or hypokalemia, can lead to weakness, lack of energy, high blood pressure, muscle cramping, gastrointestinal distress, arrhythmia and abnormal electrocardiograms.

Magnesium

The concentration of Magnesium in the body is about 250 mg/kg. The daily requirement is 300–400 mg/day and the daily intake is from 300 to 500 mg/day. It is naturally abundant in many foods. Magnesium is an activator of many enzymes including conversion of energy rich phosphates. Magnesium compounds also are involved in intracellular and plasma membranes. Membrane involved in many life supporting metabolic functions and thus deficiency can be very serious. Magnesium is widely distributed in plant and animal foods.

Calcium

Calcium, the most abundant mineral in the body is widely distributed in the throughout the body totally about 1500 g in adults. It is included in the skeleton bones but also widely distrusted in most other tissues. Calcium is essential for muscle function controlling processes like muscle contraction. Approximately 99% of the Calcium in the body is in the skeletal tissue. The Calcium in soft tissue must be maintained in a narrow physiological range for the body to function. Thus if the levels of Calcium in the blood and extracellular Calcium are not met by the diet the mineral will be reabsorbed from the bone.

Calcium has multiple roles in living cells related to its complex formation with proteins, carbohydrates and lipids. The binding of Calcium is selective based on its binding to neutral oxygens including alcohols and carbonyl groups. Because it is a divalent cation Calcium can bind simultaneously to two centers resulting it is cross-linking function in carbohydrates and proteins.

Phosphates

Phosphates are found in many different forms in foods and are both naturally occurring and as food additives. Phosphates play crucial roles in living systems. For example adenosine triphosphate (ATP) is the primary energy source in living cells. Phosphoproteins (ferritins) are critical for iron storage. Calcium as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) comprises the primary structure in bone. Phospholipids are the major components in membranes and sugar phosphates are critical in carbohydrate metabolism. Extensive discussions of phosphates can be found in reviews by Ellinger (1972) and Mollins (1990).

Several phosphate are approved as food additives and their structures can be found in Fig. 5.7. Phosphate food additives provide many functions in food including acidification of soft drinks, buff-

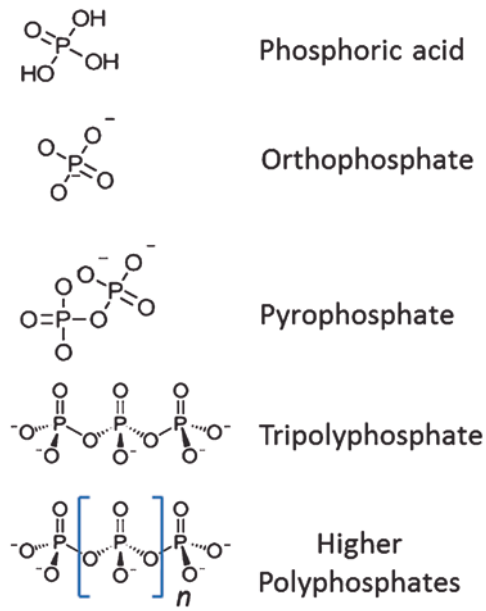

Fig. 5.7 Structures of phosphoric acid and phosphate ions used as food additives

ering of beverages, anti-caking, stabilizing, leavening, emulsification and antioxidants. At pH ranges in most foods, phosphates are negatively charges and polyphosphates are polyelectrolytes. The negative charges on these molecules result in the phosphates acting like Lewis bases and as a result they exhibit strong metal binding characteristics.

Minerals in Milk

The normal levels of the major mineral constituents of cow's milk are listed in Table 5.3. These are average values; there is a considerable natural variation in the levels of these constituents. A number of factors influence the variations in salt composition, such as feed, season, breed and individuality of the cow, stage of lactation, and udder infections. In all but the last case, the variations in individual mineral constituents do not affect the milk's osmotic pressure. The ash content of milk is relatively constant at about 0.7%. An important difference between milk and blood plasma is the relative levels of sodium and potassium. Blood plasma contains 330 mg/100 mL of sodium and only 20 mg/100 mL of potassium. In contrast, the potassium level in milk is about three times as high as that of sodium. Some of the mineral salts of milk are present at levels exceeding their solubility and therefore occur in the colloidal form. Colloidal particles in milk contain calcium, magnesium, phosphate, and citrate. These colloidal particles precipitate with the curd when milk is coagulated with rennin. Dialysis and ultrafiltration are other methods used to obtain a serum free from these colloidal particles. In milk the salts of the weak acids (phosphates, citrates, and carbonates) are distributed among the various possible ionic forms. As indicated by Jenness and Patton (1959), the ratios of the ionic species can be calculated by using the Henderson–Hasselbach equation,

$$\mathrm{pH} = pK_{\alpha} + \log \frac{[\mathrm{salt}]}{[\mathrm{acid}]}$$

The values for the dissociation constants of the three acids are listed in Table 5.4. When these values are substituted in the Henderson–Hasselbach equation for a sample of milk at pH 6.6, the following ratios will be obtained:

$$\frac{\mathrm{Citrate}^{-}}{\mathrm{Citric\,acid}} = 3000 \quad \frac{\mathrm{Citrate}^{=}}{\mathrm{Citrate}^{-}} = 72$$

$$\frac{\mathrm{Citrate}^{\equiv}}{\mathrm{Citrate}^{=}} = 16$$

From these ratios we can conclude that in milk at pH 6.6 no appreciable free citric acid or monocitrate ion is present and that tricitrate and dicitrate are the predominant ions, present in a ratio of about 16 to 1. For phosphates, the following ratios are obtained:

$$\frac{\mathrm{H_2PO_4^-}}{\mathrm{H_3PO_4}} = 43,600 \quad \frac{\mathrm{HPO_4^=}}{\mathrm{H_2PO_4^-}} = 0.30$$

$$\frac{\mathrm{PO_4^{\equiv}}}{\mathrm{HPO_4^=}} = 0.000002$$

This indicates that mono- and diphosphate ions are the predominant species. For carbonates the ratios are as follows:

$$\frac{\mathrm{HCO_3^-}}{\mathrm{H_2CO_3}} = 1.7$$

$$\frac{\mathrm{CO_3^=}}{\mathrm{HCO_3^-}} = 0.0002$$

Table 5.3 Average values for major mineral content of cow's milk (skim milk)

Constituent	Normal level (mg/100 mL)
Sodium	50
Potassium	145
Calcium	120
Magnesium	13
Phosphorus (total)	95
Phosphorus (inorganic)	75
Chloride	100
Sulfate	10
Carbonate (as CO_2)	20
Citrate (as citric acid)	175

Table 5.4 Dissociation constants of weak acids

Acid	pK_1	pK_2	pK_3
Citric	3.08	4.74	5.40
Phosphoric	1.96	7.12	10.32
Carbonic	6.37	10.25	–

The predominant forms are bicarbonates and the free acid.

Note that milk contains considerably more cations than anions; Jenness and Patton (1959) have suggested that this can be explained by assuming the formation of complex ions of calcium and magnesium with the weak acids. In the case of citrate [Cit] the following equilibria exist:

$$\mathrm{H[Cit]} \rightarrow \mathrm{[Cit]^{-3}}$$
$$\mathrm{[Cit]^{-3} + Ca^{-2} \rightarrow Ca[Cit]^{-1}}$$
$$\mathrm{Ca[Cit]^{-1} + H^{+} \rightarrow CaH[Cit]}$$
$$\mathrm{2Ca[Cit]^{-1} + Ca^{+2} \rightarrow Ca_3[Cit]_2}$$

Soluble complex ions such as Ca^{+2} can account for a considerable portion of the calcium and magnesium in milk, and analogous complex ions can be formed with phosphate and possibly with bicarbonate.

Treatments of casein micelles results in a number of changes in the water, Calcium, phosphate and other ions which migrate in or out of the micelle depending on the physiochemical changes. Gaucheron (2005) has reviewed the changes in detail. Figure 5.8 illustrates some of the important changes. Heat causes a concentration of Calcium phosphate in the micelle whereas cooling results in migration of calcium phosphate out of the micelle. Addition of sodium chloride results in phosphate coming out of the micelle and water migrating into the micelle. Lowering the pH causes calcium phosphate and water to come out of the micelle along with some of the caseins prior to isoelectric precipitation.

The equilibria levels of total and soluble calcium and phosphorus are listed in Table 5.5. The mineral equilibria in milk have been extensively studied because the ratio of ionic and total calcium exerts a profound effect on the stability of the caseinate particles in milk. Processing conditions such as heating and evaporation change the salt equilibria and therefore the protein stability. When milk is heated, calcium and phosphate change from the soluble to the colloidal phase. Changes in pH result in profound changes of all of the salt equilibria in milk. Decreasing the pH results in changing calcium and phosphate from the colloi-

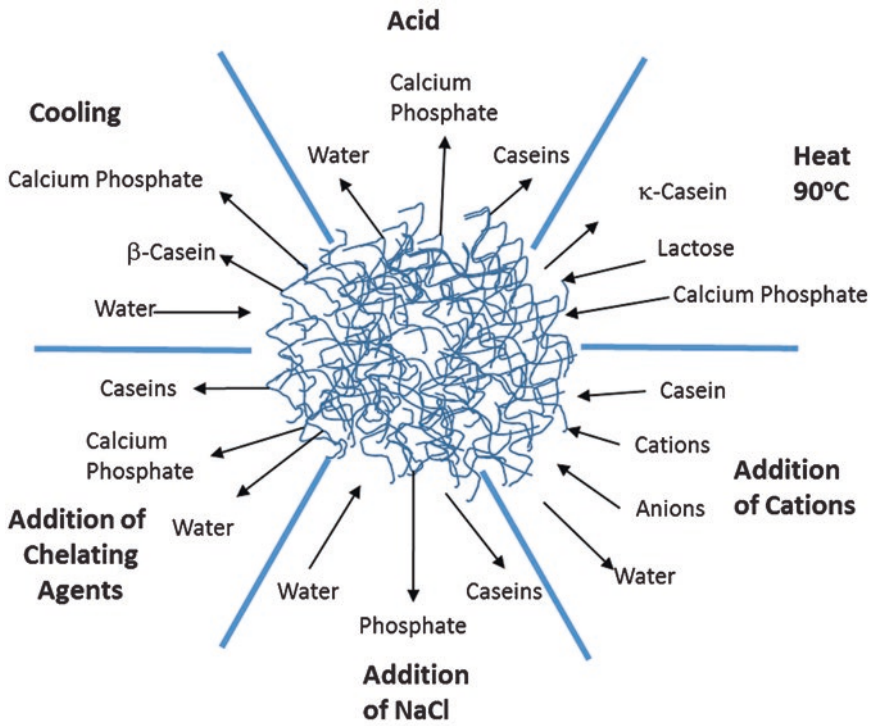

Fig. 5.8 Changes in salt equilibrium of casein micelles resulting from physio-chemical treatments (modified from Gaucheron (2005))

Table 5.5 Total and soluble calcium and phosphorus content of milk

Constituent	mg/100 mL
Total calcium	112.5
Soluble calcium	35.2
Ionic calcium	27.0
Total phosphorus	69.6
Soluble phosphorus	33.3

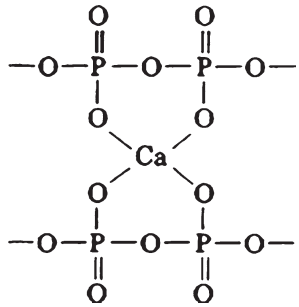

Fig. 5.9 Calcium chelate of a polyphosphate

dal to the soluble form. At pH 5.2, all of the calcium and phosphate of milk becomes soluble. An equilibrium change results from the removal of CO_2 as milk leaves the cow's udder. This loss of CO_2 by stirring or heating results in an increased pH. Concentration of milk results in a dual effect. The reduction in volume leads to a change of calcium and phosphate to the colloidal phase, but this also liberates hydrogen ions, which tend to dissolve some of the colloidal calcium phosphate. The net result depends on initial salt balance of the milk and the nature of the heat treatment.

The stability of the caseinate particles in milk can be measured by a test such as the heat stability test, rennet coagulation test, or alcohol stability test. Addition of various phosphates—especially polyphosphates, which are effective calcium complexing agents—can increase the caseinate stability of milk. Addition of calcium ions has the opposite effect and decreases the stability of milk. Calcium is bound by polyphosphates in the form of a chelate, as shown in Fig. 5.9.

Minerals in Meat

The major mineral constituents of meat are listed in Table 5.6. Sodium, potassium, and phosphorus are present in relatively high amounts. Muscle tissue contains much more potassium than sodium. Meat also contains considerably more magnesium than calcium. Table 5.4 also provides information about the distribution of these minerals between the soluble and non-soluble forms. The non-soluble minerals are associated with the proteins. Since the minerals are mainly associated with the non-fatty portion of meat, the leaner meats usually have a higher mineral or ash content. When liquid is lost from meat (drip loss), the major element lost is sodium and, to a lesser extent, calcium, phosphorus, and potassium. Muscle tissue consists of about 40% intracellular fluid, 20% extracellular fluid, and 40% solids. The potassium is found almost entirely in the intracellular fluid, as are magnesium, phosphate, and sulfate. Sodium is mainly present in the extracellular fluid in association with chloride and bicarbonate. During cooking, sodium may be lost, but the other minerals are well retained. Processing does not usually reduce the mineral content of meat. Many processed meats are cured in brine that contains mostly sodium chloride. As a result, the sodium content of cured meats may be increased.

Table 5.6 Mineral constituents in meat (beef)

Constituent	mg/100 g
Total calcium	8.6
Soluble calcium	3.8
Total magnesium	24.4
Soluble magnesium	17.7
Total citrate	8.2
Soluble citrate	6.6
Total inorganic phosphorus	233.0
Soluble inorganic phosphorus	95.2
Sodium	168
Potassium	244
Chloride	48

Ionic equilibria play an important role in the water-binding capacity of meat (Hamm 1971). The normal pH of rigor or post-rigor muscle (pH 5.5) is close to the isoelectric point of actomyosin. At this point the net charge on the protein is at a minimum. By addition of an acid or base, a cleavage of salt cross-linkages occurs, which increases the electrostatic repulsion (Fig. 5.10), loosens the protein network, and thus permits more water to be taken up. Addition of neutral salts such as sodium chloride to meat

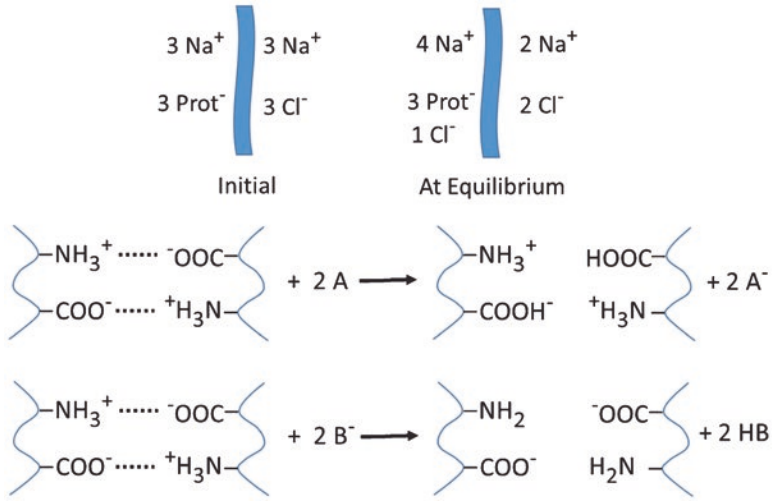

Fig. 5.10 Schematic representation of the addition of acid (HA) or base (B^-) to an isoelectric protein. The isoelectric protein has equal numbers of positive and negative charges. The acid HA donates protons, the base B^- accepts protons

increases water-holding capacity and swelling. The swelling effect has been attributed mainly to the chloride ion. The existence of intra- and extracellular fluid components has been described by Merkel (1971) and may explain the effect of salts such as sodium chloride. The proteins inside the cell membrane are non-diffusible, whereas the inorganic ions may move across this semipermeable membrane. If a solution of the sodium salt of a protein is on one side of the membrane and sodium chloride on the other side, diffusion will occur until equilibrium has been reached. This can be represented as follows:

At equilibrium the product of the concentrations of diffusable ions on the left side of the membrane must be equal to the product on the right side, shown as follows:

$$\left[\mathrm{Na}^+\right]_\mathrm{L}\left[\mathrm{Cl}^-\right]_\mathrm{L} = \left[\mathrm{Na}^+\right]_\mathrm{R}\left[\mathrm{Cl}^-\right]_\mathrm{R}$$

In addition, the sum of the cations on one side must equal the sum of anions on the other side and vice versa:

$$\left[\mathrm{Na}^+\right]_\mathrm{L} = \left[\mathrm{Pr}^-\right]_\mathrm{L} + \left[\mathrm{Cl}^-\right]_\mathrm{L} \text{ and } \left[\mathrm{Na}^+\right]_\mathrm{R} = \left[\mathrm{Cl}^-\right]_\mathrm{R}$$

This is called the Gibbs–Donnan equilibrium and provides an insight into the reasons for the higher concentration of sodium ions in the intracellular fluid.

Table 5.7 Major mineral element components in wheat grain

Element	mg/100 g
Potassium	363
Phosphorus	357
Calcium	34
Magnesium	137
Sodium	2

Source: USDA nutrient database

Struvite

Occasionally, phosphates can form undesirable crystals in foods. The most common example is struvite, a magnesium-ammonium phosphate of the composition $Mg(NH_4)PO_4{\cdot}6H_2O$. Struvite crystals are easily mistaken by consumers for broken pieces of glass. Most reports of struvite formation have been related to canned seafood, but occasionally the presence of struvite in other foods has been reported. It is assumed that in canned seafood, the struvite is formed from the magnesium of sea water and ammonia generated by the effect of heat on the fish or shellfish muscle protein.

Minerals in Plant Products

Plants generally have a higher content of potassium than of sodium. The major minerals in wheat are listed in Table 5.7 and include potassium,

Table 5.8 Mineral components in endosperm and bran fractions of red winter wheat

	P (%)	K (%)	Na (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	Cu (ppm)
Total endosperm	0.10	0.13	0.0029	0.017	0.016	2.4	13	8
Total bran wheat kernel	0.38	0.35	0.0067	0.032	0.11	32	31	11
Center section	0.35	0.34	0.0051	0.025	0.086	29	40	7
Germ end	0.55	0.52	0.0036	0.051	0.13	77	81	8
Brush end	0.41	0.41	0.0057	0.036	0.13	44	46	12
Entire kernel	0.44	0.42	0.0064	0.037	0.11	49	54	8

Source: From V.H. Morris et al., Studies on the Composition of the Wheat Kernel. II. Distribution of Certain Inorganic Elements in Center Sections, *Cereal Chem*., Vol. 22, pp. 361–372, 1945

phosphorus, calcium, magnesium, and sulfur (Schrenk 1964). Sodium in wheat is present at a level of only about 80 ppm and is considered a trace element in this case. The minerals in a wheat kernel are not uniformly distributed; rather, they are concentrated in the areas close to the bran coat and in the bran itself. The various fractions resulting from the milling process have quite different ash contents. The ash content of flour is considered to be related to quality, and the degree of extraction of wheat in milling can be judged from the ash content of the flour. Wheat flour with high ash content is darker in color; generally, the lower the ash content, the whiter the flour. This general principle applies, but the ash content of wheat may vary within wide limits and is influenced by rainfall, soil conditions, fertilizers, and other factors. The distribution of mineral components in the various parts of the wheat kernel is shown in Table 5.8.

High-grade patent flour, which is pure endosperm, has an ash content of 0.30–0.35%, whereas whole wheat meal may have an ash content from 1.35 to 1.80%.

The ash content of soybeans is relatively high, close to 5%. The ash and major mineral levels in soybeans are listed in Table 5.9. Potassium and phosphorus are the elements present in greatest abundance. About 70–80% of the phosphorus in soybeans is present in the form of phytic acid, the phosphoric acid ester of inositol (Fig. 5.11). Phytin is the calcium-magnesium-potassium salt of inositol hexaphosphoric acid or phytic acid. The phytates are important because of their effect on protein solubility and because they may interfere with absorption of calcium from the diet. Phytic acid is present in many foods of plant origin.

Table 5.9 Mineral content of soybeans (green raw)

Mineral	Range (%)
Iron	3.5
Potassium	620
Calcium	197
Magnesium	65
Phosphorus	194
Sodium	15

Source: USDA nutrient database

A major study of the mineral composition of fruits was conducted by Zook and Lehmann (1968). Some of their findings for the major minerals in fruits are listed in Table 5.10. Fruits are generally not as rich in minerals as vegetables are. Apples have the lowest mineral content of the fruits analyzed. The mineral levels of all fruits show great variation depending on growing region.

The rate of senescence of fruits and vegetables is influenced by the calcium content of the tissue (Poovaiah 1986.) When fruits and vegetables are treated with calcium solutions, the quality and storage life of the products can be extended.

Chloride

Originally, nine of the trace elements were considered to be essential to humans: cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, selenium, and zinc. Recently, chromium, silicon, and nickel have been added to this list (Reilly et al. 1996). These are mostly metals; some are metalloids. In addition to essential trace elements, several trace elements have no known essentiality and some are toxic (such as lead, mercury, and cadmium). These toxic trace elements, which are classified as contaminants, are dealt with in Chap. 11.

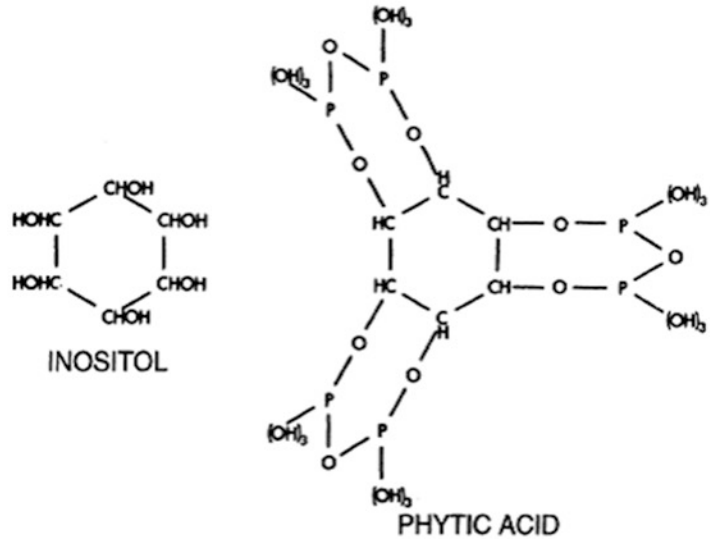

Fig. 5.11 Inositol and phytic acid

Table 5.10 Mineral content of some fruits

	Minerals (mg/100 g)						
Fruit	Na	Ca	Mg	Fe	Zn	P	K
Orange (Navel)	1	43	11	0.13	0.08	23	166
Apple (raw with skin)	1	6	5	0.12	0.04	11	107
Grape (Thompson)	2	10	7	0.36	0.07	20	191
Cherry (sweet raw)	0	13	11	0.036	0.07	21	222
Pear (fresh raw)	1	9	7	0.18	0.10	12	116
Banana (raw)	1	5	27	0.26	0.15	22	358
Pineapple (Puerto Rico)	1	13	12	0.25	0.08	9	125

Source: USDA nutrient database

Trace Elements

Because trace metals are ubiquitous in our environment, they are found in all of the foods we eat. In general, the abundance of trace elements in foods is related to their abundance in the environment. Table 5.11 presents the recommended daily allowance (RDA) for some trace elements and their common food sources.

Trace elements get into foods by different pathways. The most important source is from the soil, by absorption of elements in aqueous solution through the roots. Another, minor, source is foliar penetration. This is usually associated with industrial air pollution and vehicle emissions. Other possible sources are fertilizers, agricultural chemicals, and sewage sludge. Sewage sludge is a good source of nitrogen and phosphate but may contain high levels of trace minerals, many of these originating from industrial activities such as electroplating. Trace minerals may also originate from food processing and handling equipment, food packaging materials, and food additives.

Iron

Iron is a component of the heme pigments and of some enzymes. In spite of the fact that some foods have high iron levels, much of the population has frequently been found to be deficient in this element. Animal food products may have high levels that are well absorbed; liver may

Table 5.11 Dietary allowances and sources of some important trace elements in the human diet

Element	RDA	Common sources
Chromium	50–200 mcg	Whole grains, brewer's yeast, nuts and dark chocolate
Copper	1.5–3 mg	Seafood, nuts, legumes, green leafy vegetables, dried fruits (such as prunes and cocoa), yeast, organ meats, nuts, potatoes, grains, beans
Iodine	150 mcg	Seafood, Iodized salt
Iron	10–15 mg	Heme iron is only present in animal flesh. Beef, liver, clams and oysters. Non-heme iron is in tofu, legumes, spinach, raisins
Manganese	2.5–5.0 mg	Coffee, tea, nuts, whole grains, legumes and some fruits and vegetables
Molybdenum	75–250 mcg	Peas, legumes and some breakfast cereals
Selenium	55–70 mcg	Seafoods and organ meats, muscle meats, cereals and other grains, and dairy
Fluoride	0.7–3 mg	Fluoridated water, tea, sardines, chicken

contain several thousand ppm of iron. The iron from other foods such as vegetables and eggs is more poorly absorbed. In the case of eggs the uptake is poor because the ferric iron is closely bound to the phosphate of the yolk phosphoproteins. Iron is used as a food additive to enrich flour and cereal products. The form of iron used significantly determines how well it will be taken up by the body. Ferrous sulfate is very well absorbed, but will easily discolor or oxidize the food to which it is added. Elemental iron is also well absorbed and is less likely to change the food. For these reasons, it is the preferred form of iron for the enrichment of flour.

Iron bioavailability may be increased in the presence of meat (Politz and Clydesdale 1988). This is the so-called meat factor. The exact mechanism of this effect is not known, but it has been suggested that amino acids or polypeptides that result from digestion are able to chelate nonheme iron. These complexes would facilitate the absorption of iron. In nitrite-cured meats some factors promote iron bioavailability (the meat factor), particularly heme iron and ascorbic acid or erythorbic acid. Negative factors may include nitrite and nitrosated heme (Lee and Greger 1983).

Cobalt

Cobalt is an integral part of the only metal containing vitamin B_{12}. The level of cobalt in foods varies widely, from as little as 0.01 ppm in corn and cereals to 1 ppm in some legumes. The human requirement is very small and deficiencies do not occur.

Copper

Copper is present in foods as part of several copper-containing enzymes, including the polyphenolases. Copper is a very powerful prooxidant and catalyzes the oxidation of unsaturated fats and oils as well as ascorbic acid. The normal daily diet contains from 2 to 5 mg of copper, more than ample to cover the daily requirement of 0.6–2 mg.

Zinc

Zinc is the second most important of the essential trace elements for humans. It is a constituent of some enzymes, such as carbonic anhydrase. Zinc is sufficiently abundant that deficiencies of zinc are unknown. The highest levels of zinc are found in shellfish, which may contain 400 ppm. The level of zinc in cereal grains is 30–40 ppm. When acid foods such as fruit juices are stored in galvanized containers, sufficient zinc may be dissolved to cause zinc poisoning. The zinc in meat is tightly bound to the myofibrils and has been speculated to influence meat's water-binding capacity (Hamm 1972).

Manganese

Manganese is present in a wide range of foods but is not easily absorbed. This metal is associated with the activation of a number of enzymes. In wheat, a manganese content of 49 ppm has been reported (Schrenk 1964). This is mostly

concentrated in the germ and bran; the level in the endosperm is only 2.4 ppm. Information on the manganese content of seafoods has been supplied by Meranger and Somers (1968). Values range from a low of 1.1 ppm in salmon to a high of 42 ppm in oyster.

Molybdenum

Molybdenum plays a role in several enzyme reactions. Some of the molybdenum-containing enzymes are aldehyde oxidase, sulfite oxidase, xanthine dehydrogenase, and xanthine oxidase. This metal is found in cereal grains and legumes; leafy vegetables, especially those rich in chlorophyll; animal organs; and in relatively small amounts, less than 0.1 ppm, in fruits. The molybdenum content of foods is subject to large variations.

Selenium

Selenium has recently been found to protect against liver necrosis. It usually occurs bound to organic molecules. Different selenium compounds have greater or lesser protective effect. The most active form of selenium is selenite, which is also the least stable chemically. Many selenium compounds are volatile and can be lost by cooking or processing. Kiermeier and Wigand (1969) found about a 5% loss of selenium as a result of drying of skim milk. The variation in selenium content of milk is wide and undoubtedly associated with the selenium content of the soil. The same authors report figures for selenium in milk in various parts of the world ranging from 5 to 1270 µg/kg. The selenium in milk is virtually all bound to the proteins. Morris and Levander (1970) determined the selenium content of a wide variety of foods. Most fruits and vegetables contain less than 0.01 µg/g. Grain products range from 0.025 to 0.66 µg/g, dried skim milk from 0.095 to 0.24 µg/g, meat from 0.1 to 1.9 µg/g, and seafood from 0.4 to 0.7 µg/g.

Fluorine

Fluorine is a constituent of skeletal bone and helps reduce the incidence of dental caries. The fluorine content of drinking water is usually below 0.2 mg/L but in some locations may be as high as 5 mg/L. The optimal concentration for dental health is 1 mg/L. The fluoride content of vegetables is low, with the exception of spinach, which contains 280 µg/100 g. Milk contains 20 µg/100 g and beef about 100 µg/100 g. Fish foods may contain up to 700 µg/100 g and tea about 100 µg/g.

Iodine

Iodine is not present in sufficient amounts in the diet in several areas of the world; an iodine deficiency results in goiter. The addition of iodine to table salt has been extremely effective in reducing the incidence of goiter. The iodine content of most foods is in the area of a few mg/100 g and is subject to great local variations. Fish and shellfish have higher levels. Saltwater fish have levels of about 50–150 mg/100 g and shellfish may have levels as high as 400 mg/100 g.

Chromium

Recent well-controlled studies (Anderson 1988) have found that dietary intake of chromium is in the order of 50 µg/day. Refining and processing of foods may lead to loss of chromium. As an example, in the milling of flour, recovery of chromium in white flour is only 35–44% of that of the parent wheat (Zook et al. 1970). On the other hand, the widespread use of stainless steel equipment in food processing results in leaching of chromium into the food products (Offenbacher and Pi-Sunyer 1983). No foods are known to contain higher-than-average levels of chromium. The average daily intake of chromium from various food groups is shown in Table 5.12. It has been suggested that the dietary intake of chromium in most normal individuals is suboptimal and can lead to nutritional problems (Anderson 1988).

Table 5.12 Chromium intake from various food groups

Food group	Average daily intake (μg)	Comments
Cereal products	3.7	55% from wheat
Meat	5.2	55% from pork
		25% from beef
Fish and seafood	0.6	
Fruits, vegetables, nuts	6.8	70% from fruits and berries
Dairy products, eggs, margarine	6.2	85% from milk
Beverages, confectionery, sugar, and condiments	6.6	45% from beer, wine, and soft drinks
Total	29.1	

Source: Reprinted with permission from R.A. Anderson, Chromium, in *Trace Minerals in Foods*, K.T. Smith, ed., p. 238, 1988, by courtesy of Marcel Dekker, Inc.

Silicon is ubiquitous in the environment and present in many foods. Foods of animal origin are relatively low in silicon; foods of plant origin are relatively high. Good plant sources are unrefined grains, cereal products, and root crops. The dietary intake of silicon is poorly known but appears to be in the range of 20–50 μg/day. Although silicon is now regarded as an essential mineral for humans, a minimum requirement has not been established. Silicon *Foods*, K.T. Smith, ed., p. 385, 1988

Additional Information on Trace Elements

The variations in trace elements in vegetables may be considerable (Warren 1972) and may depend to a large extent on the nature of the soil in which the vegetables are grown. Table 5.13 illustrates the extent of the variability in the content of copper, zinc, lead, and molybdenum of a number of vegetables. The range of concentrations of these metals frequently covers one order of magnitude and occasionally as much as two orders of magnitude. Unusually high concentrations of certain metals may be associated with the incidence of diseases such as multiple sclerosis and cancer in humans.

Aluminum, which has been assumed to be nonnutritious and nontoxic, has come under increasing scrutiny. Its presence has been suggested to be involved in several serious conditions, including Alzheimer's disease (Greger 1985). Since aluminum is widely used in utensils and packaging materials, there is great interest in the aluminum content of foods. Several aluminum salts are used as food additives, for example, sodium aluminum phosphate as a leavening agent and aluminum sulfate for pH control. The estimated average daily intake of aluminum is 26.5 mg, with 70% coming from grain products (Greger 1985).

Fruits contain relatively high levels of organic acids, which may combine with metal ions. It is now generally agreed that these compounds may form chelates of the general formula $M_yH_pL_m(OH)_x$, where M and L represent the metal and the ligand, respectively. According to Pollard and Timberlake (1971), cupric ions form strong complexes with acids containing α-hydroxyl groups. The major fruit acids, citric, malic, and tartaric, are multidendate ligands capable of forming polynuclear chelates. Cupric and ferric ions form stronger complexes than ferrous ions. The strongest complexes are formed by citrate, followed by malate and then tartrate.

Metal Uptake in Canned Foods

Canned foods may take up metals from the container, tin and iron from the tin plate, and tin and lead from the solder. There are several types of internal can corrosion. *Rapid detinning is* one of the most serious problems of can corrosion. With most acid foods, when canned in the absence of oxygen, tin forms the anode of the tin-iron couple. The tin under these conditions goes into solution at an extremely slow rate and can provide product protection for 2 years or longer. There are, however, conditions where iron forms the anode, and in the presence of depolarizing or oxidizing agents the dissolution of tin is greatly accelerated. The food is protected until most of

Table 5.13 Extreme variation in the content of copper, zinc, lead, and molybdenum in some vegetables

	"Normal" content in ppm wet weight	Minimum as fraction of "Normal"	Maximum as multiple of "Normal"	Extreme range
Copper				
Lettuce	0.74	1/15	8	1–120
Cabbage	0.26	1/6	2.5	1–15
Potato	0.92	1/9	4	1–36
Bean (except broad)	0.56	2/5	2.5	1–22
Carrot	0.52	1/9	2.5	1–22
Beet	0.78	1/9	2.5	1–20
Zinc				
Lettuce	4.9	1/6	15	1–90
Cabbage	1.9	½	6	1–12
Potato	2.9	½	5	1–10
Bean (except broad)	3.6	½	2	1–4
Carrot	3.4	½	8	1–48
Beet	4.1	1/4	12	1–16
Lead				
Lettuce	0.25	1/10	30	1–300
Cabbage	0.10	1/8	2.5	1–20
Potato	0.40	1/10	15	1–150
Bean (except broad)	0.24	1/5	4	1–20
Carrot	0.22	1/3	9	1–27
Beet	0.20	1/6	11	1–66
Molybdenum				
Lettuce	0.06	1/8	12	1–96
Cabbage	0.20	1/30	8	1–240
Potato	0.15	1/16	7.5	1–120
Bean (except broad)	0.48	1/30	7	1–210
Carrot	0.22	1/4	3.5	1–14
Beet	0.04	1/30	10	1–300

Source: From H.V. Warren, Variations in the Trace Element Contents of Some Vegetables, *J. Roy. Coll. Gen. Practit*., Vol. 22, pp. 56–60, 1972

the tin is dissolved; thereafter, hydrogen is produced and the can swells and becomes a *springer*. Some foods are more likely to involve rapid detinning, including spinach, green beans, tomato products, potatoes, carrots, vegetable soups, and certain fruit juices such as prune and grapefruit juice.

Another corrosion problem of cans is sulfide staining. This may happen when the food contains the sulfur-containing amino acids cysteine, cystine, or methionine. When the food is heated or aged, reduction may result in the formation of sulfide ions, which can then react with tin and iron to form SnS and FeS. The compound SnS is the major component of the sulfide stain. This type of corrosion may occur with foods such as pork, fish, and peas (Seiler 1968). Corrosion of tin cans depends on the nature of the canned food as well as on the type of tin plate used. Formerly, hot dipped tin plate was used, but this has been mostly replaced by electrolytically coated plate. It has been shown (McKirahan et al. 1959) that the size of the crystals in the tin coating has an important effect on corrosion resistance. Tin plate with small tin crystals easily develops hydrogen swell, whereas tin plate containing large crystals is quite resistant. Seiler (1968) found that the orientation of the different crystal planes also significantly affected the ease of forming sulfide stains.

The influence of processing techniques for grapefruit juice on the rate of can corrosion was studied by Bakal and Mannheim (1966). They found that the dissolved tin content can serve as a corrosion indicator. In Israel the maximum prescribed limit for tin content of canned food is 250 ppm. Deaeration of the juice significantly lowers tin dissolution. In a study of the in-can shelf life of tomato paste, Van der Merwe and Knock (1968) found that, depending on maturity and variety, 1 g of tomato paste stored at 22 °C could corrode tin at rates ranging from 9×10^{-6} to 68×10^{-6} g/month. The useful shelf life could vary from 24 months to as few as 3 months. Up to 95% of the variation could be related to effects of maturity and variety and the associated differences in contents of water-insoluble solids and nitrate.

Severe detinning has often been observed with applesauce packed in plain cans with enameled ends. This is usually characterized by detinning at the headspace interface. Stevenson et al. (1968) found that steam flow closure reduced the detinning problem, but the best results were obtained by complete removal of oxygen through nitrogen closure. Detinning by canned spinach was studied by Lambeth et al. (1969) and was found to be significantly related to the oxalic acid content of the fresh leaves and the pH of the canned product. High-oxalate spinach caused detinning in excess of 60% after 9 months' storage.

In some cases the dissolution of tin into a food may have a beneficial effect on food color, with iron having the opposite effect. This is the case for canned wax beans (Van Buren and Downing 1969). Stannous ions were effective in preserving the light color of the beans, whereas small amounts of iron resulted in considerable darkening. A black discoloration has sometimes been observed in canned all-green asparagus after opening of the can. This has been attributed (Lueck 1970) to the formation of a black, water-insoluble coordination compound of iron and rutin. The iron is dissolved from the can, and the rutin is extracted from the asparagus during the sterilization. Rutin is a flavonol, the 3-rutinoside of quercetin. The black discoloration occurs only after the iron has been oxidized to the ferric state. Tin forms a yellow, water-soluble complex with rutin, which does not present a color problem. The uptake of iron and tin from canned foods is a common occurrence, as is demonstrated by Price and Roos (1969), who studied the presence of iron and tin in fruit juice (Table 5.14).

Table 5.14 Iron and tin content of fruit juices

Product	Iron (ppm)	Tin (ppm)
Fresh orange juice	0.5	7.5
Bottled orange juice	2.5	25
Bottled orange juice	2.0	50
Bottled pineapple juice	15.0	50
Canned orange juice	2.5	60
Canned orange juice	0.5	115
Canned orange juice	2.5	120
Canned pineapple juice	17.5	135

Source: From W.J. Price and J.T.H. Roos, Analysis of Fruit Juice by Atomic Absorption Spectrophotometry. I. The Determination of Iron and Tin in Canned Juice, *J. Sci. Food Agric*., Vol. 20, pp. 427–439, 1969

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Color and Food Colorants

6

John W. Finley, John M. deMan,
and Chang Yong Lee

Introduction

Color is important to many foods, both those that are unprocessed and those that are manufactured. Together with flavor and texture, color plays an important role in food acceptability. In addition, color may provide an indication of chemical changes in a food, such as browning and caramelization. For a few clear liquid foods, such as oils and beverages, color is mainly a matter of transmission of light. Other foods are opaque—they derive their color mostly from reflection.

Color is the general name for all sensations arising from the activity of the retina of the eye. When light reaches the retina, the eye's neural mechanism responds, signaling color among other things. Light is the radiant energy in the wavelength range of about 400–800 nm. According to this definition, color (like flavor and texture) cannot be studied without considering the human sensory system. The color perceived when the eye views an illuminated object is related to the following three factors: the spectral composition of the light source, the chemical and physical characteristics of the object, and the spectral sensitivity properties of the eye. To evaluate the properties of the object, we must standardize the other two factors. Fortunately, the characteristics of different people's eyes for viewing colors are fairly uniform; it is not too difficult to replace the eye by some instrumental sensor or photocell that can provide consistent results. There are several systems of color classification; the most important is the CIE system (Commission International de l'Eclairage—International Commission on Illumination). Other systems used to describe food color are the Munsell, Hunter, and Lovibond systems.

When the reflectance of different colored objects is determined by means of spectrophotometry, curves of the type shown in Fig. 6.1 are obtained. White materials reflect equally over the whole visible wavelength range, at a high level. Gray and black materials also reflect equally over this range but to a lower degree. Red materials reflect in the higher wavelength range and absorb the other wavelengths. Blue materials reflect in the low-wavelength range and absorb the high-wavelength light.

CIE System

The spectral energy distribution of CIE light sources *A* and *C* is shown in Fig. 6.2. CIE illuminant *A* is an incandescent light operated at 2854 K, and illuminant *C* is the same light modified by filters to result in a spectral composition that approximates that of normal daylight. Figure 6.2 also shows the luminosity curve of the standard observer as specified by CIE. This curve indicates how the eyes of normal observers respond to the various spectral light types in the visible portion of the spectrum. By breaking

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_6

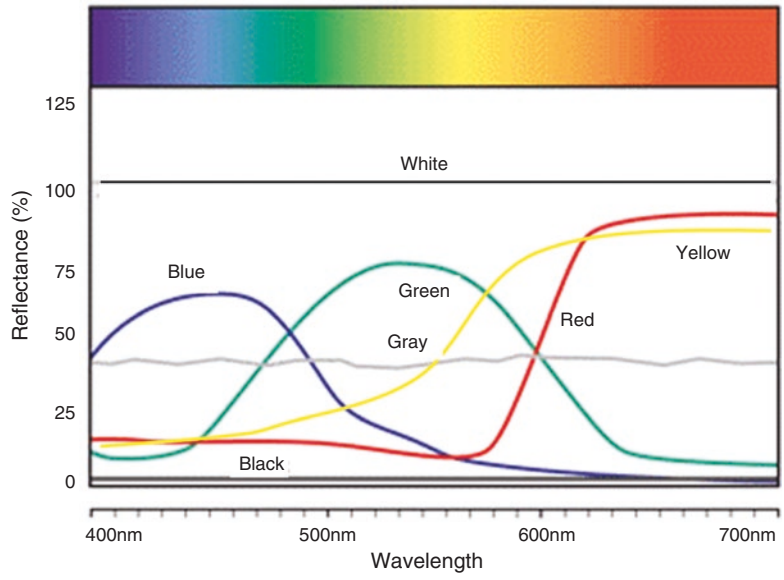


Fig. 6.1 Spectrophotometric reflectance curves of colored objects

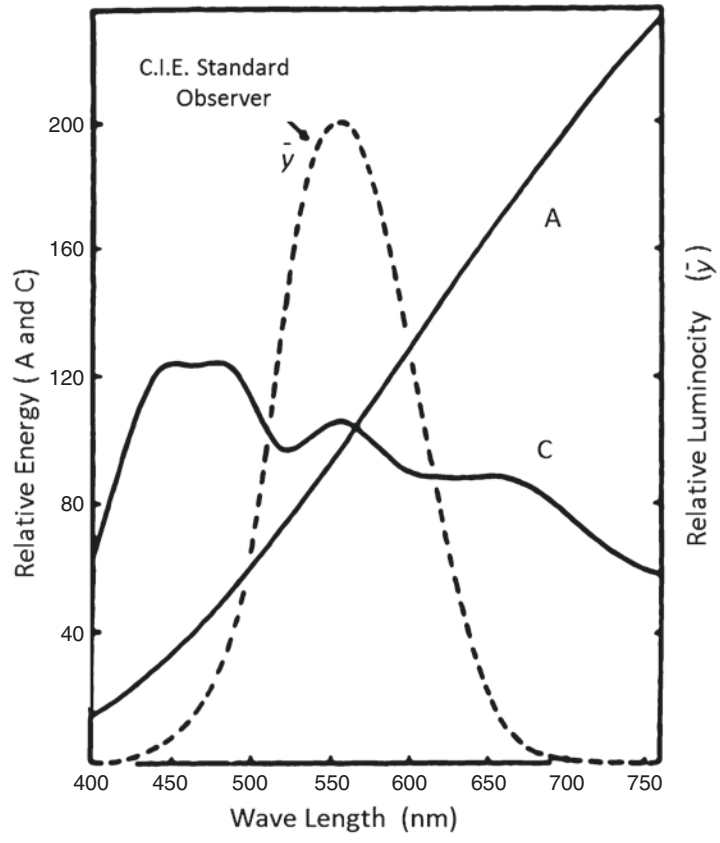


Fig. 6.2 Spectral energy distribution of light sources *A* and *C*, the CIE, and relative luminosity function γ for the CIE standard observer

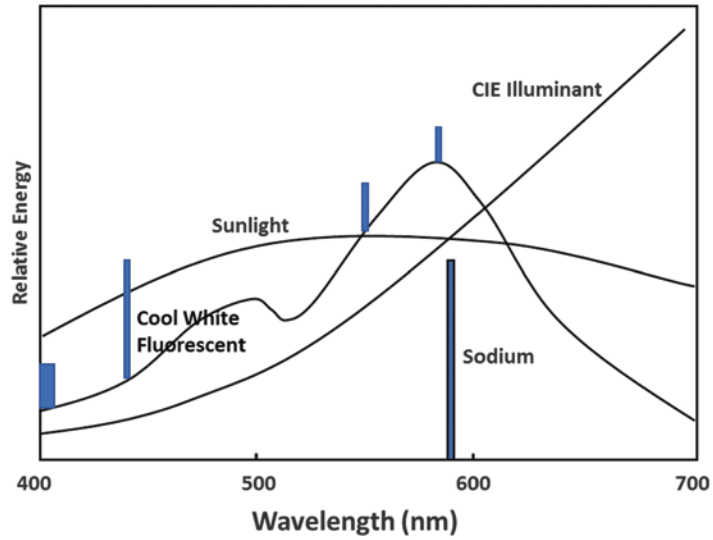


Fig. 6.3 Spectral energy distribution of sunlight, CIE illuminant, cool white fluorescent lamp, and sodium light

down the spectrum, complex light types are reduced to their component spectral light types. Each spectral light type is completely determined by its wavelength. In some light sources, a great deal of radiant energy is concentrated in a single spectral light type. An example of this is the sodium lamp shown in Fig. 6.3, which produces monochromatic light. Other light sources, such as incandescent lamps, give off a continuous spectrum. A fluorescent lamp gives off a continuous spectrum on which is superimposed a line spectrum of the primary radiation produced by the gas discharge (Fig. 6.3).

In the description of light sources, reference is sometimes made to the *black body.* This is a radiating surface inside a hollow space, and the light source's radiation comes out through a small opening. The radiation is independent of the type of material the light source is made of. When the temperature is very high, about 6000 K the maximum of the energy distribution will fall about in the middle of the visible spectrum. Such energy distribution corresponds with that of daylight on a cloudy day. At lower temperatures, the maximum of the energy distribution shifts to longer wavelengths. At 3000 K, the spectral energy distribution is similar to that of an incandescent lamp; at this temperature the energy at 380 nm is only one-sixteenth of that at 780 nm, and most of the energy is concentrated at higher wavelengths (Fig. 6.3). The uneven spectral distribution of incandescent light makes red objects look attractive and blue ones unattractive. This is called color rendition. The human eye has the ability to adjust for this effect.

The CIE system is a trichromatic system; its basis is the fact that any color can be matched by a suitable mixture of three primary colors. The three primary colors, or *primaries,* are red, green, and blue. Any possible color can be represented as a point in a triangle. The triangle in Fig. 6.4 shows how colors can be designated as a ratio of the three primaries. If the red, green, and blue values of a given light type are represented by a, b, and c, then the ratios of each to the total light are given by $a/(a + b + c)$, $b/(a + b + c)$, and $c/(a + b + c)$, respectively. Since the sum of these is one, then only two have to be known to know all three. Color, therefore, is determined by two, not three, of these mutually dependent quantities. In Fig. 6.4, a color point is represented by P. By determining the distance of P from the right angle, the quantities $a/(a + b + c)$ and $b/(a + b + c)$ are found. The quantity $c/(a + b + c)$ is then found, by first extending the horizontal dotted line through P until it crosses the hypotenuse at Q and by then constructing another right angle triangle with Q at the top. All combinations of a, b, and c will be points inside the triangle.

The relative amounts of the three primaries required to match a given color are called the *tristimulus values* of the color. The CIE primaries are imaginary, because there are no real primaries

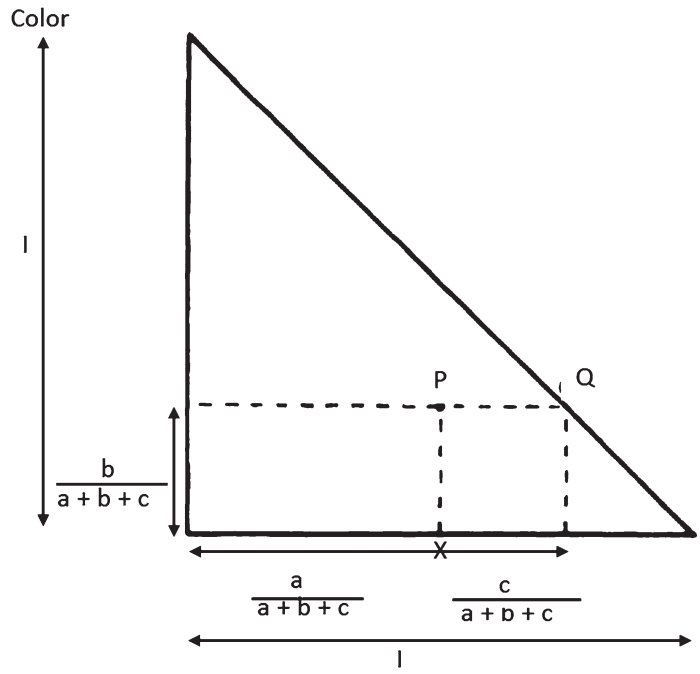


Fig. 6.4 Representation of a color as a point in a color triangle

that can be combined to match the highly saturated hues of the spectrum.

In the CIE system the red, green, and blue primaries are indicated by *X*, *Y*, and Z. The amount of each primary at any particular wavelength is given by the values $\bar{x}, \bar{y}$, and $\bar{z}$. These are called the *distribution coefficients* or the red, green, and blue factors. They represent the tristimulus values for each chosen wavelength. The distribution coefficients for the visible spectrum are presented in Fig. 6.5. The values of $\bar{y}$ correspond with the luminosity curve of the standard observer (Fig. 6.2). The distribution coefficients are dimensionless because they are the numbers by which radiation energy at each wavelength must be multiplied to arrive at the *X*, *Y*, and *Z* content. The amounts of *X*, *Y*, and Z primaries required to produce a given color are calculated as follows:

$$X = \int_{380}^{780} \bar{x}\ IRdh$$

$$XY = \int_{380}^{780} \bar{y}\, IRdh$$

$$XZ = \int_{380}^{780} \bar{z}\ IRdh$$

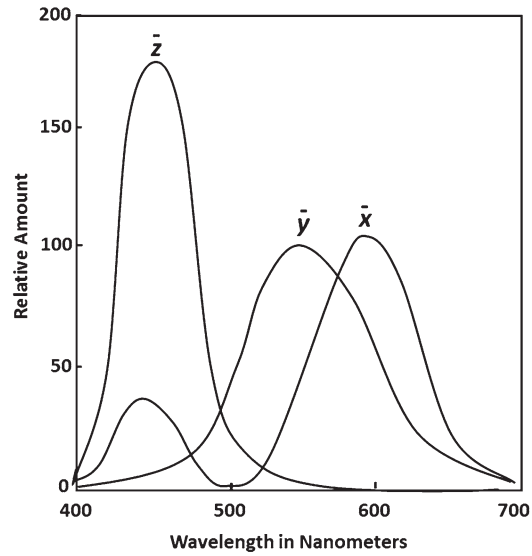


Fig. 6.5 Distribution coefficients *x*, *y*, and *z* for the visible spectrum. *Source:* From Hunter Associates Lab., Inc.

where

I = spectral energy distribution of illuminant
R = spectral reflectance of sample
dh = small wavelength interval
$\bar{x}, \bar{y}, \bar{z}$ = red, green, and blue factors

The ratios of the primaries can be expressed as

$$x = \frac{X}{X+Y+Z}$$

$$y = \frac{Y}{X+Y+Z}$$

$$z = \frac{Z}{X+Y+Z}$$

The quantities x and y are called the chromaticity coordinates and can be calculated for each wavelength from

$$x = \bar{x} / \left(\bar{x} + \bar{y} + \bar{z}\right)$$

$$y = \bar{y} / \left(\bar{x} + \bar{y} + \bar{z}\right)$$

$$z = 1 - \left(x + y\right)$$

A plot of x versus y results in the CIE chromaticity diagram (Fig. 6.6). When the chromaticities of all of the spectral colors are placed in this graph, they form a line called the locus. Within this locus and the line connecting the ends, represented by 400 and 700 nm, every point represents a color that can be made by mixing the three primaries. The point at which exactly equal amounts of each of the primaries are present is called the equal point and is white. This white point represents the chromaticity coordinates of illuminant *C*. The red primary is located at $x = 1$ and $y = 0$; the green primary at $x = 0$ and $y = 1$; and the blue primary at $x = 0$ and $y = 0$. The line connecting the ends of the locus represents purples, which are nonspectral colors resulting from mixing various amounts of red and blue. All points within the locus represent real colors. All points outside the locus are unreal, including the imaginary primaries *X*, *Y*, and *Z*. At the red end of the locus, there is only one point to represent the wavelength interval of 700–780 nm. This means that all colors in this range can be simply matched by adjustment of luminosity. In the range of 540–700 nm, the spectrum locus is almost straight; mixtures of two spectral light types along this line segment will closely match intervening colors with little loss of purity. In contrast, the spectrum locus below 540 nm is curved, indicating that a combination of two spectral lights along this portion of the locus results in colors of decreased purity.

A pure spectral color is gradually diluted with white when moving from a point on the spectrum locus to the white point *P*. Such a straight line with purity decreasing from 100 to 0% is known as a line of constant dominant wavelength. Each color, except the purples, has a dominant wavelength. The position of a color on the line connecting the locus and *P* is called excitation purity (p_e) and is calculated as follows:

$$P_e = \frac{x - x_w}{x_p - x_w} = \frac{y - y_w}{y_p - y_w}$$

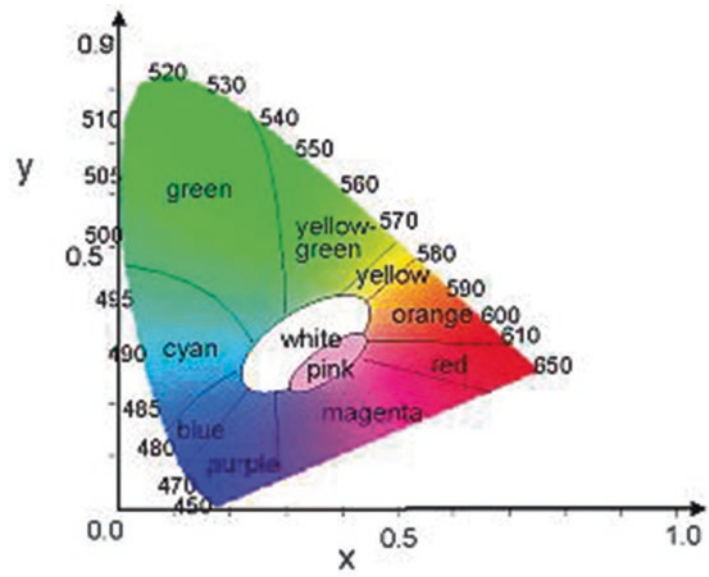


Fig. 6.6 CIE chromaticity diagram

where

x and y are the chromaticity coordinates of a color

x_w and y_w are the chromaticity coordinates of the achromatic source

x_p and y_p are the chromaticity coordinates of the pure spectral color

Achromatic colors are white, black, and gray. Black and gray differ from white only in their relative reflection of incident light. The purples are nonspectral chromatic colors. All other colors are chromatic; for example, brown is a yellow of low lightness and low saturation. It has a dominant wavelength in the yellow or orange range.

A color can be specified in terms of the tristimulus value Y and the chromaticity coordinates x and y. The Y value is a measure of luminous reflectance or transmittance and is expressed in percent simply as $Y/1000$.

Another method of expressing color is in terms of luminance, dominant wavelength, and excitation purity. These latter are roughly equivalent to the three recognizable attributes of color: lightness, hue, and saturation. Lightness is associated with the relative luminous flux, reflected or transmitted. Hue is associated with the sense of redness, yellowness, blueness, and so forth. Saturation is associated with the strength of hue or the relative admixture with white. The combination of hue and saturation can be described as chromaticity.

Complementary colors (Table 6.1) are obtained when a straight line is drawn through the equal energy point *P*. When this is done for the ends of the spectrum locus, the wavelength complementary to the 700–780 point is at 492.5 nm, and for the 380–410 point is at 567 nm. All of the wavelengths between 492.5 and 567 nm are complementary to purple. The purples can be described in terms of dominant wavelength by using the wavelength complementary to each purple, and purity can be expressed in a manner similar to that of spectral colors.

An example of the application of the CIE system for color description is shown in Fig. 6.7. The curved, dotted line originating from *C* represents the locus of the chromaticity coordinates of caramel and glycerol solutions. The chromaticity coordinates of maple syrup and honey follow the same locus. Three triangles on this curve represent the chromaticity coordinates of U.S. Department of Agriculture (USDA) glass color standards for maple syrup. These are described as light amber, medium amber, and dark amber. The six squares are chromaticity coordinates of honey, designated by USDA as water white, extra white, white, extra light amber, light amber, and amber. Such specifications are useful in describing color standards for a variety of products. In the case of the light amber standard for maple syrup, the following values apply: $x = 0.486$, $y = 0.447$, and $T = 38.9\%$. In this way, x and y provide a specification for chromaticity and T for luminous transmittance or lightness. This is easily expressed as the mixture of primaries under illuminant *C* as follows: 48.6% of red primary, 44.7% of green primary, and 6.7% of blue primary. The light transmittance is 38.9%.

The importance of the light source and other conditions that affect viewing of samples cannot be overemphasized. Many substances are metameric; that is, they may have equal transmittance or reflectance at a certain wavelength but possess noticeably different colors when viewed under illuminant *C*.

Table 6.1 Complementary colors

Wavelength (nm)	Color	Complementary color
400	Violet	Yellow
450	Blue	
500	Green	Orange
550	Yellow	Red Violet
600	Orange	Blue
650	Red	Green
700		

Munsell System

In the Munsell system of color classification, all colors are described by the three attributes of hue, value, and chroma. This can be envisaged as a three-dimensional system (Fig. 6.8). The hue scale is based on ten hues which are distributed on the circumference of the hue circle. There are five hues: red, yellow, green, blue, and purple; they are

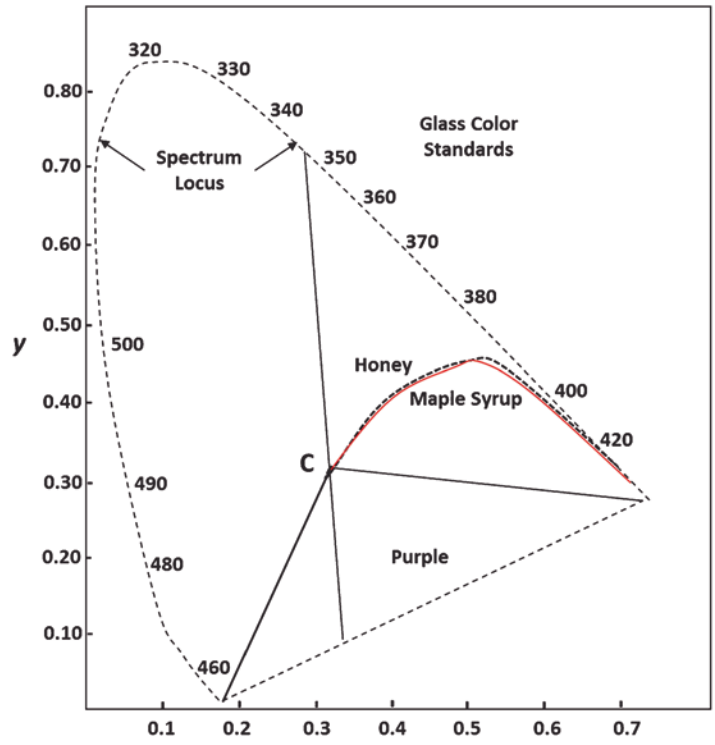


Fig. 6.7 CIE chromaticity diagram with color points for maple syrup and honey glass color standards

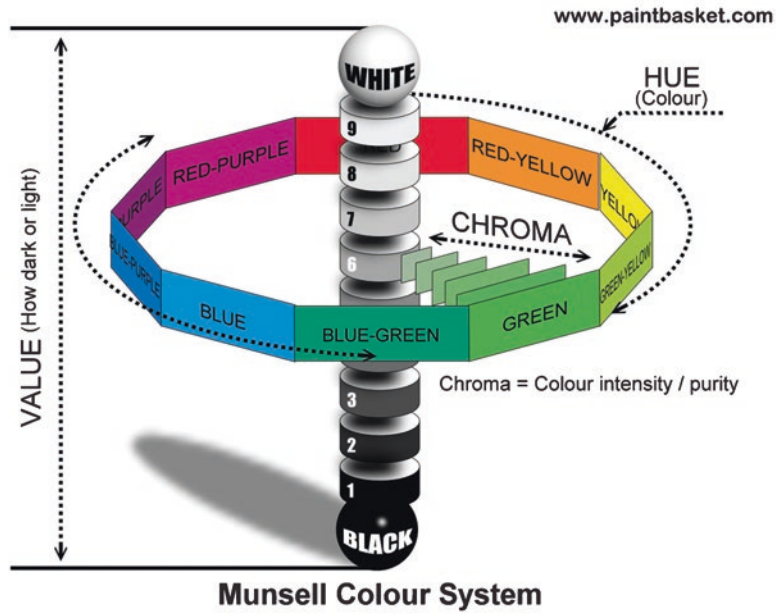


Fig. 6.8 The Munsell system of color classification

written as R, Y, G, B, and P. There are also five intermediate hues, YR, GY, BG, PB, and RP. Each of the ten hues is at the midpoint of a scale from 1 to 10. The value scale is a lightness scale ranging from 0 (black) to 10 (white). This scale is distributed on a line perpendicular to the plane of the

hue circle and intersecting its center. Chroma is a measure of the difference of a color from a gray of same lightness. It is a measure of purity. The chroma scale is of irregular length, and begins with 0 for the central gray. The scale extends outward in steps to the limit of purity obtainable by available pigments. The shape of the complete Munsell color space is indicated in Fig. 6.9. The description of a color in the Munsell system is given as *H*, *V*/*C*. For example, a color indicated as 5R 2.8/3.7 means a color with a red hue of 5R, a value of 2.8, and a chroma of 3.7. All colors that can be made with available pigments are laid down as color chips in the Munsell book of color.

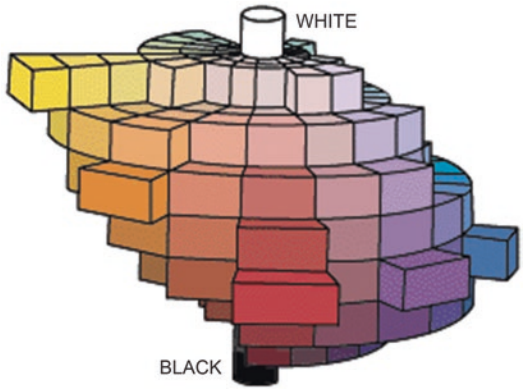


Fig. 6.9 The Munsell color space

Hunter System

The CIE system of color measurement is based on the principle of color sensing by the human eye. This accepts that the eyes contain three light-sensitive receptors—the red, green, and blue receptors. One problem with this system is that the *X, Y,* and Z values have no relationship to color as perceived, though a color is completely defined. To overcome this problem, other color systems have been suggested. One of these, widely used for food colorimetry, is the Hunter *L*, *a*, *b*, system. The so-called uniform-color, opponent-colors color scales are based on the opponent-colors theory of color vision. In this theory, it is assumed that there is an intermediate signal-switching stage between the light receptors in the retina and the optic nerve, which transmits color signals to the brain. In this switching mechanism, red responses are compared with green and result in a red-to-green color dimension. The green response is compared with blue to give a yellow-to-blue color dimension. These two color dimensions are represented by the symbols *a* and *b.* The third color dimension is lightness *L,* which is nonlinear and usually indicated as the square or cube root of *Y.* This system can be represented by the color space shown in Fig. 6.10. The *L*, *a*, *b*, color solid is similar to the

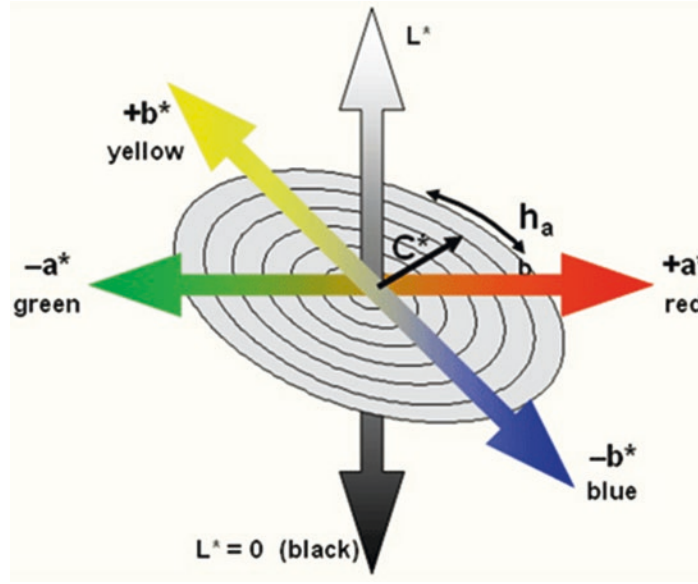


Fig. 6.10 The Hunter *L*, *a*, *b* color space. *Source:* From Hunter Associates Lab., Inc.

Munsell color space. The lightness scale is common to both. The chromatic spacing is different. In the Munsell system, there are the polar hue and chroma coordinates, whereas in the *L*, *a*, *b*, color space, chromaticity is defined by rectangular *a* and *b* coordinates. CIE values can be converted to color values by the equations shown in Table 6.2 into *L*, *a*, *b*, values and vice versa (MacKinney and Little 1962; Clydesdale and Francis 1970). This is not the case with Munsell values. These are obtained from visual comparison with color chips (called Munsell renotations) or from instrumental measurements (called Munsell renotations), and conversion is difficult and tedious.

The Hunter tristimulus data, *L* (value), *a* (redness or greenness), and *b* (yellowness or blueness), can be converted to a single color function called color difference (ΔE) by using the following relationship:

$$\Delta E = (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2$$

The color difference is a measure of the distance in color space between two colors. It does not indicate the direction in which the colors differ.

Lovibond System

The Lovibond system is widely used for the determination of the color of vegetable oils. The method involves the visual comparison of light transmitted through a glass cuvette filled with oil at one side of an inspection field; at the other side, colored glass filters are placed between the light source and the observer. When the colors on each side of the field are matched, the nominal value of the filters is used to define the color of the oil. Four series of filters are used—red, yellow, blue, and gray filters. The gray filters are used to compensate for intensity when measuring samples with intense chroma (color purity) and are used in the light path going through the sample. The red, yellow, and blue filters of increasing intensity are placed in the light path until a match with the sample is obtained. Vegetable oil colors are usually expressed in terms of red and yellow; a typical example of the Lovibond color of an oil would be R1.7 Y17. The visual determination of oil color by the Lovibond method is widely used in industry and is an official method of the American Oil Chemists' Society. Visual methods of this type are subject to a number of errors, and

Table 6.2 Mathematical relationship between color scales

To convert	To L, *a*, b	To X%, Y, Z%	To Y, x, y
From *X%, Y Z%*	$L = 10\sqrt{Y}$ $a = \frac{17.5(X\% - Y)}{\sqrt{Y}}$ $b = \frac{7.0(Y - Z\%)}{\sqrt{Y}}$		$Y = Y\%$ $x = \frac{X}{X+Y+Z}$ $y = \frac{Y}{X+Y+Z}$
From *L, a, b*		$Y = 0.01L^2$ $X\% = 0.01L^2 + \frac{aL}{175}$ $Z\% = 0.01L^2 - \frac{bL}{70}$	$Y = 0.01L^2$ $x = \frac{a + 1.75L}{5.645L + a - 3.012b}$ $y = \frac{1.786L}{5.645L + a - 3.012b}$
From *Y, x, y*	$L = 10\sqrt{Y}$ $a = 17.5\sqrt{Y}\frac{1.02x}{y} - 1$ $b = 5.929\sqrt{Y}\frac{2.181y + x - 1}{y}$	$X\% = 1.02 \times \frac{Y}{y}$ $Z\% = .847\left[1 - (x + y)\right]\frac{Y}{y}$	

Source: From Hunter Associates Lab., Inc.

the results obtained are highly variable. A study has been reported (Maes 1997) to calculate CIE and Lovibond color values of oils based on their visible light transmission spectra as measured by a spectrophotometer. A computer software has been developed that can easily convert light transmission spectra into CIE and Lovibond color indexes.

Gloss

In addition to color, there is another important aspect of appearance, namely gloss. Gloss can be characterized as the reflecting property of a material. Reflection of light can be diffused or undiffused (specular). In specular reflection, the surface of the object acts as a mirror, and the light is reflected in a highly directional manner. Surfaces can range from a perfect mirror with completely specular reflection to a surface reflecting in a completely diffuse manner. In the latter, the light from an incident beam is scattered in all directions and the surface is called matte.

Food Colorants

Color is one of the important quality attributes of foods. It is because that consumers often use color as an index of freshness, wholesomeness, and overall quality. Specific colors of fruits are often associated with maturity. Consumers use color as a way to identify a food and a way to judge the quality of a food. No matter how one can provide consumers the most nutritious, safest, and most economical foods, if they do not have attractive color, consumers will not accept them. Color influence flavor perception: red color for strawberry, raspberry, or cherry flavored; yellow to be lemon; and green to be lime flavored. Color also affects the apparent level of sweetness: strongly red-colored strawberry-flavored drinks to be sweeter than less strongly colored ones. Unfortunately, the color of a food may change during processing, storage, and preparation that are often perceived as undesirable, which is an indication of chemical changes. Some processed foods are manufactured to be brightly colored to appeal consumers' eyes, especially to children. Therefore, understanding how to preserve the natural colors of food during processing is very important for the acceptability of a food product. In addition, it should also be noted some substances such as β-carotene or riboflavin or anthocyanins are not only colorants but nutrients and nutraceuticals. Therefore, color of foods has multiple effects on foods and to the consumers. Thus controlling, changing, or stabilizing the color of foods is a major objective for food scientists.

Until the mid-eighteenth century, the only external sources of coloring used in foods were natural: animal, vegetables, and minerals including saffron, carrots, mulberries, flowers, and copper and iron ores. Since then, food manufacturers used many different chemicals to color foods. Pickles were colored green with copper sulfate. Candy was colored with salts of copper and lead. Artificial food colors have been obtained from organic coal tar in the latter part of the nineteenth century. Addition of colorants to foods has many commercial reasons: to enhance colors that occur naturally, to make food more attractive and appetizing, to offset color loss during processing and storage, to correct natural variations in color and to provide color to colorless and "fun foods" for kids. Color additives are now recognized as an important part of practically all processed foods.

However, color additives have been controversial almost since they were first introduced, due to the facts that they have the reputation of being potentially toxic, they may be used to deceive the consumers, and that their primary function is to enhance appearance rather than nutritive value, shelf life, or safety. For example, pickles were colored with copper sulfate, cheese with lead oxide, candy with compounds containing lead and mercury. Arsenic salts were also commonly used. The number of synthetic food colorants has

declined over the years as government safety regulations and toxicity testing have advanced. The FDA regulates food color additives under the authority of the Color Additives Amendment Act of 1960 to the Federal Food, Drug, and Cosmetic Act. FDA's permitted colors are classified as subject to *certification* or *exempt from certification*, both of which are subjected to rigorous safety standards prior to their approval and listing for use in foods:

+ Certified colors:
+ Color that are exempt from certification:

Certified (Synthetic) Color

Certified colors are synthetically produced dye, lakes, or pigments and used widely because they impart an intense, uniform color. They are less expensive and blend more easily to create a variety of hues. Those for food use are chemically classified as azo, xanthene, triphenylmethane, and indigoid dyes, which are synthesized mainly from raw materials obtained from petroleum. Currently, there are nine certified color additives that may be added to foods (Table 6.3 and Fig. 6.11). Most of the certified colorants used in the United States have been assigned and FD&C number as required by the Food, Drug and Cosmetic Act: FD&C indicates that these colors are approved by FDA for use in coloring foods, drugs, and cosmetics; and D&C is considered safe to use with drugs and cosmetics.

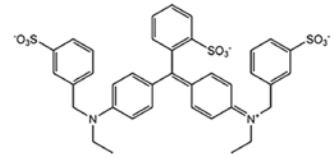
Certified colors may be used as dyes or converted to lakes. They can be manufactured as a powder, granule, or liquid. Lakes are prepared by precipitating the soluble dye onto an approved insoluble base, such as aluminum hydroxide and then dried. Since the lakes are dry products providing opacity, they are stable to heat and light, can be used in dry product and suitable to use for fats, gums, waxes, oils, and food packaging materials. They are stable to heat and light.

Colors Exempt from Certification (Natural)

Colors that are exempt from certification are a group of pigments derived from natural sources such as animals, plants, or minerals. They always have been part of our diet. They have been isolated and added back to foods for the same reasons as the certified colors. However, the exempted natural colorants are relatively unstable, easily affected

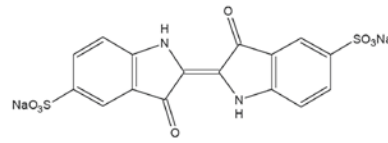
Table 6.3 Color certified for use in foods

FD&C number	Common name	Chemical class	Characteristics
FD&C Blue No. 1	Brilliant blue FCF	Triphenylmethane	Stable to heat
			Unstable to light Allura
FD&C Blue No. 2	Indigotine (royal blue)	Indigoid	Stable to light
			Unstable to water
D&C Green No. 3	Fast green FCF	Triphenylmethane	Stable, brilliant color
FD&C Red No. 3	Erythrosine (cherry red)	Xanthene	Stable to heat
FD&C Red No. 40	Allura red (orange-red)		unstable to redox agents
FD&C Yellow No. 5	Tartrazine (lemon yellow)	Azo	Stable to heat and light
			Very soluble in water
FD&C Yellow No. 6	Sunset yellow	Azo	Fair stability to heat and light
Orange		Azo	Not soluble in water
Citrus Red No. 2		Azo	Use only for orange skins



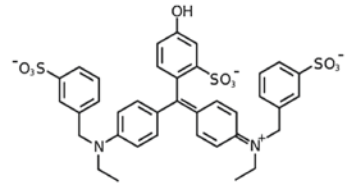
FD&C Blue No. 1 (Brilliant Blue)

Commonly used in ice cream, canned peas, icings, dairy products, sweets and drinks.



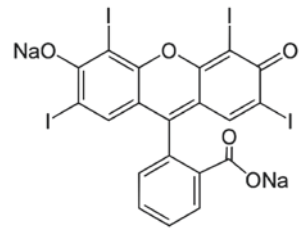
FD&C Blue No. 2 (Indigotine, royal blue)

Originally indigo was a natural dye extracted from plants. Today, nearly all indigo dye is produced by industrial syntheses. It is the blue of blue jeans.



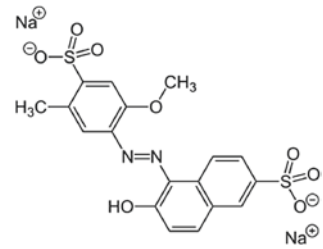
FD&C Green No. 3 (Fast Green)

It can be used for tinned green peas and other vegetables. It is the least used of the seven main FDA approved dyes. prohibited in European Union and some other countries.



FD&C Red No. 3 (Erythrosine, Cherry-red)

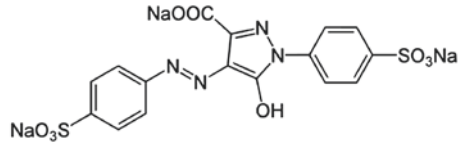
Known as a organoiodine compound. Primarily used in sweets, such as candies, cake-decorating gels. Commonly used in many countries of the world, but less commonly used in the United Stated, the second least used after Fast Green.



FD&C Red No. 40 (Allura Red AC, orange-red)

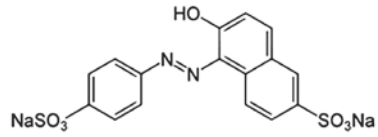
Has dark red color. Very soluble in watere. The most commonly used red colorant in the United States, especially in soft drinks.

Fig. 6.11 The chemical structures of FDA certified food colorants. (As noted, most of the FD&C colorants are sodium salts of sulfonic acids)



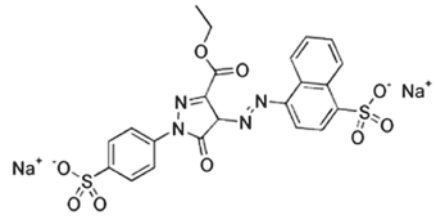
FD&C Yellow No. 5 (Tartrazine, lemon yellow)

A commonly used color all over the world for ice cream, confectionery, drink mixes, corn chips, popcorn, potato chips, mustard, pickles,….



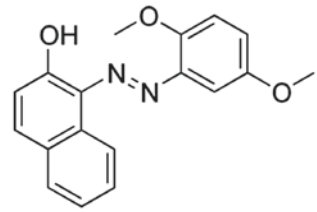
FD&C Yellow No. 6 (Sunset yellow)

It is used in candy, desserts, snacks, sauces and preserved fruits.



Orange B

Restricted use only in frankfurters and sauage casing or surfaces.



Citrus Red No. 2

Restricted use only to color the skins of orange

Fig. 6.11 (continued)

by the food matrix, such as pH, salts, and processing conditions, compared to certified synthetic colorants. However, exempt colors are well received by the consumer due to the name of "natural." Common natural colorings include annatto, beet root, caramel, carrot oil, grape skins, paprika, saffron, turmeric, and others, such as inorganic compounds, titanium dioxide. Some of these can be used only with certain restrictions (Table 6.4). Today, some of these are also well perceived by consumer as the source of antioxidants, derived from bioactive green, red, yellow, orange, and blue colors as the sources of lycopene, beta-carotene, lutein, anthocyanin and astaxanthin.

Table 6.4 Colorants exempt from certification

Exempt color name	Uses and restriction
Annatto extract	Foods generally
Astaxanthin	Salmon fish feed
Beet powder	Foods generally
Canthaxanthin	Foods generally (not to exceed 30 mg/lb), and animal feed
Caramel	Foods generally
β-apo-carotenal	Foods generally (not to exceed 15 mg/lb)
β-carotene	Foods generally
Carrot oil	Foods generally
Cochineal extract; carmine	
Corn endosperm oil	Chicken feed
Cottonseed flour	Foods generally
Grape skin extract	Carbonated drinks, alcoholic beverages
Fruit juice	Foods generally
Paprika	Foods generally
Riboflavin	Foods generally
Saffron	Foods generally
Titanium dioxide	Foods generally (not to exceed 1%)
Turmeric	Foods generally
Vegetable juices	Foods generally

Source: Griffiths (2005): CFR (2004)

With few exceptions, the naturally occurring pigments can be divided into the following four groups:

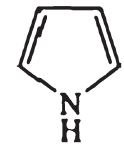
1. Tetrapyrrole compounds: chlorophylls, hemes, and bilins
2. Isoprenoid derivatives: carotenoids
3. Benzopyran derivatives: anthocyanins and flavonoids
4. Betalains and other colorants: betacyanins, betaxanthins, caramels and melanoidins.

The chlorophylls are characteristic of green vegetables and leaves. The heme pigments are found in meat and fish. The carotenoids are a large group of compounds that are widely distributed in animal and vegetable products; they are found in fish and crustaceans, vegetables and fruits, eggs, dairy products, and cereals. Anthocyanins and flavonoids are found in root vegetables and fruits such as berries and grapes. Caramels and melanoidins are found in syrups and cereal products, especially if these products have been subjected to heat treatment.

Tetrapyrrole Pigments

Myoglobins

The basic unit from which the tetrapyrrole pigments are derived is pyrrole.



The basic structure of the heme pigments consists of four pyrrole units joined together into a porphyrin ring as shown in Fig. 6.12. In the heme pigments, the nitrogen atoms are linked to a central iron atom. The color of meat is the result of the presence of two pigments, myoglobin and hemoglobin. Both pigments have globin as the protein portion, and the heme group is composed of the porphyrin ring system and the central iron atom. In myoglobin, the protein portion has a molecular weight of about 17,000. In hemoglobin, this is about 67,000—equivalent to four times the size of the myoglobin protein. The central iron in Fig. 6.12 has six coordination bonds; each bond represents an electron pair accepted by the iron from five nitrogen atoms, four from the

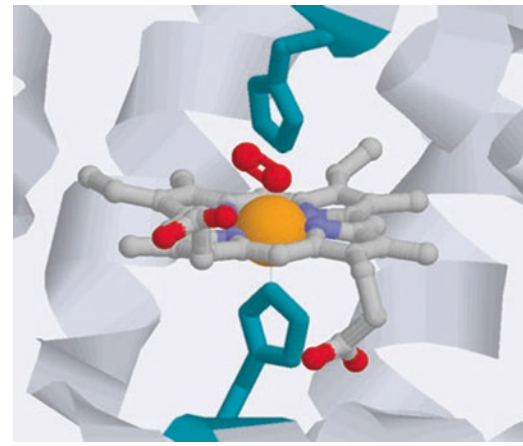


Fig. 6.12 Schematic representation of the heme complex of myoglobin (http://www.wiley.com/college/pratt/0471393878/instructor/structure/myoglobin_hemoglobin/index.html)

porphyrin ring and one from a histidyl residue of the globin. The sixth bond is available for joining with any atom that has an electron pair to donate. The ease with which an electron pair is donated determines the nature of the bond formed and the color of the complex. Other factors playing a role in color formation are the oxidation state of the iron atom and the physical state of the globin.

In fresh meat and in the presence of oxygen, there is a dynamic system of three pigments, oxymyoglobin, myoglobin, and metmyoglobin. The reversible reaction with oxygen is

$$\mathbf{Mb} + \mathbf{O_2} \rightarrow \mathbf{MbO_2}$$

In both pigments, the iron is in the ferrous form; upon oxidation to the ferric state, the compound becomes metmyoglobin. The bright red color of fresh meat is due to the presence of oxymyoglobin; discoloration to brown occurs in two stages, as follows:

$$\underset{(\text{red})}{\mathbf{MbO_2}} \rightleftarrows \underset{(\text{Purplish})}{\mathbf{Mb}} \rightleftarrows \underset{(\text{Brownish})}{\mathbf{MetMb}}$$

Oxymyoglobin represents a ferrous covalent complex of myoglobin and oxygen. The absorption spectra of the three pigments are shown in Fig. 6.13 (Bodwell and McClain 1971). Myoglobin forms an ionic complex with water in the absence of strong electron pair donors that can form covalent complexes. It shows a diffuse absorption band in the green area of the spectrum at about 555 nm and has a purple color. In metmyoglobin, the major absorption peak is shifted toward the blue portion of the spectrum at about 505 nm with a smaller peak at 627 nm. The compound appears brown.

As indicated above, oxymyoglobin and myoglobin exist in a state of equilibrium with oxygen; therefore, the ratio of the pigments is dependent on oxygen pressure. The oxidized form of myoglobin, the metmyoglobin, cannot bind oxygen. In meat, there is a slow and continuous oxidation of the heme pigments to the metmyoglobin state. Reducing substances in the tissue reduce the metmyoglobin to the ferrous form. The oxygen pressure, which is so important for the state of the equilibrium, is greatly affected by packaging materials used for meats. The maximum rate of conversion to metmyoglobin occurs

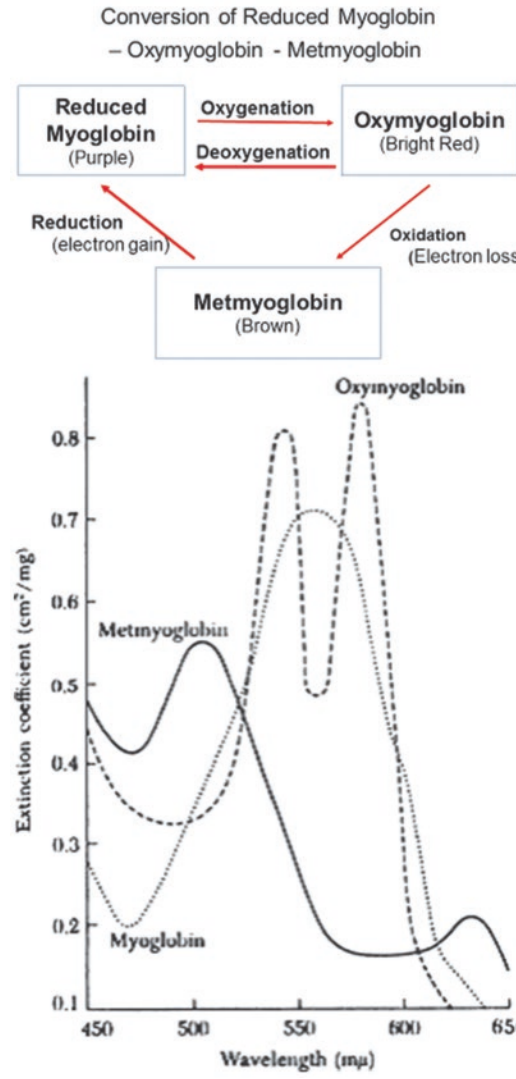


Fig. 6.13 Absorption spectra of myoglobin, oxymyoglobin, and metmyoglobin. *Source*: From C.E. Bodwell and P.E. McClain, Proteins, in *The Sciences of Meat Products,* 2nd ed., J.E. Price and B.S. Schweigert, eds., 1971, W.H. Freeman & Co.

at partial pressures of 1–20 nm of mercury, depending on pigment, pH, and temperature (Fox 1966). When a packaging film with low oxygen permeability is used, the oxygen pressure drops to the point where oxidation is favored. To prevent this, Landrock and Wallace (1955) established that oxygen permeability of the packaging film must be at least 5 L of oxygen/m^2/day/atm.

Fresh meat open to the air displays the bright red color of oxymyoglobin on the surface. In the

interior, the myoglobin is in the reduced state and the meat has a dark purple color. As long as reducing substances are present in the meat, the myoglobin will remain in the reduced form; when they are used up, the brown color of metmyoglobin will predominate. According to Solberg (1970), there is a thin layer a few nanometers below the bright red surface and just before the myoglobin region, where a definite brown color is visible. This is the area where the oxygen partial pressure is about 1.4 nm and the brown pigment dominates. The growth of bacteria at the meat surface may reduce the partial oxygen pressure to below the critical level of 4 nm. Microorganisms entering the logarithmic growth phase may change the surface color to that of the purplish-red myoglobin (Solberg 1968).

In the presence of sulfhydryl as a reducing agent, myoglobin may form a green pigment, called sulfmyoglobin. The pigment is green because of a strong absorption band in the red region of the spectrum at 616 nm. In the presence of other reducing agents, such as ascorbate, cholemyoglobin is formed. In this pigment, the porphyrin ring is oxidized. The conversion into sulfmyoglobin is reversible; cholemyoglobin formation is irreversible, and this compound is rapidly oxidized to yield globin, iron, and tetrapyrrole. According to Fox (1966), this reaction may happen in the pH range of 5–7.

Heating of meat results in the formation of a number of pigments. The globin is denatured. In addition, the iron is oxidized to the ferric state. The pigment of cooked meat is brown and called hemichrome. In the presence of reducing substances such as those that occur in the interior of cooked meat, the iron may be reduced to the ferrous form; the resulting pigment is pink hemochrome.

In the curing of meat, the heme reacts with nitrite of the curing mixture. The nitrite-heme complex is called nitrosomyoglobin, which has a red color but is not particularly stable. On heating the more stable nitrosohemochrome, the major cured meat pigment is formed, and the globin portion of the molecule is denatured. This requires a temperature of 65 °C. This molecule has been called nitrosomyoglobin and nitrosylmyoglobin, but Möhler (1974) has pointed out that the only correct name is nitric oxide myoglobin. The first reaction of nitrite with myoglobin is oxidation of the ferrous iron to the ferric form and formation of MetMb. At the same time, nitrate is formed according to the following reaction (Möhler 1974):

$$4\mathrm{MbO_2} + 4\mathrm{NO_2^-} + 2\mathrm{H_2O} \rightarrow 4\mathrm{MetMbOH} + 4\mathrm{NO_3^-} + \mathrm{O_2}$$

During the formation of the curing pigment, the nitrite content is gradually lowered; there are no definite theories to account for this loss.

The reactions of the heme pigments in meat and meat products have been summarized in the scheme presented in Fig. 6.14 (Fox 1966). Bilin-type structures are formed when the porphyrin ring system is broken.

Chlorophylls

The chlorophylls are green pigments responsible for the color of leafy vegetables and some fruits. In green leaves, the chlorophyll is broken down during senescence and the green color tends to disappear. In many fruits, chlorophyll is present in the unripe state and gradually disappears as the yellow and red carotenoids take over during ripening. In plants, chlorophyll is isolated in the chloroplastids. These are microscopic particles consisting of even smaller units, called grana, which are usually less than 1 μm in size and at the limit of resolution of the light microscope. The grana are highly structured and contain laminae between which the chlorophyll molecules are positioned.

The chlorophylls are tetrapyrrole pigments in which the porphyrin ring is in the dihydro form and the central metal atom is magnesium. There are two chlorophylls, *a* and *b,* which occur together in a ratio of about 1:25. Chlorophyll *b* differs from chlorophyll *a* in that the methyl group on carbon 3 is replaced with an aldehyde group. The structural formula of chlorophyll *a* is given in Fig. 6.15. Chlorophyll is a diester of a dicarboxylic acid (chlorophyllin); one group is esterified with methanol, the other with phytyl alcohol. The magnesium is removed very easily

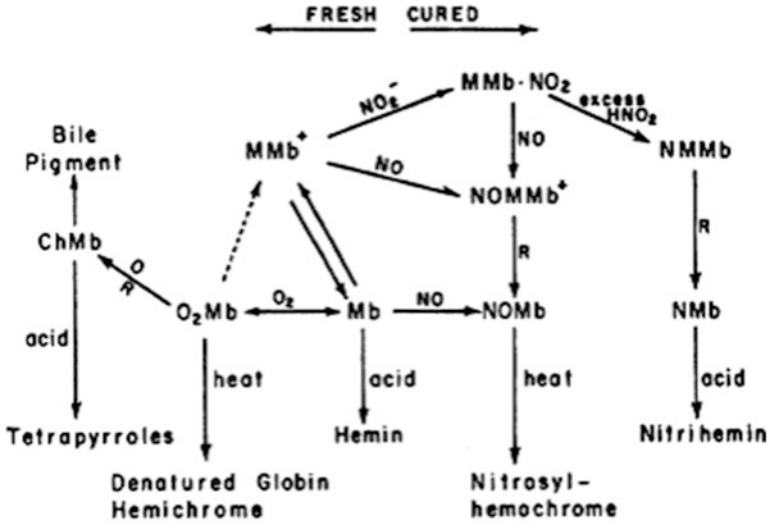


Fig. 6.14 Heme pigment reactions in meat and meat products. *ChMb* cholemyoglobin (oxidized porphyrin ring), *O_2Mb* oxymyoglobin (Fe^{+2}), *MMb* metmyoglobin (Fe^{+3}), *Mb* myoglobin (Fe^{+2}), *MMb·NO_2* metmyoglobin nitrate, *NOMMb* nitrosylmetmyoglobin, *NOMb* nitrosylmyoglobin, *NMMb* nitrimetmyoglobin, *NMb* nitrimyoglobin, the latter two being reaction products of nitrous acid and the heme portion of the molecule, *R* reductants, *O* strong oxidizing conditions. *Source:* From J.B. Fox, The Chemistry of Meat Pigments, *J. Agr. Food Chem.,* Vol. 14, no. 3, pp. 207–210, 1966, American Chemical Society

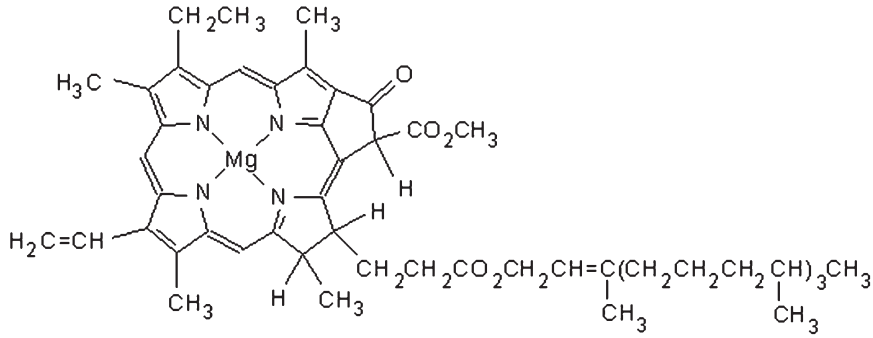


Fig. 6.15 Structure of chlorophyll *a.* (Chlorophyll *b* differs in having a formyl group at carbon 3). *Source:* Reprinted with permission from J.R. Whitaker, *Principles of Enzymology for the Food Sciences,* 1972, by courtesy of Marcel Dekker, Inc.

by acids, giving pheophytins *a* and *b.* The action of acid is especially important for fruits that are naturally high in acid. However, it appears that the chlorophyll in plant tissues is bound to lipoproteins and is protected from the effect of acid. Heating coagulates the protein and lowers the protective effect. The color of the pheophytins is olive-brown. Chlorophyll is stable in alkaline medium. The phytol chain confers insolubility in water on the chlorophyll molecule. Upon hydrolysis of the phytol group, the water-soluble methyl chlorophyllides are formed. This reaction can be catalyzed by the enzyme chlorophyllase. In the presence of copper or zinc ions, it is possible to replace the magnesium, and the resulting zinc or copper complexes are very stable. Removal of the phytol group and the magnesium results in pheophorbides. All of these reactions are summarized in the scheme presented in Fig. 6.16.

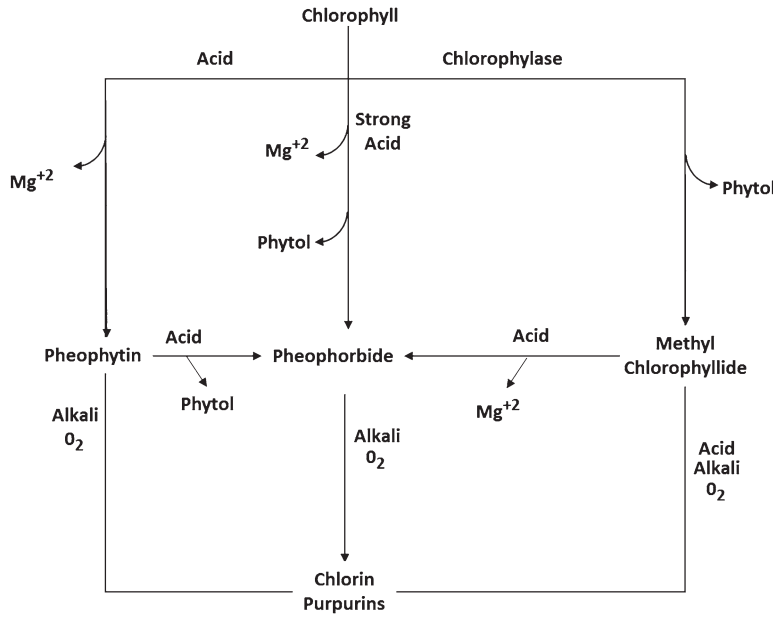


Fig. 6.16 Reactions of chlorophylls

In addition to those reactions described above, it appears that chlorophyll can be degraded by yet another pathway. Chichester and McFeeters (1971) reported on chlorophyll degradation in frozen beans, which they related to fat peroxidation. In this reaction, lipoxidase may play a role, and no pheophytins, chlorophyllides, or pheophorbides are detected. The reaction requires oxygen and is inhibited by antioxidants.

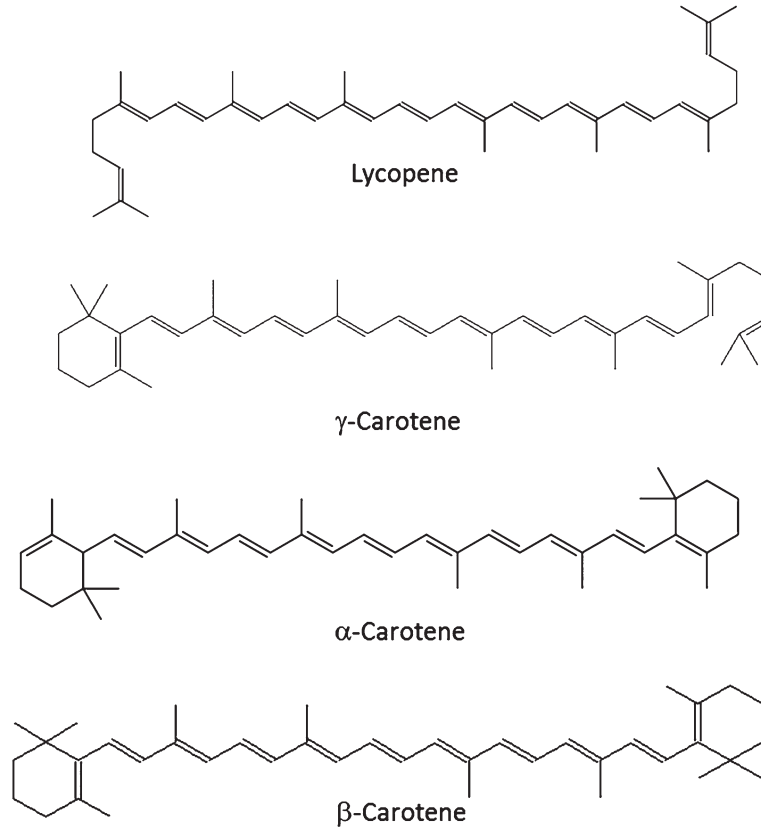
Isoprenoid Derivative Pigments

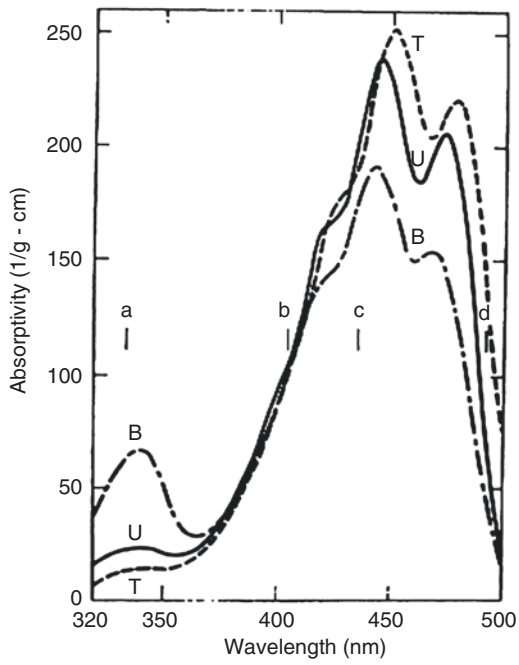
Carotenoids

The naturally occurring carotenoids, with the exception of crocetin and bixin, are tetraterpenoids. They have a basic structure of eight isoprenoid residues arranged as if two 20-carbon units, formed by head-to-tail condensation of four isoprenoid units, had joined tail to tail. There are two possible ways of classifying the carotenoids. The first system recognizes two main classes, the carotenes, which are hydrocarbons, and the xanthophylls, which contain oxygen in the form of hydroxyl, methoxyl, carboxyl, keto, or epoxy groups. The second system divides the carotenoids into three types (Fig. 6.17), acyclic, monocyclic, and bicyclic. Examples are lycopene (I)—acyclic; γ-carotene (II)—monocyclic; and α-carotene and β-carotene (III)—bicyclic.

The carotenoids take their name from the major pigments of carrot (*Daucus carota*). The color is the result of the presence of a system of conjugated double bonds. The greater the number of conjugated double bonds present in the molecule, the further the major absorption bands will be shifted to the region of longer wavelength; as a result, the hue will become more red. A minimum of seven conjugated double bonds are required before a perceptible yellow color appears. Each double bond may occur in either *cis* or *trans* configuration. The carotenoids in foods are usually of the all-*trans* type and only occasionally a mono-*cis* or *di-cis* compound occurs. The prefix *neo-* is used for stereoisomers with at least one *cis* double bond. The prefix *pro-* is for poly-*cis* carotenoids. The effect of the presence of *cis* double bonds on the absorption spectrum of β-carotene is shown in Fig. 6.18. The configuration has an effect on color. The all-*trans* compounds have the deepest color; increasing numbers of *cis* bonds result in gradual lightening of the color. Factors that cause change of bonds from *trans* to *cis* are light, heat, and acid.

Fig. 6.17 The carotenoids





In the narrower sense, the carotenoids are the four compounds shown in Fig. 6.17—α-, β-, and γ-carotene and lycopene—polyene hydrocarbons of overall composition $C_{40}H_{56}$. The relation between these and carotenoids with fewer than 40 carbon atoms is shown in Fig. 6.19. The prefix *apo-* is used to designate a carotenoid that is derived from another one by loss of a structural element through degradation. It has been suggested that some of these smaller carotenoid molecules are formed in nature by oxidative degradation of C_{40} carotenoids (Grob 1963).

Fig. 6.18 Absorption spectra of the three stereoisomers of beta carotene. *B* = neo-β-carotene; *U* = neo-β-carotene-U; *T* = all-trans-β-carotene. *a, b, c,* and *d* indicate the location of the mercury arc lines 334.1 nm, 404.7 nm, 435.8 nm and 491.6 nm, respectively. *Source:* From F. Stitt et al., Spectrophotometric Determination of Beta Carotene Stereoisomers in Alfalfa, *J. Assoc. Off. Agric. Chem.* Vol. 34, pp. 460–471, 1951

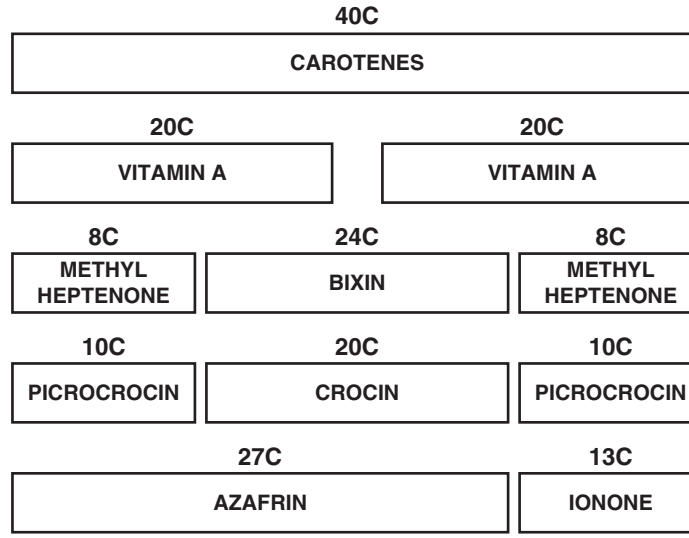


Fig. 6.19 Relationship between the carotene and carotenoids with fewer than 40 carbons

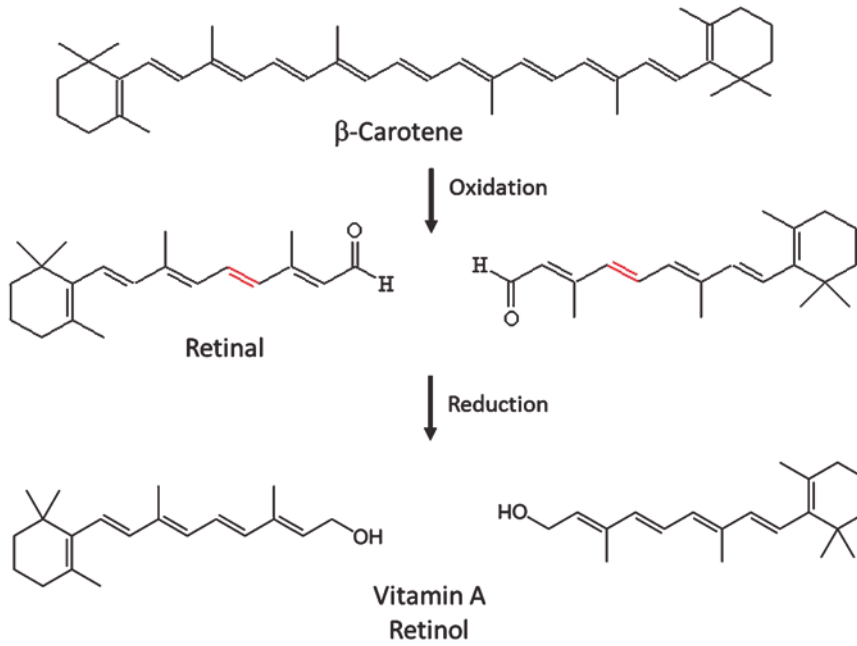


Fig. 6.20 Formation of retinal and vitamin A from β-carotene

Several examples of this possible relationship are found in nature. One of the best known is the formation of retinin and vitamin A from β-carotene (Fig. 6.20). Another obvious relationship is that of lycopene and bixin (Fig. 6.21). Bixin is a food color additive obtained from the seed coat of the fruit of a tropical brush, *Bixa orellana.* The pigment bixin is a dicarboxylic acid esterified with one methanol molecule. A pigment named crocin has been isolated from saffron. Crocin is a glycoside containing two molecules of gentiobiose. When these are

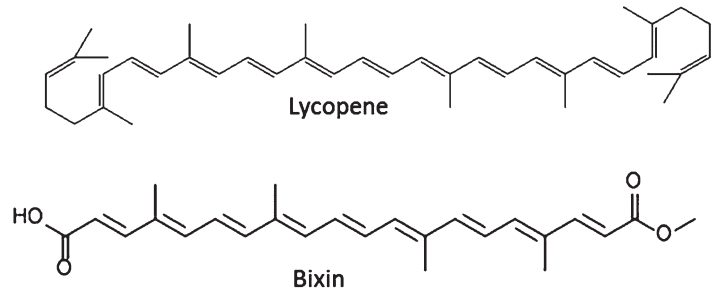


Fig. 6.21 Relationship between lycopene and bixin. *Source*: From E.C. Grob, The Biogenesis of Carotenes and Carotenoids, in *Carotenes and Carotenoids*, K. Lang, ed., 1963, Steinkopff Verlag

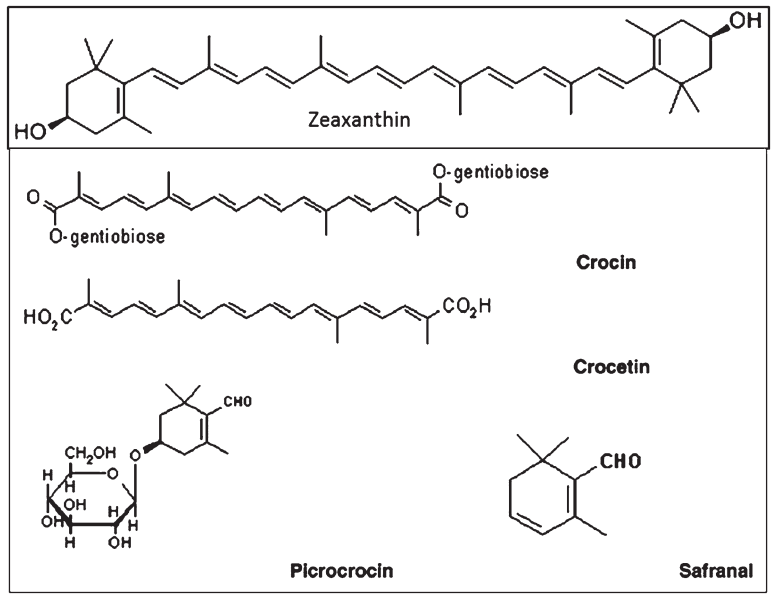


Fig. 6.22 Relationship between crocin and picrocrocin and the carotenoids

removed, the dicarboxylic acid crocetin is formed (Fig. 6.22). It has the same general structure as the aliphatic chain of the carotenes. Also obtained from saffron is the bitter compound picrocrocin. It is a glycoside and, after removal of the glucose, yields saffronal. It is possible to imagine a combination of two molecules of picrocrocin and one of crocin; this would yield protocrocin. Protocrocin, which is directly related to zeaxanthin, has been found in saffron (Grob 1963).

The structure of a number of important xanthophylls as they relate to the structure of β-carotene is given in Fig. 6.23. Carotenoids may occur in foods as relatively simple mixtures of only a few compounds or as very complex mixtures of large numbers of carotenoids. The simplest mixtures usually exist in animal products because the animal organism has a limited ability to absorb and deposit carotenoids. Some of the most complex mixtures are found in citrus fruits.

Beta-carotene as determined in fruits and vegetables is used as a measure of the provitamin A content of foods. The column chromatographic procedure, which determines this content, does not separate α-carotene, β-carotene, and cryptoxanthin. Provitamin A values of some foods are given in Table 6.5. Carotenoids are not synthesized by animals, but they may change ingested carotenoids into animal carotenoids—as in, for example, salmon, eggs, and crustaceans. Usually carotenoid content of foods does not exceed 0.1% on a dry weight basis.

In ripening fruit, carotenoids increase at the same time chlorophylls decrease. The ratio of carotenes to xanthophylls also increases.

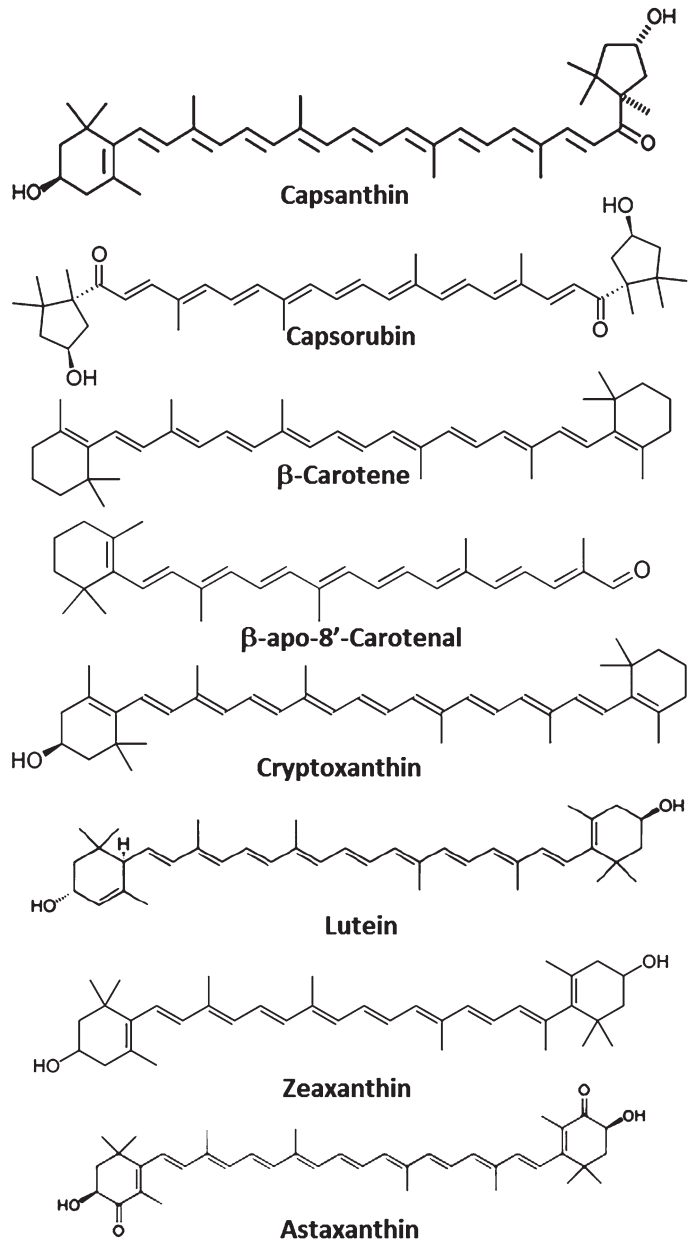


Fig. 6.23 Structure of some of the important carotenoids. *Source*: From B. Borenstein and R.H. Bunnell, Carotenoids: Properties, Occurrence, and Utilization in Foods, in *Advances in Food Research,* Vol. 15, C.O. Chichester et al., eds., 1967, Academic Press

Common carotenoids in fruits are α- and γ-carotene and lycopene. Fruit xanthophylls are usually present in esterified form. Oxygen, but not light, is required for carotenoid synthesis and the temperature range is critical. The relative amounts of different carotenoids are related to the characteristic color of some fruits. In the sequence of peach, apricot, and tomato, there is an increasing proportion of lycopene and increasing redness. Many peach varieties are devoid of lycopene. Apricots may have about 10% and tomatoes up to 90%. The lycopene content of tomatoes increases during ripening. As the chlorophyll breaks down during ripening, large amounts of carotenoids are formed (Table 6.6).

Table 6.5 Provitamin A value of various fruits and vegetables

Product	IU/100 g
Carrots, mature	20,000
Carrots, young	10,000
Spinach	13,000
Sweet potato	6000
Broccoli	3500
Apricots	2000
Lettuce	2000
Tomato	1200
Asparagus	1000
Bean, french	1000
Cabbage	500
Peach	800
Brussels sprouts	700
Watermelon	550
Banana	400
Orange juice	200

Source: From B. Borenstein and R.H. Bunnell, Carotenoids: Properties, Occurrence, and Utilization in Foods, in *Advances in Food Research,* Vol. 15, C.O. Chichester et al., eds., 1967, Academic Press

Table 6.6 Development of pigments in the ripening tomato

Pigment	Green (mg/100 g)	Half-ripe (mg/100 g)	Ripe (mg/100 g)
Lycopene	0.11	0.84	7.85
Carotene	0.16	0.43	0.73
Xanthophyll	0.02	0.03	0.06
Xanthophyll ester	0	0.02	0.10

Color is an important attribute of citrus juice and is affected by variety, maturity, and processing methods. The carotenoid content of oranges is used as a measure of total color. Curl and Bailey (1956) showed that the 5,6-epoxides of fresh orange juice isomerize completely to 5,8-epoxides during storage of canned juice. This change amounts to the loss of one double bond from the conjugated double bond system and causes a shift in the wavelength of maximum absorption as well as a decrease in molar absorbance. In one year's storage at 70 °F, an apparent carotenoid loss of 20–30% occurs.

Table 6.7 Composition of the carotenes in crude palm oil

Carotene	% of total carotenes
Phytoene	1.27
Cis-β-carotene	0.68
Phytofluene	0.06
β-carotene	56.02
α-carotene	35.06
ζ-carotene	0.69
γ-carotene	0.33
δ-carotene	0.83
Neurosporene	0.29
β-zeacarotene	0.74
α-zeacarotene	0.23
Lycopene	1.30

Source. Reprinted with permission from Choo Yuen May, Carotenoids from Palm Oil, *Palm Oil Developments,* Vol. 22, pp. 1–6, Palm Oil Research Institute of Malaysia

Peaches contain violaxanthin, cryptoxanthin, β-carotene, and persicaxanthin as well as 25 other carotenoids, including neoxanthin. Apricots contain mainly β- and γ-carotene, lycopene, and little if any xanthophyll. Carrots have been found to have an average of 54 ppm of total carotene (Borenstein and Bunnell 1967), consisting mainly of α-, β, and ζ-carotene and some lycopene and xanthophyll. Canning of carrots resulted in a 7–12% loss of provitamin A activity because of *cis-trans* isomerization of α- and β-carotene (Weckel 1962). In dehydrated carrots, carotene oxidation and off-flavor development have been correlated (Falconer et al. 1964). Corn contains about one-third of the total carotenoids as carotenes and two-thirds xanthophylls. Compounds found in corn include zeaxanthin, cryptoxanthin, β-carotene, and lutein.

One of the highest known concentrations of carotenoids occurs in crude palm oil. It contains about 15–300 times more retinol equivalent than carrots, green leafy vegetables, and tomatoes. All of the carotenoids in crude palm oil are destroyed by the normal processing and refining operations. Recently, improved gentler processes have been developed that result in a "red palm oil" that retains most of the carotenoids. The composition of the carotenes in crude palm oil with a total carotene concentration of 673 mg/kg is shown in Table 6.7.

Milkfat contains carotenoids with seasonal variation (related to feed conditions) ranging from 2 to 13 ppm. Egg yolk contains lutein, zeaxanthin, and cryptoxanthin. The total carotenoid content ranges from 3 to 89 ppm. Crustaceans contain carotenoids bound to protein resulting in a blue or blue-gray color. When the animal is immersed in boiling water, the carotenoid-protein bond is broken and the orange-red color of the free carotenoid appears. Widely distributed in crustaceans is astaxanthin. Red fish contain astaxanthin, lutein, and taraxanthin.

Common unit operations of food processing are reported to have only minor effects on the carotenoids (Borenstein and Bunnell 1967). The carotenoid-protein complexes are generally more stable than the free carotenoids. Because carotenoids are highly unsaturated, oxygen and light are major factors in their breakdown. Blanching destroys enzymes that cause carotenoid destruction. Carotenoids in frozen or heat-sterilized foods are quite stable. The stability of carotenoids in dehydrated foods is poor, unless the food is packaged in inert gas. A notable exception is dried apricots, which keep their color well. Dehydrated carrots fade rapidly.

Several of the carotenoids are now commercially synthesized and used as food colors. A possible method of synthesis is described by Borenstein and Bunnell (1967). Beta-ionone is obtained from lemon grass oil and converted into a C14 aldehyde. The C14 aldehyde is changed to a C16 aldehyde, then to a C19 aldehyde. Two moles of the C19 aldehyde are condensed with acetylene dimagnesium bromide and, after a series of reactions, yield β-carotene.

Three synthetically produced carotenoids are used as food colorants, β-carotene, β-apo-8′-carotenal (apocarotenal), and canthaxanthin. Because of their high tinctorial power, they are used at levels of 1–25 ppm in foods (Dziezak 1987). They are unstable in light but otherwise exhibit good stability in food applications. Although they are fat soluble, water-dispersible forms have been developed for use in a variety of foods. Beta-carotene imparts a light yellow to orange color, apocarotenal a light orange to reddish-orange, and canthaxanthin, orange-red to red. The application of these compounds in a variety of foods has been described by Counsell (1985). Natural carotenoid food colors are annatto, oleoresin of paprika, and unrefined palm oil.

Benzopyran Derivative Pigments

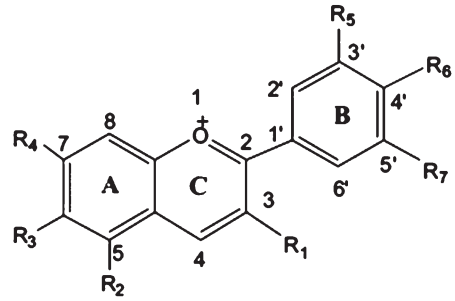
Anthocyanins and Flavonoids

The anthocyanin pigments are present in the sap of plant cells; they take the form of glycosides and are responsible for the red, blue, and violet colors of many fruits and vegetables. When the sugar moiety is removed by hydrolysis, the aglucone remains and is called anthocyanidin. The sugar part usually consists of one or two molecules of glucose, galactose, and rhamnose. The basic structure consists of 2-phenyl-benzopyrylium or flavylium with a number of hydroxy and methoxy substituents. Most of the anthocyanidins are derived from 3,5,7-trihydroxy-flavylium chloride (Fig. 6.24) and the sugar moiety is usually attached to the hydroxyl group on carbon 3. The anthocyanins are highly colored, and their names are derived from those of flowers. The structure of some of the more important anthocyanidins is shown in Fig. 6.25, and the occurrence of anthocyanidins in some fruits and vegetables is listed in Table 6.8. Recent studies have indicated that some anthocyanins contain additional components such as organic acids and metals (Fe, Al, Mg).

Substitution of hydroxyl and methoxyl groups influences the color of the anthocyanins. This effect has been shown by Braverman (1963) (Fig. 6.26). Increase in the number of hydroxyl groups tends to deepen the color to a more bluish shade. Increase in the number of methoxyl groups increases redness. The anthocyanins can occur in different forms. In solution, there is an equilibrium between the colored cation R^+ or oxonium salt and the colorless pseudobase ROH, which is dependent on pH.

$$R^+ + H_2O \rightleftharpoons ROH + H^+$$

As the pH is raised, more pseudobase is formed and the color becomes weaker. However, in addition to pH, other factors influence the color of anthocyanins, including metal chelation and combination with other flavonoids and tannins.

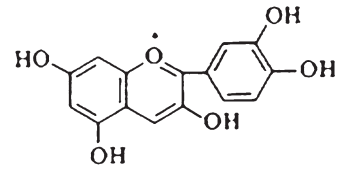


General anthocyanins structure

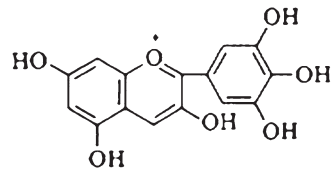
Name	Abbreviation	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Cyanidin	Cy	OH	OH	H	OH	OH	OH	H
Delphinidin	Dp	OH	OH	H	OH	OH	OH	OH
Malvidin	Mv	OH	OH	H	OH	OMe	OH	OMe
Pelargonidin	Pg	OH	OH	H	OH	H	OH	H
Peonidin	Pn	OH	OH	H	OH	OMe	OH	H
Petunidin	Pt	OH	OH	H	OH	OMe	OH	OH

Fig. 6.24 Chemical structure of fruit anthocyanidins

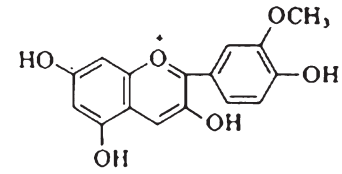
Fig. 6.25 Structure of some important anthocyanidins



Cyanidin



Delphinidin



Peonidin

Anthocyanidins are highly colored in strongly acid medium. They have two absorption maxima—one in the visible spectram at 500–550 nm, which is responsible for the color, and a second in the ultraviolet (UV) spectrum at 280 nm. The absorption maxima relate to color. For example, the relationship in 0.01% HC1 in methanol is as follows: at 520 nm pelargonidin is scarlet, at 535 nm cyanidin is crimson, and at 546 nm delphinidin is blue-mauve (Macheix et al. 1990).

About 16 anthocyanidins have been identified in natural products, but only the following six of these occur frequently and in many different products: pelargonidin, cyanidin, delphinidin, peonidin, malvidin, and petunidin. The anthocyanin pigments of Red Delicious apples were found to contain mostly cyanidin-3-galactoside, cyanidin-3-arabinoside, and cyanidin-7-arabinoside (Sun and Francis 1968). Bing cherries contain primarily cyanidin-3-rutinoside, cyanidin-3-glucoside,

Table 6.8 Anthocyanidins occurring in some fruits and vegetables

Fruit or vegetable	Anthocyanidin
Apple	Cyanidin
Black currant	Cyanidin and delphinidin
Blueberry	Cyanidin, delphinidin, malvidin, petunidin, and peonidin
Cabbage (red)	Cyanidin
Cherry	Cyanidin and peonidin
Grape	Malvidin, peonidin, delphinidin, cyanidin, petunidin, and pelargonidin
Orange	Cyanidin and delphinidin
Peach	Cyanidin
Plum	Cyanidin and peonidin
Radish	Pelargonidin
Raspberry	Cyanidin
Strawberry	Pelargonidin and a little cyanidin

Source: From P. Markakis, Anthocyanins, in *Encyclopedia of Food Technology,* A.H. Johnson and M.S. Peterson, eds., 1974, AVI Publishing Co.

and small amounts of the pigments cyanidin, peonidin, peonidin-3-glucoside, and peonidin-3-rutinoside (Lynn and Luh 1964). Cranberry anthocyanins were identified as cyanidin-3-monogalactoside, peonidin-3-monogalactoside, cyanidin monoarabinoside, and peonidin-3-monoarabinoside (Zapsalis and Francis 1965). Cabernet Sauvignon grapes contain four major anthocyanins: delphinidin-3-monoglucoside, petunidin-3-monoglucoside, malvidin-3-monoglucoside, and malvidin-3-monoglucoside acetylated with chlorogenic acid. One of the major pigments is petunidin (Somaatmadja and Powers 1963).

Anthocyanin pigments can easily be destroyed when fruits and vegetables are processed. High temperature, increased sugar level, pH, and ascorbic acid can affect the rate of destruction (Daravingas and Cain 1965). These authors studied the change in anthocyanin pigments during the processing and storage of raspberries.

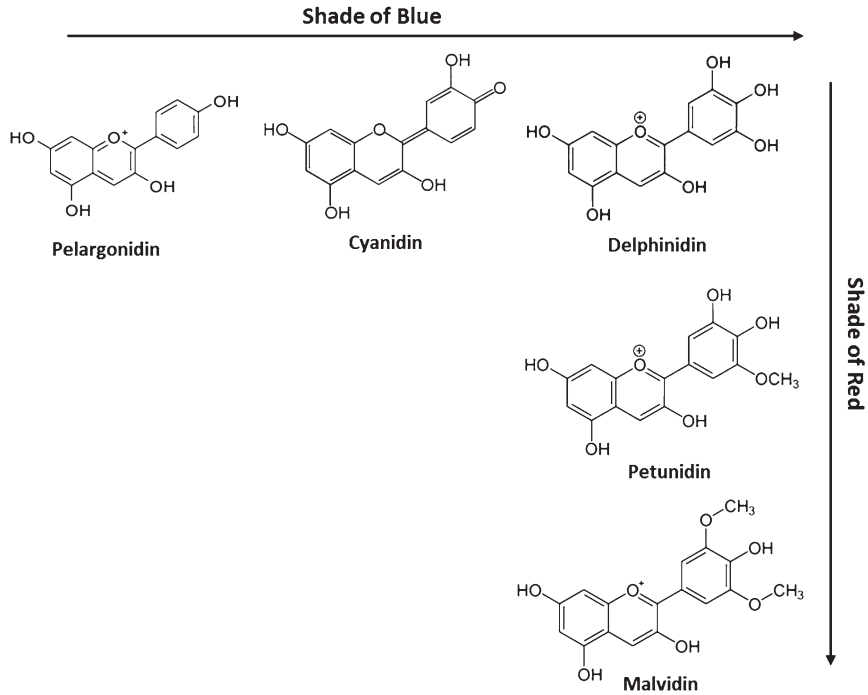


Fig. 6.26 Effect of substituents on the color of anthocyanidins. *Source*: Reprinted with permission from J.B.S. Braverman, *Introduction to the Biochemistry of Foods,* © 1963, Elsevier Publishing Co.

During storage, the absorption maximum of the pigments shifted, indicating a change in color. The level of pigments was lowered by prolonged times and higher temperatures of storage. Higher concentration of the ingoing sugar syrup and the presence of oxygen resulted in greater pigment destruction.

The stability of anthocyanins is increased by acylation (Dougall 1997). These acylated anthocyanins may occur naturally as in the case of an anthocyanin from the purple yam (Yoshida et al. 1991). This anthocyanin has one sinapic residue attached through a disaccharide and was found to be stable at pH 6.0 compared to other anthocyanins without acylation. Dougall (1997) were able to produce stable anthocyanins by acylation of carrot anthocyanins in cell cultures. They found that a wide range of aromatic acids could be incorporated into the anthocyanin.

Anthocyanins can form purplish or slategray pigments with metals, which are called lakes. This can happen when canned foods take up tin from the container. Anthocyanins can be bleached by sulfur dioxide. According to Jurd (1964), this is a reversible process that does not involve hydrolysis of the glycosidic linkage, reduction of the pigment, or addition of bisulfite to a ketonic, chalcone derivative. The reactive species was found to be the anthocyanin carbonium ion (R^+), which reacts with a bisulfite ion to form a colorless chromen-2(or 4)-sulfonic acid (R–SO_3H), similar in structure and properties to an anthocyanin carbinol base (R–OH). This reaction is shown in Fig. 6.27.

The colors of the anthocyanins at acid pH values correspond to those of the oxonium salts. In slightly alkaline solutions (pH 8–10), highly colored ionized anhydro bases are formed. At pH 12, these hydrolyze rapidly to fully ionized chalcones (Fig. 6.28). Leuco bases are the reduced form of the anthocyanins. They are usually without much color but are widely distributed in fruits and vegetables. Under the influence of oxygen and acid hydrolysis, they may develop the characteristic color of the carbonium ion. Canned pears, for example, may show "pinking"—a change from the leuco base to the anthocyanin.

The flavonoids or anthoxanthins are glycosides with a benzopyrone nucleus. The flavones have a double bond between carbons 2 and 3. The flavonols have an additional hydroyxl group at carbon 3, and the flavanones are saturated at carbons 2 and 3 (Fig. 6.29). The flavonoids have low coloring power but may be involved in discolorations; for example, they can impart blue and green colors when combined with iron. Some of these compounds are also potential substrates for enzymic browning and can cause undesirable discoloration through this mechanism. The most ubiquitous flavonoid is quercetin, a 3,5,7,3′,4′-pentahydroxy flavone (Fig. 6.30). Many flavonoids contain the sugar rutinose, a disaccharide of glucose and rhamnose. Hesperidin is a flavanone occurring in citrus fruits and, at pH 12, the inner ring opens to form a chalcone in a similar way as shown for the anthocyanins. The chalcones are yellow to brown in color.

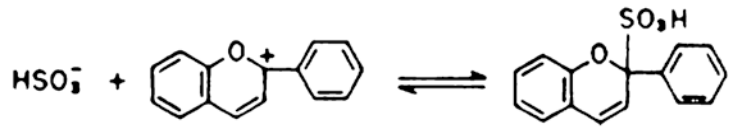


Fig. 6.27 Reaction of bisulfite with the anthocyanin carbonium ion

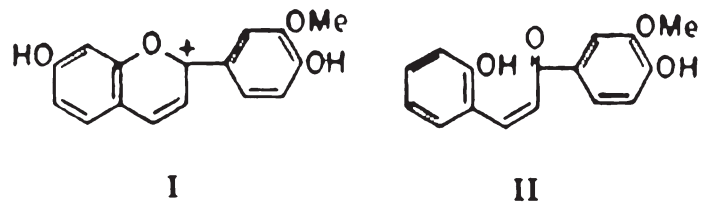


Fig. 6.28 Structure of anhydro base (I) and chalcone (II)

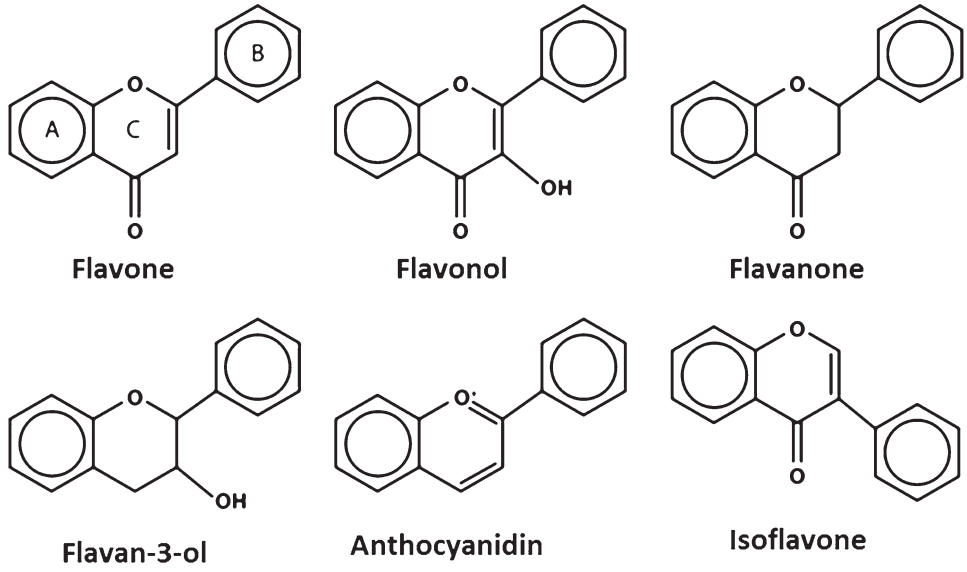


Fig. 6.29 Structure of flavones, flavonals, flavanones, flavanonols, and isoflavones

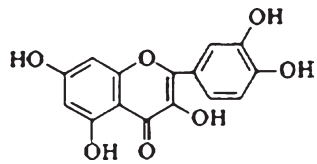


Fig. 6.30 Structure of quercetin

Tannins

Tannins are polyphenolic compounds present in many fruits. They are important as color compounds and also for their effect on taste as a factor in astringency (see Chap. 7). Tannins can be divided into two classes—hydrolyzable tannins and nonhydrolyzable or condensed tannins. The tannins are characterized by the presence of a large number of hydroxyl groups, which provide the ability to form reversible bonds with other macromolecules, polysaccharides, and proteins, as well as other substances such as alkaloids. This bond formation may occur during the development of the fruit or during the mechanical damage that takes place during processing.

Hydrolyzable tannins are composed of phenolic acids and sugars that can be broken down by acid, alkaline, or enzymic hydrolysis. They are polyesters based on gallic acid and/or hexahydroxydiphenic acid (Fig. 6.31). The usual sugar is D-glucose and molecular weights are in the range of 500–2800. Gallotannins release gallic acid on hydrolysis, and ellagitannins produce ellagic acid. Ellagic acid is the lactone form of hexahydroxydiphenic acid, which is the compound originally present in the tannin (Fig. 6.31).

Nonhydrolyzable or condensed tannins are also named proanthocyanidins. These are polymers of flavan-3-ols, with the flavan bonds most commonly between C4 and C8 or C6 (Fig. 6.24) (Macheix et al. 1990). Many plants contain tannins that are polymers of (+)-catechin or (−)-epicatechin. These are hydrogenated forms of flavonoids or anthocyanidins. Other monomers occupying places in condensed fruit tannins have trihydroxylation in the B-ring: (+)-gallocat-echin and (−)-epigallocatechin. Oligomeric and polymeric procyanidins are formed by addition of more flavan-3-ol units and result in the formation of helical structures. These structures can form bonds with proteins.

Tannins are present in the skins of red grapes and play an important part in the flavor profile of red wine. Tannins in grapes are usually estimated in terms of the content of gallic acid (Amerine and Joslyn 1970).

Oxidation and polymerization of phenolic compounds as a result of enzymic activity of phenoloxidases or peroxidases may result in the formation of brown pigments. This can take place during the growth of fruits (e.g., in dates) or during mechanical damage in processing.

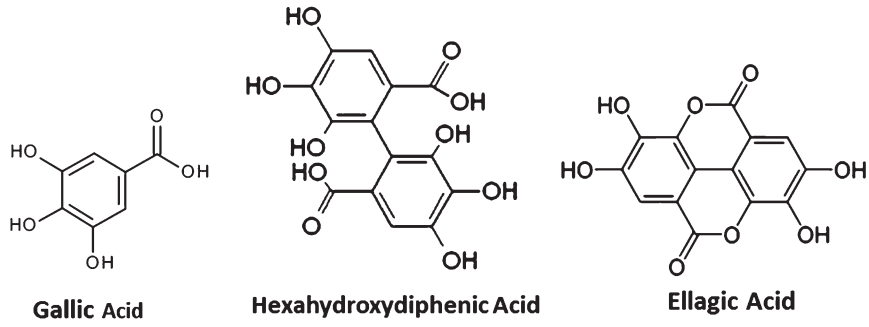


Fig. 6.31 Structure of components of hydrolyzable tannins

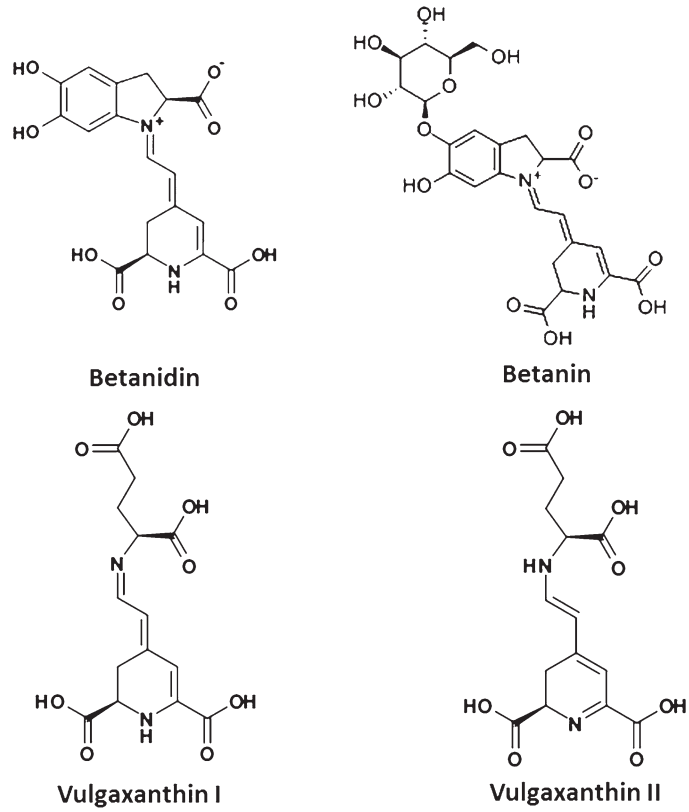


Fig. 6.32 Structure of naturally occurring betalains in red beets. *Source:* From J.H. Von Elbe and I.-Y. Maing, Betalains as Possible Food Colorants of Meat Substitutes, *Cereal Sci. Today*, Vol. 18, pp. 263–264, 316–317, 1973

Other Pigments

Betalains

Table beets are a good source of red pigments; these have been increasingly used for food coloring. The red and yellow pigments obtained from beets are known as betalains and consist of the red betacyanins and the yellow betaxanthins (Von Elbe and Maing 1973). The structures of the betacyanins are shown in Fig. 6.32. The major betacyanin is betanin, which accounts for 75–95% of the total pigments of beets. The remaining pigments contain isobetanin, prebetanin, and isoprebetanin. The latter two are sulfate monoesters of betanin and isobetanin,

respectively. The major yellow pigments are vulgaxanthin I and vulgaxanthin. II Betanin is the glucoside of betanidin, and isobetanin is the C-15 epimer of betanin.

Betanidin has three carboxyl groups ($pk_a = 3.4$), two phenol groups ($pH_a = 8.5$), and asymmetric carbons at positions 2 and 15. The 15-position is easily isomerized under acid or basic conditions in the absence of oxygen to yield isobetanidin. Under alkaline conditions and in the presence of glutamine or glutamic acid, betanin can be converted to vulgaxanthin (Mabry 1970).

The color of betanin solutions is influenced by pH. In the range of 3.5–7.0, the spectrum shows a maximum of 537 nm (Fig. 6.33). Below pH 3, the intensity of this maximum decreases and a slight increase in the region of 570–640 nm occurs and the color shifts toward violet. At pH values over 7, a shift of the maximum occurs to longer wavelength. At pH 9, the maximum is about 544 nm and the color shifts toward blue. Von Elbe et al. (1974) found that the color of betanin is most stable between pH 4.0 and 6.0. The thermostability is greatest between pH 4.0 and 5.0. Light and air have a degrading effect on betanin, and the effect is cumulative.

Caramels and Melanoidins

Caramel color can be produced from a variety of carbohydrate sources, but usually corn sugar syrup is used. Corn starch is first hydrolyzed with acid to a DE of 8–9, followed by hydrolysis with bacterial α-amylase to a DE of 12–14, then with fungal amyloglucosidase up to a DE of 90–95. Several types of caramel are produced. The largest amount is electropositive or positive caramel, which is made with ammonia. Electronegative or negative caramel is made with ammonium salts. A slightly electronegative caramel is soluble in alcohol and is used for coloring beverages (Greenshields 1973). The composition and coloring power of caramel depends on the type of raw materials and the process used. The melanoidins are formed from the reactions between reducing sugars and basic nitrogenous compounds, often called the Maillard reaction. Both Maillard-type reactions and pure caramelizing reactions are thought to be involved commonly in most of heat

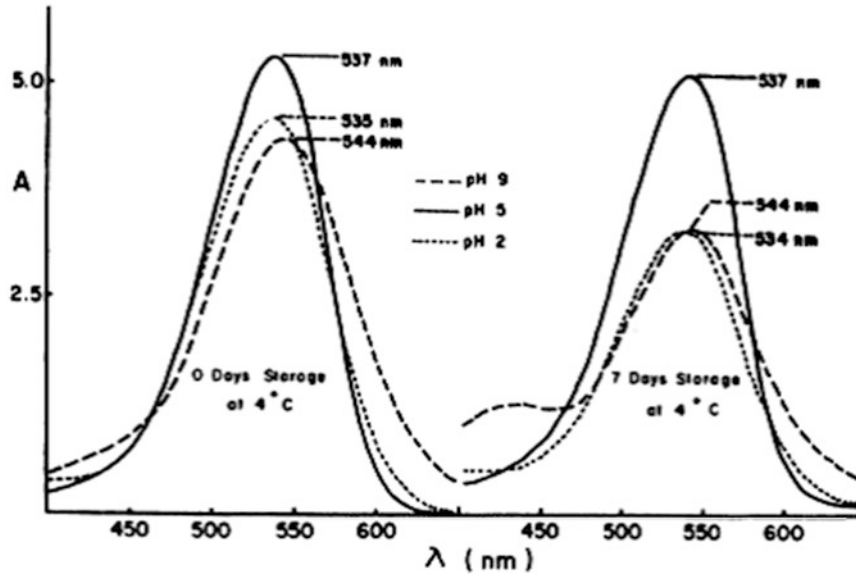


Fig. 6.33 Visible spectra of betanin at pH values of 2.0, 5.0, and 9.0. *Source*: From J.H. Von Elbe, I.-Y. Maing, and C.H. Amundson, Color Stability of Betanin, *Journal of Food Science,* Vol. 39, pp. 334–337, 1974, Institute of Food Technologists

(at high temperatures) processed food products. The reaction products are extremely complex in composition with high and low molecular weight colored compounds, as well as a variety of volatile components.

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Flavor

7

Han-Seok Seo, John W. Finley,
and John M. deMan

Our response to the food when we consume is a combination of visual, tactile, thermal, taste and aroma. Frequently our first interaction with a food is visual or to the aroma of the food. When we put it in our mouth we respond to the temperature, texture (tactile) and taste response. In this chapter we will consider the taste and aroma responses. In recent years, the understanding of taste and smell has increased exponentially.

Hall (1968) defined flavor as follows: "Flavor is the sensation produced by a material taken in the mouth, perceived principally by the senses of taste and smell, and also by the general pain, tactile and temperature receptors in the mouth. Flavor denotes the sum of the characteristics of the material which produce that sensation." More recently, the International Organization for Standardization (2008) characterized flavor as a "complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting. The flavor may be influenced by tactile, thermal, painful and/or kinaesthetic effects." Although the senses of taste and smell are the principal systems for distinguishing flavor in foods, other sensory cues contribute to the overall sensation of flavor. For example, texture has a very definite effect on our perception of taste. Smoothness, roughness, granularity, and viscosity can all influence flavor, as can hotness of spices, coolness of menthol, brothiness or fullness of certain amino acids, and the tastes described as metallic and alkaline. In addition, flavor perception is affected by visual cues (colors and images) as well as auditory cues (background sound and biting/drinking-induced sound) (DuBose et al. 1980; Spence 2012).

The senses of taste and smell give animals or humans the ability to evaluate what they eat and drink. This evaluation helps animals and humans to promote ingestion of nutritious substances and prevent consumption of potential poisons or toxins. Animals, including humans, develop taste and smell preferences, which is the ability to choose certain types of food in *preference* to others. Taste and smell preferences can change with differing body needs and dietary interactions. The senses of taste and smell also motivate us to eat by seeking the nutrients and energy such as fat and sugar. However, likings of sugar and fat vary with genotype, as well as individual experiences and environmental factors. Animals often develop food aversions, particularly if they become ill soon after eating a certain food, even though that food was not the cause of the illness. Food preferences and aversions involve the senses of taste and smell, and these phenomena are almost certainly mediated through the central nervous system. In addition, by sniffing off-odors or tasting bitterness or sourness, the senses of taste and smell help us to avoid ingesting harmful foods containing toxic, microbes, microbial by products, or chemical contamination (Reed and Knaapila 2010).

Traditionally we are taught that there are five basic taste responses on the tongue; salt, acid,

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_7

sweet, bitter, and umami. Each taste quality has a specific role in the detection of nutritious as well as poisonous substances; sweet taste for carbohydrate sources of calories, umami for protein and amino acid contents, salty for mineral contents, and sour for fruits ripeness and spoiled foods, and bitter for harmful compounds (Iwata et al. 2014). Many earlier textbooks and journal articles cite the "tongue map" suggesting that different areas of the tongue are sensitive to specific tastes as show in Fig. 7.1. However, all taste qualities are sensitive across the area of tounge.

The interactions of foods with saliva can also have a major influence on our perception of tasting substances. Saliva acts as a solvent for taste substances as well as a diffuser of the solutes to the taste receptor sites. Salivar also acts as a buffer for acidic foods and may bind with bitter taste substances. In addition, some salivary constitutents alter taste sensitivity by continuously stimulating the taste receptors, and salivar also protect the taste receptors from dryness, bacterial infection, and disuse atrophy (Matsuo 2000).

Taste is the response to dissolved molecules and ions called tastants. Taste is detected when tastants interact with taste receptor cells. These cells are clustered in taste buds on the tongue and scattered in other areas of the body, for example the nasal epithelium, the trachea, the stomach, and the intestines (Finger and Kinnamon 2011). Sweet taste receptors (T1Rs) are found in cells of the duodenum. When sugars reach the duodenum, the cells respond by releasing incretins, causing the pancreas beta cells, located in the islets of Langerhans, to increase the release of insulin (Laffitte et al. 2014). Bitter taste receptors (T2Rs) are found in the cilia of human bronchial and sinonasal epithelial cells where they can serve to cause a response to expel inhaled irritants (Shah et al. 2009).

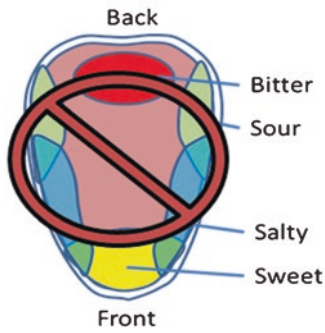


Fig. 7.1 Misinterpreted tongue map suggesting four basic tastes are sensitive on specific regions of the tongue. The basic tastes are sensitive on every part of the tongue

Traditionally various areas of the tongue were considered to be responsible for the perception of the basic tastes. More recent studies have demonstrated that taste buds contain 50–120 taste cells with multiple receptors for all five basic tastes. Each taste cell has receptors on its apical surface that are transmembrane proteins. These proteins admit the ions that give rise to the sensations of salty and sour tastes as well as bind to the molecules that elicit the sensations of sweet, bitter, and umami tastes (Engelen 2010).

The various types of taste cells are located within taste buds. These structures are predominantly located on the tongue and soft palate. Most of the taste buds on the tongue are located within tiny projections on the tongue called papillae. The predominant papillae on the tongue are the filiform or threadlike structures that do not contain taste buds. The filiform structures are involved in somatosensory and mechanical functions. The taste buds are found on the fungiform papillae on the anterior two-thirds of the tongue. The fungiform papillae are the most noticeable and typically contain one or more taste buds. The circumvallate papillae are located on the dorsal side of the tounge and the foliate papillae are found in small trenches located on the sides of the rear of the tongue (Fig. 7.2).

Taste buds are onion-shaped structures composed of between 50 and 100 taste cells. Each taste cell has finger-like projection called a microvilli. The microvilli protrude through an opening at the top of the taste bud referred to as the taste pore. Food chemicals called tastants which are dissolved in the saliva contact the taste cells through the taste pore. They then interact with proteins on the surfaces of the cells known as taste receptors or with pore like proteins called ion channels. These interactions cause electrical changes in the taste cells that send chemical signals to adjacent neurons ultimately resulting in

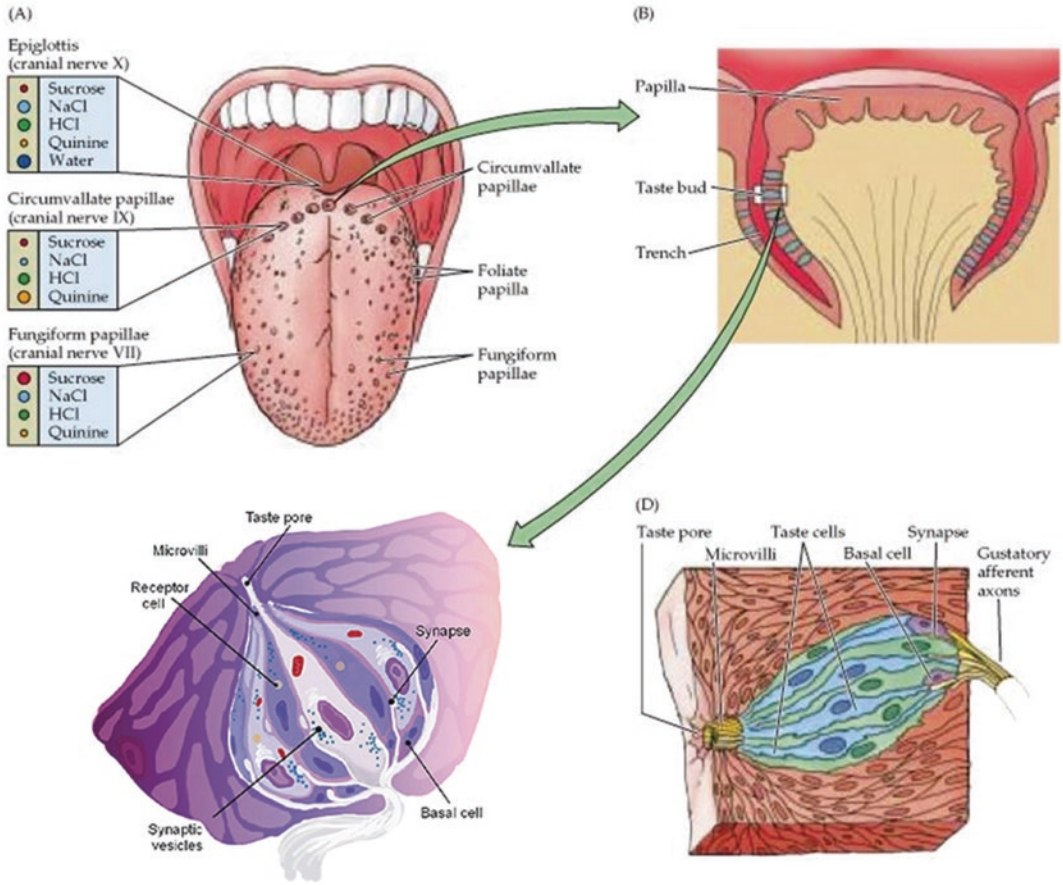


Fig. 7.2 Taste buds and the cranial nerves of the tongue. (**a**) Distribution of taste papillae on the dorsal surface of the tongue. Different responses to sweet, salty, sour, and bitter tastants recorded in the three cranial nerves (VII, IX, and X) that innervate the tongue and epiglottis are indicated at *left* (**a**). The size of the circles representing sucrose, NaCl, HCl, quinine, and water corresponds to the relative response of the papillae to these stimuli. (**b**) Diagram of a circumvallate papilla showing location of individual taste buds. (**c**) Light micrograph of a taste bud. (**d**) Diagram of a taste bud, showing various types of taste cells and the associated gustatory nerves. The apical surfaces of the receptor cells have microvilli that are oriented toward the taste pore (reference source of Fig. 7.2 is missing: http://www.uth.tmc.edu/courses/dental/smell-taste/taste.html?)

impulses to the brain. The electrical changes in the taste cells that result in signals to the brain are dependent on the concentrations of the ions. Like neurons, taste cells have a net negative charge internally and a net positive charge externally. Tastants bind to the taste cells, the concentration of positive ions inside cells increases, eliminating the net charge differences internally and externally. This depolarization causes the taste cells to release chemical signals called neurotransmitters, which cause neurons connected to the taste cells to transmit electrical messages to the brain.

The responses to bitter and sweet tastes by the taste cells are not always closely correlated with the chemical structure of the tastant molecule. Manny carbohydrates, particularly simple sugars are sweet, but others are not sweet at all. Many non-carbohydrate molecules result in a sweet taste response. For example, chloroform, stevaside, saccharin and aspartame all cause sweet responses. None of these molecules has any common structure with sweet sugars. Compounds that result in salty or sour tastes are less diverse because they are generally ions such as hydrogen

ions for sour and sodium for salty taste. The chemicals that produce salty and sour tastes act directly through ion channels, whereas those responsible for sweet and bitter tastes bind to surface of the receptor. The receptors then signal the cells which cause the opening and closing of ion channels. Gustducin is a G-protein that converts the electrical impulse to a signal. Gustducin is referred to as a G-protein, which is found on the underside of many different receptors. The term G-protein is used because the activity of such proteins is regulated by guanosine triphosphate, GTP. When a tastant molecule binds to a taste cell receptor, it prompts the subunits of gustducin to split apart and carry out biochemical reactions that ultimately open and close ion channels and make the cell interior more positively charged.

Within a taste bud, there is a network of dendrites of sensory nerves called "taste nerves." Taste cells are stimulated by the binding of chemicals to their receptors causing the taste cell to become depoloraized, this depolarization is transmitted to the taste nerve fibers resulting in a potential that is ultimately transmitted to the brain. The nerve transmission rapidly adapts after the initial stimulus, and a strong discharge is observed in the taste nerve fibers but within a few seconds. That response diminishes to a steady-state level of much lower amplitude. We know that binding between stimulus and receptor is a weak one because no irreversible effects have been observed. A mechanism of taste stimulation with electrolytes has been proposed by Beidler (1957); it is shown in Fig. 7.3. The time required for taste response to take place is in the order of 25 ms. The taste molecule is weakly adsorbed, thereby creating a disturbance in the molecular geography of the surface and allowing an interchange of ions across the surface. This reaction is followed by an electrical depolarization that initiates a nerve impulse.

The taste receptor mechanism has been more fully described by Kurihara (1987). The process from chemical stimulation to transmitter release is schematically presented in Fig. 7.4. The receptor membranes contain voltage-dependent calcium channels. Taste compounds contact the taste cells and depolarize the receptor membrane; this depolarization spreads to the synaptic area, activating the voltage-dependent calcium channels. Influx of calcium triggers the release of the transmitter norepinephrine.

The relationship between stimulus concentration and neural response is not simple. As the stimulus concentration increases, the response increases at a decreasing rate until a point is reached where further increase in stimulus concentration does not produce a further increase in response. Beidler (1954) proposed the following equation relating magnitude of response and stimulus concentration:

$$\frac{C}{R} = \frac{C}{R_s} + \frac{1}{KR_s}$$

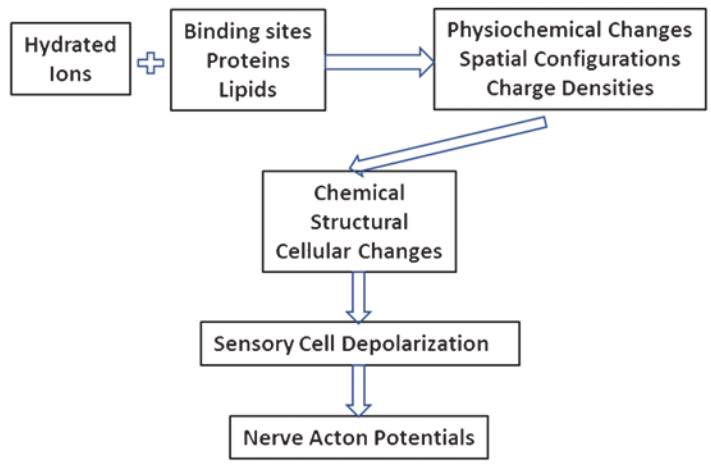


Fig. 7.3 Mechanism of taste stimulation as proposed by Beidler. *Source*: From L.M. Beidler, Facts and Theory on the Mechanism of Taste and Odor Perception, in *Chemistry of Natural Food Flavors*, 1957, Quartermaster Food and Container Institute for the Armed Forces

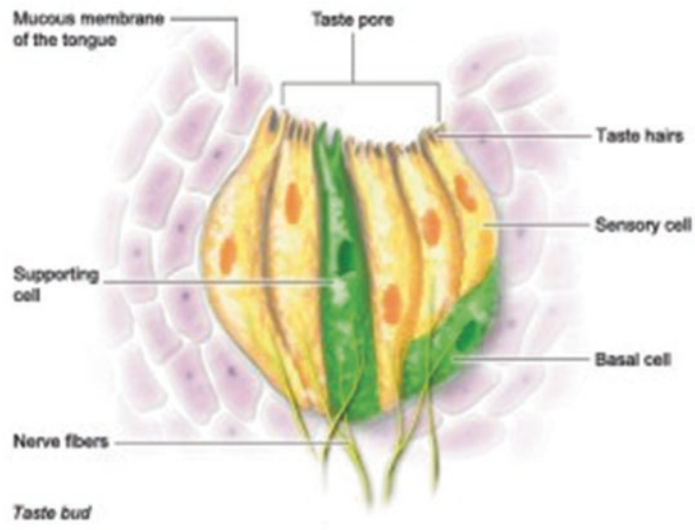


Fig. 7.4 Diagram of a taste cell (reference source: http://scienceinit.in/2016/03/31/how-do-we-taste)

where
C = stimulus concentration
R = response magnitude
R_s = maximum response
K = equilibrium constant for the stimulus- receptor reaction

K values reported by Beidler for many substances are in the range of 5–15.

It appears that the initial step in the stimulus-receptor reaction is the formation of a weak complex, as evidenced by the small values of K. The complex formation results in the initiation of the nerve impulse. Because of the decreasing rate of response, we know that the number of receptor sites is finite. The taste response is a function of the proportion of sites occupied by the stimulus compound.

According to Beidler (1957), the threshold value of a substance depends on the equilibrium constant and the maximum response. Since K and R_s both vary from one substance to another and from one species to another, the threshold also varies between substances and species. The concentration of the stimulus can be increased in steps just large enough to elicit an increase in response. This amount is called the just noticeable difference (JND).

There appear to be significant age- or sex-related differences in taste sensitivity, and especially heavy smoking (more than 20 cigarettes per day) results in a deterioration in taste responsiveness with age.

Differences in taste perception between individuals seem to be common. Peryam (1963) found that sweet and salt are usually well recognized. However, with sour and bitter taste some difficulty is experienced. Some tasters ascribe a bitter quality to citric acid and a sour quality to caffeine.

Recent studies have demonstrated that taste-signalling molecules are distributed not only in the gustatory epithelium, but also in other tissues, including the gastrointestinal tract, airways, testes and brain. Taste signalling mechanisms in the gastrointestinal tract have been found to participate in detecting sweet, umami and bitter compounds. It has been proposed that tastant/nutrient detection by other systems contributes to the behavioural responses to food intake (Iwatsuki and Torii 2012).

The bitter taste receptor (TAS2R)-family of G-protein-coupled receptors has been identified on the tongue as detectors of bitter taste. In the last few years, they have been discovered in extra-oral tissues, including the airways, the gut, the brain and even the testis. In tissues that contact the exterior, protective functions for

TAS2Rs have been proposed, in analogy to their function on the tongue as toxicity detector. However, TAS2Rs have also been found in internal organs, suggesting other roles for these receptors, perhaps involving as yet unidentified endogenous ligands. The current review gives an overview of the different proposed functions for TAS2Rs in tissues other than the oral cavity; from appetite regulation to the treatment of asthma, regulation of gastrointestinal motility and control of airway innate immunity (Avau and Depoortere 2016).

Once taste signals are transmitted to the brain, several neural pathways are activated that influence digestive function. Tasting food is followed rapidly by increased salivation and by low level secretory activity in the stomach.

Taste Sensations

The sense of taste is equivalent to excitation of taste receptors. Taste receptors for a large number of specific chemicals have been identified which contribute to the reception of taste. Five basic types of tastes are recognized by humans:

- Sweet—usually indicates energy rich nutrients.
- Bitter—allows sensing of diverse natural toxins.
- Salty—allows modulating diet for electrolyte balance.
- Sour—typically the taste of acids.
- Umami—the taste of amino acids (e.g., meat broth or aged cheese).

None of these tastes are elicited by a single chemical. There are thresholds for detection of taste that differ among chemicals that deliver similar taste. For example, sucrose, 1-propyl-2 amino-4-nitrobenzene and lactose all taste sweet to humans, but the sweet taste is elicited by these chemicals at concentrations of roughly 10 mM, 2 uM and 30 mM respectively—a range of potency of roughly 15,000-fold. Substances sensed as bitter typically have very low thresholds. Table 7.1 illustrates the relative threshold concentrations of various types of tastants.

Table 7.1 Taste thresholds for basic taste sensations

Examples of human taste thresholds		
Taste	Substance	Threshold for tasting
Salty	NaCl	0.01 M
Sour	HCl	0.0009 M
Sweet	Sucrose	0.01 M
Bitter	Quinine	0.000008 M
Umami	Glutamate	0.0007 M

(Source: rbowen@colostate.edu

The taste cells transduce the stimuli from tastants and provide the identity, concentration, and pleasant or unpleasant quality of the tastant. This information is translated to the gastrointestinal system causing salivation and swallowing (or gagging and regurgitation if the substance is noxious). The temperature and texture of food is relayed from the mouth via somatic sensory receptors from the trigeminal and other sensory cranial nerves to the thalamus and somatic sensory cortices. Food is not simply eaten for nutritional value; taste perception also depends on cultural backgrounds and psychological factors (Purves et al. 2001).

Chemical Structure and Taste

A first requirement for a substance to produce a taste is that it be water soluble. The relationship between the chemical structure of a compound and its taste is more easily established than that between structure and smell. In general, all acid substances are sour. Sodium chloride and other salts are salty, but as constituent atoms get bigger, a bitter taste develops. Potassium bromide is both salty and bitter, and potassium iodide is predominantly bitter. Sweetness is a property of sugars and related compounds but also of lead acetate, beryllium salts, and many other substances such as the artificial sweeteners saccharin and cyclamate. Bittemess is exhibited by alkaloids such as quinine, picric acid, and heavy metal salts.

Minor changes in chemical structure may change the taste of a compound from sweet to bitter or tasteless. For example, Beidler (1966) has examined saccharin and its substitution compounds. Saccharin is 500 times sweeter than sugar (Fig. 7.5). Introduction of a methyl group or of chloride in the *para* position reduces the

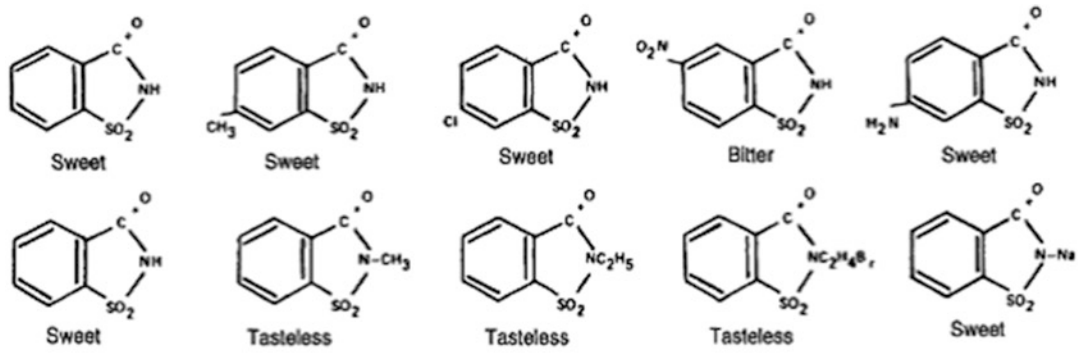


Fig. 7.5 The effect of substitutions in saccharin on sweetness. *Source*: From L.M. Beidler, Chemical Excitation of Taste and Odor Receptors, in *Flavor Chemistry*, I. Hornstein, ed., 1966, American Chemistry Society

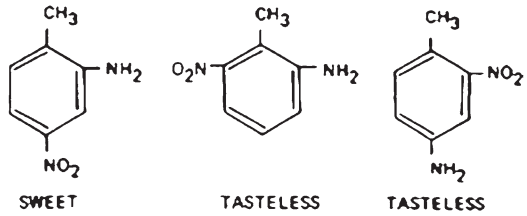


Fig. 7.6 Taste of nitrotoluidine isomers

sweetness by half. Placing a nitro group in the *meta* position makes the compound very bitter. Introduction of an amino group in the *para* position retains the sweetness. Substitutions at the imino group by methyl, ethyl, or bromoethyl groups all result in tasteless compounds. However, introduction of sodium at this location yields sodium saccharin, which is very sweet.

The compound 5-nitro-*o*-toluidine is sweet. The positional isomers 3-nitro-*o*-toluidine and 3-nitro-*p*-toluidine are both tasteless (Fig. 7.6). Teranishi et al. (1971) provided another example of change in taste resulting from the position of substituent group: 2-amino-4-nitro-propoxybenzene is 4000 times sweeter than sugar, 2-nitro-4-amino-propoxybenzene is tasteless, and 2,4-dinitro-propoxybenzene is bitter (Fig. 7.7). Dulcin (*p*-ethoxyphenylurea) is extremely sweet, the thiourea analog is bitter, and the *o*-ethoxyphenylurea is tasteless (Fig. 7.8).

Just as positional isomers affect taste, so do different stereoisomers. There are eight amino acids that are practically tasteless. A group of three has varying tastes; except for glutamic acid, these are probably derived from sulfur-containing decomposition products. Seven amino acids have a bitter taste in the L form or a sweet taste in the D form, except for L-alanine, which has a sweet taste (Table 7.2). Solms et al. (1965) reported on the taste intensity, especially of aromatic amino acids. L-tryptophan is about half as bitter as caffeine; D-tryptophan is 35 times sweeter than sucrose and 1.7 times sweeter than calcium cyclamate. L-phenylalanine is about one-fourth as bitter as caffeine; the D form is about seven times sweeter than sucrose. L-tyrosine is about one-twentieth as bitter as caffeine, but D-tyrosine is still 5.5 times sweeter than sucrose.

Some researchers claim that differences exist between the L and D forms of some sugars. They propose that L-glucose is slightly salty and not sweet, whereas D-glucose is sweet. There is even a difference in taste between the two anomers of D-mannose. The α form is sweet as sugar, and the β form is bitter as quinine.

Optical isomers of carvone have totally different flavors. The D+ form is characteristic of caraway; the L− form is characteristic of spearmint.

The ability to taste certain substances is genetically determined and has been studied with phenylthiourea. At low concentrations, about 25% of subjects tested do not taste this compound; for the other 75%, the taste is bitter. The inability to taste phenylthiourea is probably due to a recessive gene. The compounds by which tasters and nontasters can be differentiated all contain the following isothiocyanate group:

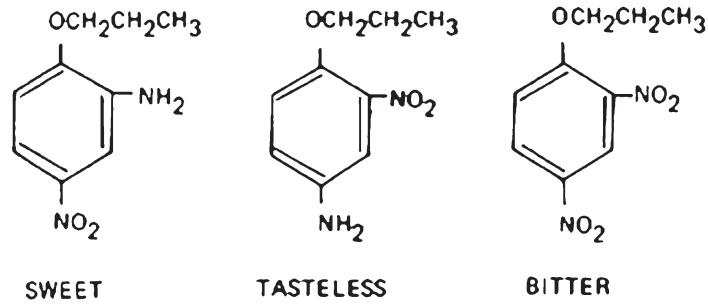


Fig. 7.7 Taste of substituted propoxybenzenes

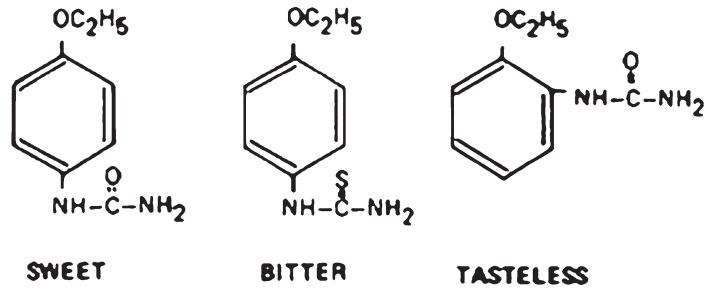
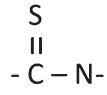


Fig. 7.8 Taste of substituted ethoxybenenes

Table 7.2 Difference in taste between the L-and D-forms of amino acids

Amino acid	Taste of L isomer	Taste of D isomer
Asparagine	Insipid	Sweet
Glutamic acid	Unique	Almost tasteless
Phenylalanine	Faintly bitter	Sweet, bitter aftertaste
Leucine	Flat, faintly bitter	Strikingly sweet
Valine	Slightly sweet, bitter	Strikingly sweet
Serine	Faintly sweet, stale after taste	Strikingly sweet
Histidine	Tasteless to bitter	Sweet
Isoleucine	Bitter	Sweet
Methionine	Flat	Sweet
Tryptophan	Bitter	Very sweet



These compounds—phenylthiourea, thiourea, and thiouracil—are illustrated in Fig. 7.9. The corresponding compounds that contain the group,

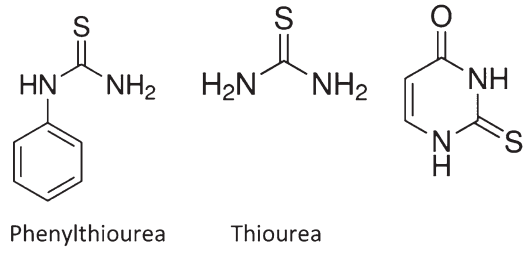
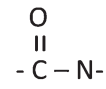


Fig. 7.9 Compounds containing the $-\overset{S}{\overset{\|}{C}}-N-$ group by which tasters and nontasters can be differentiated



phenylurea, urea, and uracil, do not show this phenomenon. Another compound containing the isothiocyanate group has been found in many species of the Cruciferae family; this family includes cabbage, turnips, and rapeseed and is well known for its goitrogenic effect. The compound is goitrin, 5-vinyloxazolidine-2-thione (Fig. 7.10).

Sweet Taste

Many investigators have attempted to relate the chemical structure of sweet tasting compounds to the taste effect, and a series of theories have been proposed (Shallenberger 1971). Shallenberger and Acree (1967, 1969) proposed a theory that can be considered a refinement of some of the ideas incorporated in previous theories. According to this theory, called the AH,B theory, all compounds that bring about a sweet taste response possess an electronegative atom A, such as oxygen or nitrogen. This atom also possesses a proton attached to it by a single covalent bond; therefore, AH can represent a hydroxyl group, an imine or amine group, or a methine group. Within a distance of about 0.3 nm from the AH proton, there must be a second electronegative atom B, which again can be oxygen or nitrogen (Fig. 7.11). Investigators have recognized that sugars that occur in a favored chair conformation yield a glycol unit conformation with the proton of one hydroxyl group at a distance of about 0.3 nm from the oxygen of the next hydroxyl group; this unit can be considered as an AH,B system. It was also found that the π bonding cloud of the benzene ring could serve as a B moiety. This explains the sweetness of benzyl alcohol and the sweetness of the *anti* isomer of anisaldehyde oxime, as well as the lack of sweetness of the *syn* isomer. The structure of these compounds is given in Fig. 7.12. The AH,B system present in sweet compounds is, according to Shallenberger, able to react with a similar AH,B unit that exists at the taste bud receptor site through the formation of simultaneous hydrogen bonds. The relatively strong nature of such bonds could explain why the sense of sweetness is a lingering sensation. According to the AH,B theory, there should not be a difference in sweetness between the L and D isomers of sugars. Experiments by Shallenberger (1971) indicated that a panel could not distinguish among the sweet taste of the enantiomorphic forms of glucose, galactose, mannose, arabinose, xylose, rhamnose, and glucoheptulose. This suggests that the notion that L sugars are tasteless is a myth.

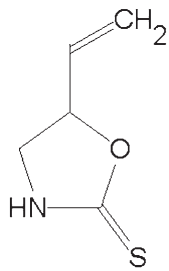


Fig. 7.10 5-vinyloxazoli ine-2-thione

Spillane (1996) has pointed out that the AH,B theory appears to work quite well, although spatial, hydrophobic/hydrophilic, and electronic effects are also important. Shallenberger (1998) describes the initiation of sweetness as being due to a concerted intermolecular, antiparallel hydrogen-bonding interaction between the glycophore (Greek *glyks,* sweet; *phoros,* to carry) and receptor dipoles. The difficulty in explaining the sweetness of compounds with different chemical structures is also covered by Shallenberger (1998) and how this has resulted in alternative taste theories. The application of sweetness theory is shown to have important applications in the food industry.

Extensive experiments with a large number of sugars by Birch and Lee (1971) support Shallenberger's theory of sweetness and indicate that the fourth hydroxyl group of glucopyranosides is of unique importance in determining

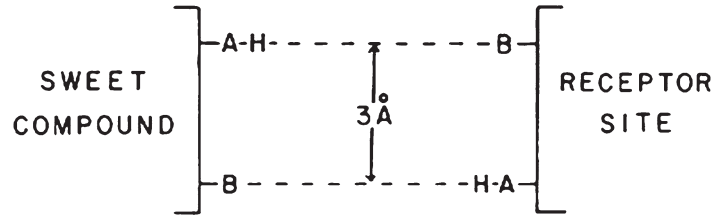


Fig. 7.11 The AH,B theory of sweet taste perception

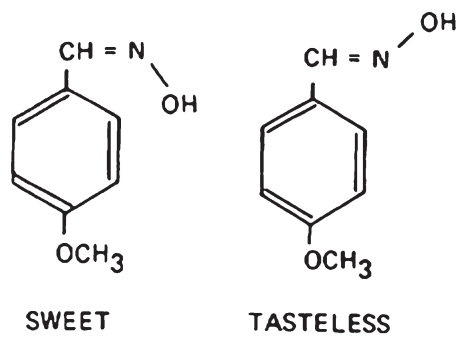


Fig. 7.12 *Anti*-anisaldehyde oxime, sweet; and *syn*-anisaldehyde oxime, tasteless

sweetness, possibly by donating the proton as the AH group. Apparently the primary alcohol group is of little importance for sweetness. Substitution of acetyl or azide groups confers intense bitterness to sugars, whereas substitution of benzoyl groups causes tastelessness.

As the molecular weight of saccharides increases, their sweetness decreases. This is best explained by the decrease in solubility and increase in size of the molecule. Apparently, only one sugar residue in each oligosaccharide is involved in the interaction at the taste bud receptor site.

The relative sweetness of a number of sugars and other sweeteners has been reported by Solms (1971) and is given in Table 7.3. These figures apply to compounds tasted singly and do not necessarily apply to sugars in foods, except in a general sense. The relative sweetness of mixtures of sugars changes with the concentration of the components. Synergistic effects may increase the sweetness by as much as 20–30% in such mixtures (Stone and Oliver 1969).

Steroidal alkaloids (SAs) and their glycosylated forms (SGAs) found in the nightshade family are toxic to humans and animals. These compounds are produced by members of the Solanaceae and Liliaceae plant families. In the plants these metabolites serves as a chemical barriers against a broad range of pests and pathogens. In humans and animals, SAs are considered anti-nutritional factors because they affect the digestion and absorption of nutrients from food and in some cases they can cause poisoning (Cardenas et al. 2015).

Table 7.3 Relative sweetness of sugars and other sweeteners

Compound	Relative sweetness
Sucrose	1
Lactose	0.27
Maltose	0.5
Sorbitol	0.5
Galactose	0.6
Glucose	0.5–0.7
Mannitol	0.7
Glycerol	0.8
Fructose	1.1–1.5
Cyclamate	30–80
Glycyrrhizin	50
Aspartyl-phenylalanine methylester	100–200
Stevioside	300
Naringin dihydrochalcone	300
Saccharin	500–700
Neohesperidin dihydrochalcone	1000–1500

Source: From J. Solms, Nonvolatile Compounds and the Flavor of Foods, in *Gustation and Olfaction,* G Ohloff and A.F. Thomas, eds., 1971, Academic Press

Sour Taste

Although it is generally recognized that sour taste is a property of the hydrogen ion, there is no simple relationship between sourness and acid concentration. Acids have different tastes; the sourness as experienced in the mouth may depend on the nature of the acid group, pH, titratable acidity, buffering effects and the presence of other compounds, especially sugars. Organic acids have a greater taste effect than inorganic acids (such as hydrochloric acid) at the same pH. Information on a number of the most common acids found in foods and phosphoric acid (which is also used in soft drinks) has been collected by Solms (1971) and compared with hydrochloric acid. This information is presented in Table 7.4.

According to Beatty and Cragg (1935), relative sourness in unbuffered solutions of acids is not a function of molarity but is proportional to the amount of phosphate buffer required to bring the pH to 4.4. Ough (1963) determined relative sourness of four organic acids added to wine and

Table 7.4 Properties of some acids, arranged in order of decreasing acid taste and with tartaric acid as reference

Acid	Properties of 0.05 N solutions			Ionization constant	Taste sensation	Found In
	Taste	Total acid (g/L)	pH			
Hydrochloric	+1.43	1.85	1.70	–	–	–
Tartaric	0	3.75	2.45	1.04 × 10–3	Hard	Grape
Malic	−0.43	3.35	2.65	3.9 × 10–4	Green	Apple, pear, prune, grape, cherry, apricot
Phosphoric	−1.14	1.65	2.25	7.52 × 10–3	Intense	Orange, grapefruit
Acetic	−1.14	3.00	2.95	1.75 × 10–5	Vinegar	–
Lactic	−1.14	4.50	2.60	1.26 × 10–4	Sour, tart	–
Citric	−1.28	3.50	2.60	8.4 × 10–4	Fresh	Berries, citrus, pineapple
Propionic	−1.85	3.70	2.90	1.34 × 10–5	Sour, cheesy	–

Source: From J. Solms, Nonvolatile Compounds and the Flavor of Foods, in *Gustation and Olfaction*, G. Ohloff and A.F. Thomas, eds., 1971, Academic Press

also preference for these acids. Citric acid was judged the most sour, fumaric and tartaric about equal, and adipic least sour. The tastes of citric and tartaric acids were preferred over those of fumaric and adipic acids.

Pangborn (1963) determined the relative sourness of lactic, tartaric, acetic, and citric acid and found no relation between pH, total acidity, and relative sourness. It was also found that there may be considerable differences in taste effects between sugars and acids when they are tested in aqueous solutions and in actual food products.

Buffering action appears to help determine the sourness of various acids; this may explain why weak organic acids taste more sour than mineral acids of the same pH. It is suggested that the buffering capacity of saliva may play a role, and foods contain many substances that could have a buffering capacity.

Wucherpfennig (1969) examined the sour taste in wine and found that alcohol may decrease the sourness of organic acids. He examined the relative sourness of 17 organic acids and found that the acids tasted at the same level of undissociated acid have greatly different intensities of sourness. Partially neutralized acids taste more sour than pure acids containing the same amount of undissociated acids. The change of malic into lactic acid during the malolactic fermentation of wines leads to a decrease in sourness, thus making the flavor of the wine milder.

Salty Taste

The salty taste is best exhibited by sodium chloride. It is sometimes claimed that the taste of salt by itself is unpleasant and that the main purpose of salt as a food component is to act as a flavor enhancer or flavor potentiator. The taste of salts depends on the nature of both cation and anion. As the molecular weight of either cation or anion—or both—increases, salts are likely to taste bitter. The lead and beryllium salts of acetic acid have a sweet taste. The taste of a number of salts is presented in Table 7.5.

The current trend of reducing sodium intake in the diet has resulted in the formulation of low-sodium or reduced-sodium foods. It has been shown (Gillette 1985) that sodium chloride enhances mouthfeel, sweetness, balance, and saltiness, and also masks or decreases off-notes. Salt substitutes based on potassium chloride do not enhance mouthfeel or balance and increase bitter or metallic off-notes.

Some individuals are sensitive are sensitive and need to reduce the sodium content of their diet. Salt sensitivity individuals experience increases in blood pressure in response to salt intake, Salt sensitive individuals are more likely to have high blood pressure than those who are resistant to salt. Salt-sensitive individuals are at higher risk for high blood pressure, cardiovascular disease and lower survival rate later in life if

Table 7.5 Taste sensations of salts

Taste	Salts
Salty	LiCl, LiBr, Lil, NaNO3, NaCl, NaBr, Nal, KNO3, KCl
Salty and bitter	KBr, NH4l, KCl
Bitter	CsCl, CsBr, Kl, MgSO4
Sweet	Lead acetate,[a] beryllium acetate[a]
Taste	Salts

[a]Extremely toxic

Table 7.6 Percentage of salt-sensitive people in different populations (data from Sullivan 1991)

	Population	
Blood pressure	White (%)	Black (%)
Normal	15	27
Hypertension	29	50

they continuously live an unhealthy lifestyle or have a high-sodium diet (Weinberger et al. 2001). A study by Sullivan 1991), salt-sensitive individuals are more likely to have hypertension, as are blacks more than whites (Table 7.6). Another study reports that approximately 60% of Chinese who have high blood pressure are salt-sensitive (Li 2012).

Sodium homeostasis in the human body is regulated mainly by the renin-angiotensin-aldosterone system. This system operates mainly in the kidney and in vascular smooth muscle cells. Variations in this system, due to genetic background, age, race, gender and medical history, cause the kidney of salt-sensitive individuals to handle excess sodium less efficiently. Asian or African ancestry, older age, female gender, high blood pressure, and kidney disease are all associated with salt-sensitivity.

Salt sensitive individuals exhibit variations in genes involved in the renin-angiotensin-aldosterone system which predispose them to salt sensitivity (Sanada et al. 2011). About 38% of the general population carries an ACE gene variant that causes increased activity of the system leading to blood pressure increase in response to higher sodium levels in the blood. This part of the population becomes salt-sensitive. Two other genes associated with salt sensitivity are the NOS3 gene, and the AGT gene, Table 7.7 lists the frequency of risk variants associated with increased risk for salt sensitivity and hypertension.

To help consumers reduce or control sodium intake, many salt substitutes with low sodium content have been designed to reduce the risk of high blood pressure and cardiovascular disease associated with a high intake of sodium chloride, while delivering similar taste [Scientific Advisory Committee on Nutrition Salt and Health (2003)]. The increase in sodium consumption is considered a potential health threat for some individuals. The Institute of Medicine of the National Academy of Sciences has established adequate daily intakes (AIs) for sodium and potassium and a tolerable upper intake level (UL) for sodium, based on its effects on blood pressure (Table 7.8; IOM 2004). Persons with a greater risk for hypertension (adults who are Black, over 40 years old, or already have hypertension or prehypertension) have been urged to consume no more than the AI level of sodium each day (CDCP 2009; Doyle and Glass 2010). These products are predominantly potassium chloride (KCl). Potassium Chloride's toxicity is similar to Sodium Chloride in healthy individuals; the LD50 is about 2.5 g/kg. Potassium lactate is frequently used to reduce sodium levels in meat and poultry products. The recommended daily allowance of potassium is higher than that for sodium (Caggiula et al. 1985).

Sodium is an essential micronutrient and, via salt taste, appetitive. High consumption of sodium is, however, related to negative health effects such as hypertension, cardiovascular diseases and stroke. In industrialized countries, about 75% of sodium in the diet comes from processed foods and foods eaten away from home. Reducing sodium in processed foods will be, however, challenging due to sodium's specific functionality in terms of flavor and associated palatability of foods (i.e., increase of saltiness, reduced bitterness, enhancement of sweetness and other congruent flavors). Salt has many beneficial properties for both preservation and multiple culinary benefits. Salt improves the sensory properties of nearly all foods. The principle reason for adding salt to food is that enhances the positive sensory attributes of foods. Salt makes

Table 7.7 Percentage gene variants associated with salt sensitivity in different populations

Gene symbol	All (%)	African (%)	American (%)	Asian (%)	European (%)
ACE	38	17	40	31	56
ADD1	27	17	19	50	20
ADRB1	30	40	21	21	34
AGT	66	88	64	83	41
AGTR1	16	3	23	7	27
CYP11B2	36	17	43	31	49
GNB3	48	79	42	47	31
NOS3	26	50	50	20	50

(Source: http://www.gbhealthwatch.com/Trait-Salt-Sensitivity.php?)
ALL general population, *AFR* Africans, *AMR* Americans, *ASN* Asians, *EUR* Europeans. Data are from 1000 genome project

Table 7.8 Daily sodium and potassium intakes and recommended intakes in the U.S. (IOM 2004)

	Sodium	Sodium chloride (g)	Potassium
AI (adequate intake): 19–50 years	1.5 g/d (65 mmol)	3.8	4.7 g/d (120 mmol)
AI: 51–70 years	1.3 g/d (55 mmol)	3.3	4.7 g/d (120 mmol)
AI: >71 years	1.2 g/d (50 mmol)	3	4.7 g/d (120 mmol)
UL: tolerable upper intake level	2.3 g/d (95 mmol)	5.8	Not established
Median intake (males)	4.2 g (183 mmol)	10.6	2.9–3.2 g/d (74–82 mmol)
Median intake (females)	3.3 g (142 mmol)	8.3	2.1–2.3 g/d (54–59 mmol)
AI (adequate intake): 19–50 years	1.5 g/d (65 mmol)	3.8	4.7 g/d (120 mmol)

(Source: IOM 2004)

foods "taste" better. Consumers who are accustomed to higher levels of salt in their foods find foods without salt unpalatable. Reductions in levels of salt in their food therefore must be gradual. In order to lower salt consumption in the population as a whole, it will be necessary to reduce salt levels in the human food supply with careful attention to their flavor-enhancing properties (Liem et al. 2011).

Rama et al. (2013) demonstrated that salt crystal size impacted upon the rate of initial response and perceived saltiness. They studied three different sizes of salt crystals on potato crisps to measure the rate of solubilisation of the salt crystals. A single sample of salt was ground in a mortar and pestle and mechanically sieved to produce three sizes of salt particles: S1 (<106 μm), S2 (106–425 μm), S3 (425–710 μm). The smallest crystal size salt dissolved and diffused throughout the mouth to the tongue saliva faster than the medium and the larger crystals ones; the smallest crystal size delivered the highest maximum concentration and greatest total sodium in the saliva. The results correlated l with the sensory perceived saltiness, where the smallest crystal size fraction resulted in the fastest salty perception, highest maximum saltiness intensity and maximum total saltiness. The different delivery rates can be explained by differential dissolution kinetics and enhanced mass transfer of sodium into the saliva. The sodium concentration in the saliva from the various crystal sizes salt are shown in Fig. 7.13.

The results demonstrate that when salt is placed on the surface of foods the total salt added can be reduced by using smaller crystal size salt. Salt substitutes offer alternatives to enhance flavor while reducing sodium content of the food. Frequently low-sodium products have been formulated with a blend of sodium and potassium chlorides, but potassium causes bitter and metallic tastes. Many methods have been developed to improve foods made with low or reduced sodium.

Some food manufacturers have reduced sodium in foods like salty snacks. AkzoNobel (www.akzonobel.com/saltspecialties) developed

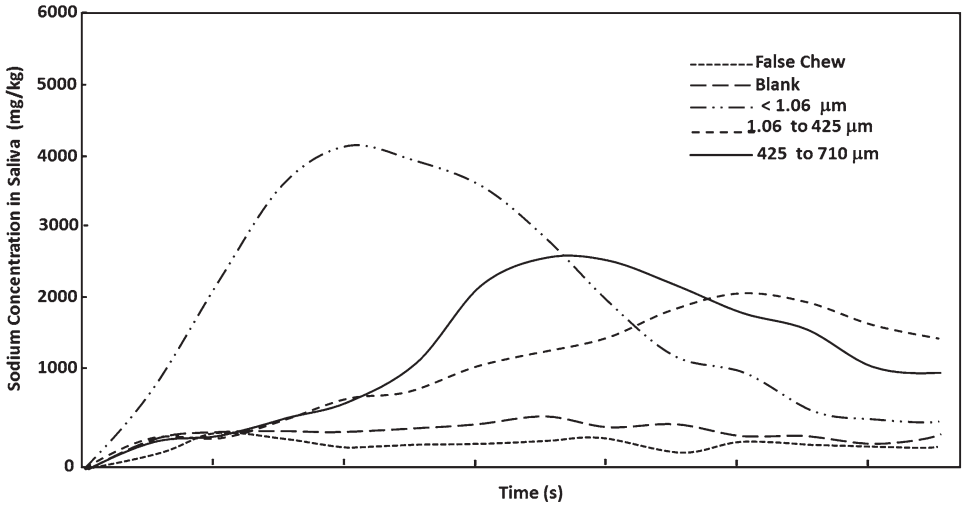


Fig. 7.13 Salivary sodium concentration after chewing crisps with differing salt crystal fractions, S1 (<106 μm), S2 (106–425 μm), and S3 (425–710 μm), Blank (no salt) and false chew (n = 8). Error bars indicate standard deviation. (Adapted from: Rama et al. 2013)

its OneGrain technology to combine regular salt, a salt replacer, and taste-enhancing flavors in single salt grains to achieve up to 50% sodium reduction. Suprasel Loso OneGrain, produced using the technology, can provide a one-for-one replacement for regular salt, and the company says that the ingredient is a genuine replacement for salt in terms of taste and functionality (Nachay 2013).

Reducing sodium in baked goods is challenging because of the important roles that sodium plays. Innophos (www.innophos.com) offers calcium phosphates and sodium aluminum phosphates that can be used to reduce sodium in chemically leavened bakery products. These ingredients like Cal-Rise® calcium acid pyrophosphate, Regent 12XX® monocalcium phosphate, monohydrate, Levair® sodium aluminum phosphate, and more replace some or all of the traditional leavening agents in a variety of baked goods applications. Each ingredient has its own benefits, some of which are improved texture, resilient crumb structure, and better stability (Nachay 2013).

Morton Salt (www.mortonsalt.com) offers Morton® LiteSalt™ Mixture, a blend of sodium chloride and potassium chloride that contains 50% less sodium than regular salt, and Morton Salt Balance® Salt Blend, a blend of sodium chloride and potassium chloride with 25% less sodium than regular salt (Nachay 2013).

Tate & Lyle (www.tateandlyle.com) offers SODA-LO™, which is manufactured using proprietary technology that turns salt crystals into free-flowing crystalline microspheres. The benefit of this ingredient, according to the company, is that the smaller crystals optimize saltiness perception in foods by maximizing the surface area relative to volume, allowing for an up to 50% reduction in sodium in some applications. The company also emphasizes that since the ingredient is made from salt, it does not impart any off-tastes. It functions well in breads, breadings, and coatings, and salty snacks (Nachay 2013).

Bitter Taste

Bitter taste is characteristic of many foods and can be attributed to a great variety of inorganic and organic compounds. Many substances of plant origin are bitter. Although bitter taste by itself is usually considered to be unpleasant, it is a component of the taste of many foods, usually those foods that are sweet or sour. Inorganic salts can have a bitter taste (Table 7.5). Some amino acids may be bitter (Table 7.2). Bitter peptides

Table 7.9 Taste of some selected peptides

Taste	Composition of peptides
Flat	L-Lys-L-Glu, L-PhE-L-Phe, Gly-Gly-Gly-Gly
Sour	L-Ala-L-Asp, γ-L-Glu-L-Glu, Gly- L-Asp-L-Ser-Gly
Bitter	L-Leu-L-Leu, L-Arg-L-Pro, L-Val- L-Val-L-Val
Sweet	L-Asp-L-Phe-OMe, L-Asp-L- Met-OMe
Biting	γ-L-Glutamyl-S-(prop-1-enyl)-L-cysteln

Source: From J. Solms, Nonvolatile Compounds and the Flavor of Foods, in *Gustation and Olfaction*, G. Ohloff and A. F. Thomas, eds., 1971, Academic Press

may be formed during the partial enzymic hydrolysis of proteins—for example, during the ripening of cheese. Solms (1969) has given a list of peptides with different taste sensations (Table 7.9).

Bitter taste is an important evolutionary system that helps prevents mammal from ingesting food containing bitter-tasting toxins, which include a wide range of structurally diverse molecules. Bitter taste mediated by a family of heptahelical G protein-coupled receptors, called taste 2 receptors or TAS2Rs or T2Rs. The ability of TAS2Rs to recognize a broad range of bitter compounds provides us with the ability to detect the wide range of bitter substances in foods and beverages. Individual TAS2Rs possess only one binding site, in which they accommodate their ligands by contacting different but overlapping sets of amino acids on the protein in the transmembrane portion of the cell. There is a large genetic variability in TAS2Rss in humans including single nucleotide polymorphisms, variations in copy numbers and receptor functionally which cause variability in the sensitivity to the bitterness of specific compounds (Meyerhof et al. 2011).

Food preferences are influenced by many factors including personal experiences, cultural adaptations and perceived health benefits. Taste is the most important determinant effecting whether a food is liked or disliked. Based on the response to bitter-tasting compounds, such as phenylthiocarbamide (PTC) or 6-n-propylthiouracil (PROP), individuals can be classified as supertasters, tasters, or nontasters. Genetic differences in bitter taste perception may account for many individual differences in food preferences. Other factors such as age, sex and ethnicity may also modify the

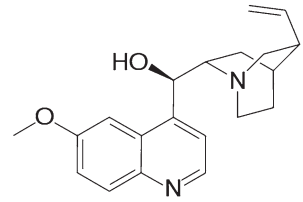


Fig. 7.14 Structure of quinine. This has an intensely bitter taste

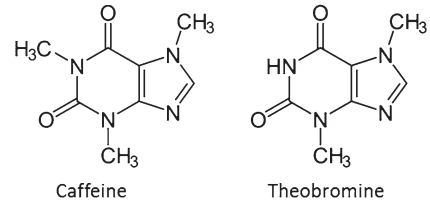


Fig. 7.15 Caffeine and theobromine

response to bitter-tasting compounds (Bartoshuk et al. 1994). There are several members of the TAS2R receptor gene family that encode taste receptors on the tongue, and genetic polymorphisms of TAS2R38 have been associated with differences in the perception of PTC and PROP (El-Sohemy et al. 2007; Hayes and Keast 2011).

Alkaloids and glycosides are the most common bitter compounds in foods. Alkaloids are basic nitrogen-containing organic compounds that are derived from pyridine, pyrrolidine, quinoline, isoquinoline, or purine. Quinine is often used as a standard for testing bitterness (Fig. 7.14). The bitterness of quinine hydrochloride is detectable in a solution as dilute as 0.00004 M, or 0.0016%. If 5-mL of this solution is tasted, the amount of substance a person detects would be 0.08 mg (Moncrieff 1951). Our sensitivity to bitterness is more extreme than our sensitivity to other tastes; the order of sensitivity is from bitter to sour to salty and our least sensitivity is to sweet taste. Threshold values reported by Moncrieff are as follows: sour—0.007% HC1; salt—0.25% NaCl; and sweet—0.5% sucrose. If the artificial sweeteners such as saccharine are considered, the sweet sensitivity is second to bitter. Quinine is used as a component of some soft drinks to produce bitterness. Other alkaloids occurring as natural bitter constituents of foods are caffeine and theobromine (Fig. 7.15), which are derivatives of purine.

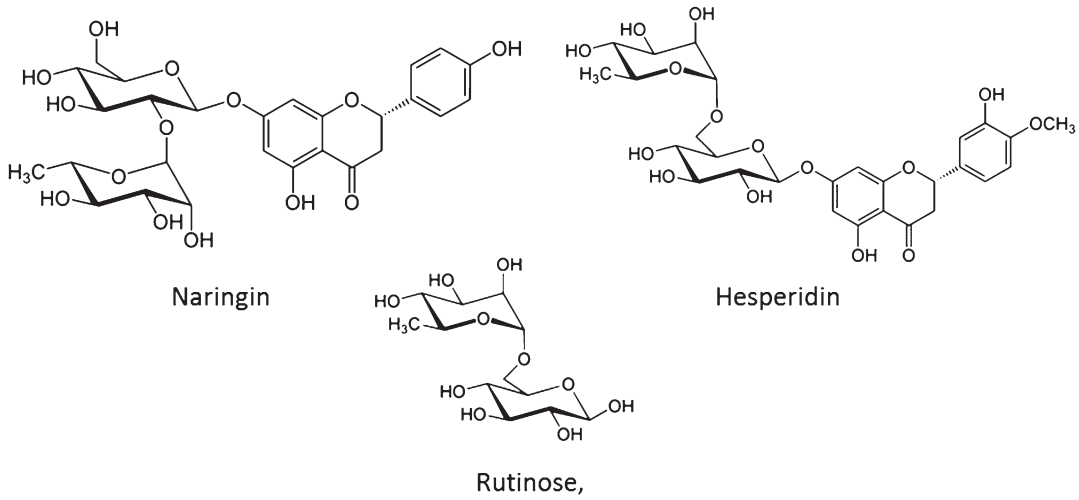


Fig. 7.16 Naringin, hesperidin, rutinose, 6-O-α-L-rharnnopyranosyl-D-glucopyranose

Another naturally occurring bitter substance is the glycoside naringin, which occurs in grapefruit and some other citrus fruits. Naringin in pure form is more bitter than quinine and can be detected in concentrations of less than 0.002%. Naringin (Fig. 7.16) contains the sugar moiety rutinose (L-rhamnose-D-glucose), which can be removed by hydrolysis with boiling mineral acid. The aglucose is called naringenin, and it lacks the bitterness of naringin. Since naringin is only slightly soluble in water (0.05% at 20 °C), it may crystallize out when grapefruit is subjected to below-freezing temperatures. Hesperidin (Fig. 7.16) occurs widely in citrus fruits and is also a rutinose glycoside. It occurs in oranges and lemons. Dried orange peel may contain as much as 8% hesperidin. The aglycone of hesperidin is called hesperetin. The sugar moiety is attached to carbon 7. Horowitz and Gentili (1969) have studied the relationship between bitterness and the structure of 7-rhamnoglycosides of citrus fruits; they found that the structure of the disaccharide moiety plays an important role in bitterness. The point of attachment of rhamnose to glucose determines whether the substance will be bitter or tasteless. Thus, neohesperidin contains the disaccharide neohesporidose, which contains rhamnose linked 1 → 2 to glucose; therefore, the sugar moiety is 2-*O*-α-L-rhamnopyranosyl-D-glucose. Glycosides containing this sugar, including neohesperidin, have a bitter taste. When the linkage between rhamnose and glucose is 1 → 6, the compound is tasteless as in hesperidin, where the sugar part, rutinose, is 6-*O*-α-L-rhamnopyranosyl-D-glucose.

Bitterness occurs as a defect in dairy products as a result of casein proteolysis by enzymes that produce bitter peptides. Bitter peptides are produced in cheese because of an undesirable pattern of hydrolysis of milk casein (Habibi-Najafi and Lee 1996). According to Ney (1979), bitterness in amino acids and peptides is related to hydrophobicity. Each amino acid has a hydrophobicity value (Δf), which is defined as the free energy of transfer of the side chains and is based on solubility properties (Table 7.10). The average hydrophobicity of a peptide, Q, is obtained as the sum of the Δf of component amino acids divided by the number of amino acid residues. Ney (1979) reported that bittemess is found only in peptides with molecular weights below 6000 Da when their Q value is greater than 1400. These findings indicate the importance of molecular weight and hydrophobicity. In a more detailed study of the composition of bitter peptides, Kanehisa (1984) reported that at least six amino acids are required for strong bitterness. A bitter peptide requires the presence of a basic amino acid at the N-terminal position and a hydrophobic one at the C-terminal position. It appears that at least two hydrophobic amino acids are required in the C-terminal area of the peptide to produce intense bitterness. The high hydrophobicity of leucine and the number of

leucine and possibly proline residues in the peptide probably play a role in the bitterness.

Flavan-3-ols and their condensation products are the most common flavonoids consumed in the American diet. The flavan-3-ols and their polymeric condensation products, the proanthocyanidins, are regarded as functional ingredients in various beverages, whole and processed foods, herbal remedies and supplements. They are presence in food influence several taste parameters including astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation. Some foods contain only monomeric flavan-3-ols [(−)-epicatechin predominates] and dimeric proanthocyanidins, most foods contain oligomers of d.p. values ranging from 1 to 10 or greater than 10. Flavan-3-ols have been reported to exhibit several health beneficial effects by acting as antioxidant, anticarcinogen, cardiopreventive, antimicrobial, anti-viral, and neuro-protective agents Aron and Kennedy (2008).

Beer flavonoids such as the flavan-3-ols and their condensed products, the proanthocyanidins are easily oxidized. As a result they are capable of hindering or preventing the oxididation of other components present in beer. Flavan-3-ols and proanthocyanidin improve oxidative stability in food systems, and thus theyalso can function as beer flavor modifiers and/or stabilizers. The polyphenols can also bind with protein contributing to haze in beer (Aron and Shellhammer 2010).

The antioxidant capacity of polyphenols from green tea, grape juice, and chocolate, as well as a wide range of fresh fruits and vegetables is well established. Fresh produce is a good source of polyphenols which influence the sensory and nutritional qualities of produce. The astringency and bitterness of foods and beverages are largely due to their polyphenolic content. Considerable variation is found in measuring the polyphenolic content of produce. The polyphenolic content of produce seems to be primarily influenced by genetics, but numerous other factors including degree of ripeness, climate, storage and processing can also influence phenolic content (Hughes 2010).

Table 7.10 Hydrophobicity values (Δf) of the side chains of amino acids

Amino acid	Abbreviation	Δf (cal/mol)
Glycine	Gly	0
Serine	Ser	40
Threonine	Thr	440
Histidine	His	500
Aspartic acid	Asp	540
Glutamic acid	Glu	550
Arginine	Arg	730
Alanine	Ala	730
Methionine	Met	1300
Lysine	Lys	1500
Valine	Val	1690
Leucine	Leu	2420
Proline	Pro	2620
Phenylalanine	Phe	2650
Tyrosine	Tyr	2870
Isoleucine	lle	2970
Tryptophan	Trp	3000

Other Aspects of Taste

The basic sensations—sweet, sour, salty, and bitter—account for the major part of the taste response. However, it is generally agreed that these basic tastes alone cannot completely describe taste. In addition to the four individual tastes, there are important interrelationships among them. One of the most important in foods is the interrelationship between sweet and sour. The sugar-acid ratio plays an important part in many foods, especially fruits. Kushman and Ballinger (1968) have demonstrated the change in sugar-acid ratio in ripening blueberries (Table 7.11). Sugar-acid ratios play an important role in the flavor quality of fruit juices and wines

Table 7.11 Change in sugar-acid ratio during ripening of blueberries[a]

	Unripe	Ripe	Overripe
Total sugar (%)	5.8	7.9	12.4
pH	2.83	3.91	3.76
Titratable acidity	23.9	12.9	7.5
Sugar-acid ratio	3.8	9.5	25.8

Source: From L.J. Kushman and W.E. Ballinger, Acid and Sugar Changes During Ripening in Wolcott Blueberries, *Proc. Amer. Soc. Hort. Soc*., Vol. 92, pp. 290–295, 1968

[a]The sugars are mainly glucose and fructose, and the acidity is expressed as citric acid

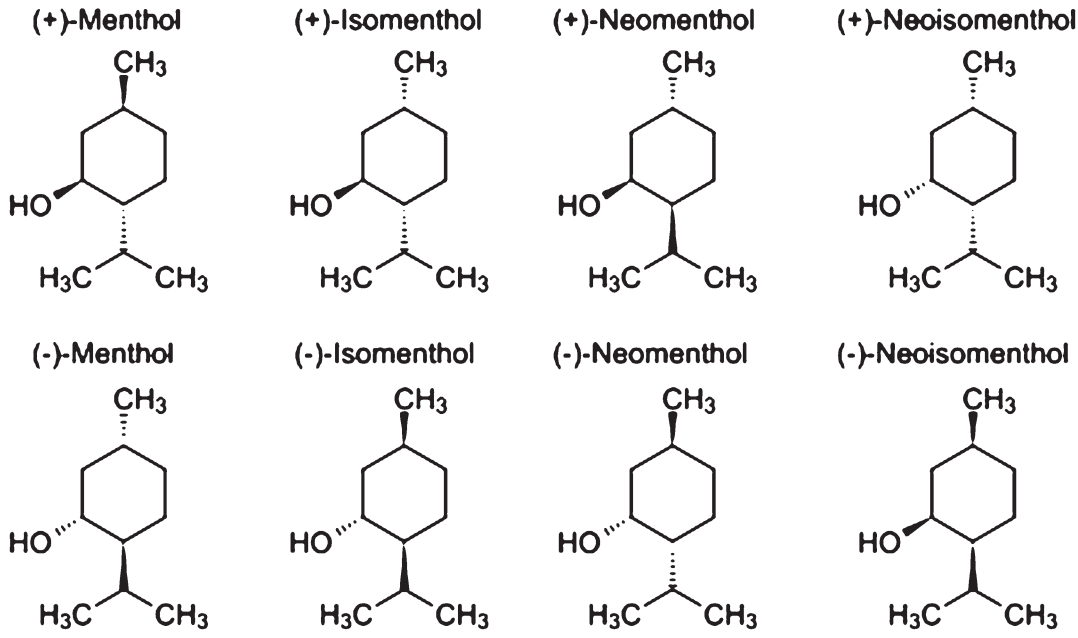


Fig. 7.17 Isomeric forms of menthol

(Ough 1963). Alkaline taste has been attributed to the hydroxyl ion. Caustic compounds can be detected in solutions containing only 0.01% of the alkali. Probably the major effect of alkali is irritation of the general nerve endings in the mouth. Another effect that is difficult to describe is astringency. Borax is known for its ability to produce this effect, as are the tannins present in foods, especially those that occur in tea. Even if astringency is not considered a part of the taste sense, it must still be considered a feature of food flavor.

Another important taste sensation is coolness, which is a characteristic of menthol. The cooling effect of menthol is part of the mint flavor complex and is exhibited by only some of the possible isomeric forms. Only (−) and (+) menthol show the cooling effect, the former to a higher degree than the latter, but the isomers isomenthol, neomenthol, and neoisomenthol do not give a cooling effect (Fig. 7.17) (Kulka 1967). Hotness is a property associated with spices and is also referred to as pungency. The compound primarily responsible for the hotness of black pepper is piperine (Fig. 7.18). In red pepper or capsicum, nonvolatile amides are responsible for the heat effect. The heat effect of spices and their constituents

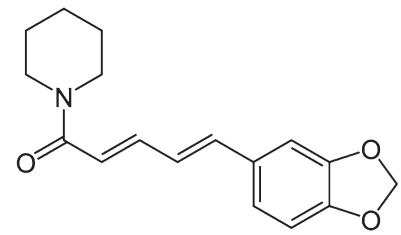


Fig. 7.18 Pipeline, responsible for the hotness of pepper

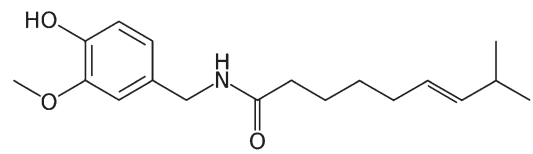


Fig. 7.19 Capsaicin, the pungent principle of red pepper

can be measured by an organoleptic threshold method (Rogers 1966) and expressed in heat units. The pungent principle of capsicum is capsaicin. The structure of capsaicin is given in Fig. 7.19. Capsaicin shows similarity to the compound zingerone, the pungent principle of ginger (Fig. 7.20).

Govindarajan (1979) has described the relationship between pungency and chemical struc-

ture of pungent compounds. There are three groups of natural pungent compounds—the capsaicinoids, piperine, and the gingerols. These have some common structural aspects, including an aromatic ring and an alkyl side chain with a carbonyl function (Figs. 7.19 and 7.20). Structural variations in these compounds affect the intensity of the pungent response. These structural variations include the length of the alkyl side chain, the position of the amide group near the polar aromatic end, the nature of the groupings at the alkyl end, and the unsaturation of the alkyl chain.

The metallic taste has been described by Moncrieff (1964). There are no receptor sites for this taste or for the alkaline and meaty tastes. However, according to Moncrieff, there is no doubt that the metallic taste is a real one. It is observable over a wide area of the surface of the tongue and mouth and, like irritation and pain, appears to be a modality of the common chemical sense. The metallic taste can be generated by salts of metals such as mercury and silver (which are most potent) but normally by salts of iron, copper, and tin. The threshold concentration is in the order of 20–30 ppm of the metal ion. In canned foods, considerable metal uptake may occur and the threshold could be exceeded in such cases. Moncrieff (1964) also mentions the possibility of metallic ion exchange between the food and the container. The threshold concentration of copper is increased by salt, sugar, citric acid, and alcohol. Tannin, on the other hand, lowers the threshold value and makes the copper taste more noticeable. The metallic taste is frequently observed as an aftertaste. The lead salt of saccharin gives an impression of intense sweetness, followed by a metallic aftertaste. Interestingly, the metallic taste is frequently associated with oxidized products. Tressler and Joslyn (1954) indicate that 20 ppm of copper is detectable by taste in orange juice. Copper is well known for its ability to catalyze oxidation reactions. Stark and Forss (1962) have isolated and identified oct-1-en-3-one as the compound responsible for the metallic flavor in dairy products.

Taste Inhibition and Modification

Some substances have the ability to modify our perception of taste qualities. Two such compounds are gymnemagenin, which is able to suppress the ability to taste sweetness, and the protein from miracle fruit, which changes the perception of sour to sweet. Both compounds are obtained from tropical plants.

The leaves of the tropical plant *Gymnema sylvestre,* when chewed, suppress the ability to taste sweetness. The effect lasts for hours, and sugar seems like sand in the mouth. The ability to taste other sweeteners such as saccharin is equally suppressed. There is also a decrease in the ability to taste bitterness. The active principle of leaves has been named gymnemic acid and has been found (Stöcklin et al. 1967) to consist of four components, designated as gymnemic acids, A_1, A_2, A_3, and A_4. These are D-glucuronides of acetylated gymnemagenins. The unacetylated gymnemagenin is a hexahydroxy pentacyclic triterpene; its structure is given in Fig. 7.21.

The berries of a West African shrub (*Synsepalum dulcificum)* contain a substance that has the ability to make sour substances taste sweet. The berry, also known as miracle fruit, has been shown

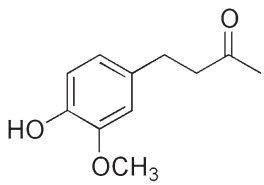


Fig. 7.20 Zingerone, the pungent principle of ginger

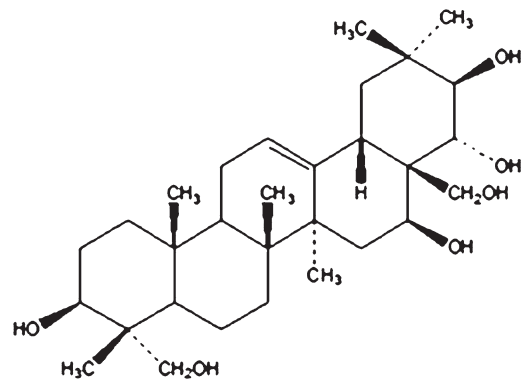


Fig. 7.21 Structure of gymnemagenin

to contain a taste-modifying protein (Kurihara and Beidler 1968, 1969). The protein is a basic glycoprotein with a molecular weight of 44,000. It is suggested that the protein binds to the receptor membrane near the sweet receptor site. The low pH changes the conformation of the membrane so that the sugar part of the protein fits into the sweet receptor site. The taste-modifying protein was found to contain 6.7% of arabinose and xylose.

These taste-modifying substances provide an insight into the mechanism of the production of taste sensations and, therefore, are a valuable tool in the study of the interrelationship between taste and chemical structure.

Flavor Enhancement—Umami

A number of compounds have the ability to enhance or improve the flavor of foods. It has often been suggested that these compounds do not have a particular taste of their own. Evidence now suggests that there is a basic taste response to amino acids, especially glutamic acid. This taste is sometimes described by the word *umami,* derived from the Japanese for deliciousness (Kawamura and Kare 1987). It is suggested that a primary taste has the following characteristics:

- The receptor site for a primary taste chemical is different from those of other primary tastes.
- The taste quality is different from others.
- The taste cannot be reproduced by a mixture of chemicals of different primary tastes.

From these criteria, we can deduce that the glutamic acid taste is a primary taste for the following reasons:

- The receptor for glutamic acid is different from the receptors for sweet, sour, salty, and bitter.
- Glutamic acid does not affect the taste of the four primary tastes.
- The taste quality of glutamic acid is different from that of the four primary tastes.
- Umami cannot be reproduced by mixing any of the four primary tastes.

Monosodium glutamate has long been recognized as a flavor enhancer and is now being considered a primary taste, umami. The flavor potentiation capacity of monosodium glutamate in foods is not the result of an intensifying effect of the four primary tastes. Glutamate may exist in the L and D forms and as a racemic mixture. The L form is the naturally occurring isomer that has a flavor-enhancing property. The D form is inert. Although glutamic acid was first isolated in 1866, the flavor-enhancing properties of the sodium salt were not discovered until 1909 by the Japanese chemist Kikunae Ikeda. Almost immediately, commercial production of the compound started and total production for the year 1954 was estimated at 13000,000 pounds. The product as first described by Ikeda was made by neutralizing a hydrolysate of the seaweed *Laminaria japonica* with soda. Monosodium glutamate is now produced from wheat gluten, beet sugar waste, and soy protein and is used in the form of the pure crystallized compound. It can also be used in the form of protein hydrolysates derived from proteins that contain 16% or more of glutamic acid. Wheat gluten, casein, and soy flour are good sources of glutamic acid and are used to produce protein hydrolysates. The glutamic acid content of some proteins is listed in Table 7.12 (Hall 1948). The protein is hydrolyzed with hydrochloric acid, and the neutralized hydrolysate is used in liquid form or as a dry powder. Soy sauce,

Table 7.12 Glutamic acid content of some proteins

Protein source	Glutamic acid (%)
Wheat gluten	36.0
Corn gluten	24.5
Zein	36.0
Peanut flour	19.5
Cottonseed flour	17.6
Soybean flour	21.0
Casein	22.0
Rice	24.1
Egg albumin	16.0
Yeast	18.5

Source: From L.A. Hall, Protein Hydrolysates as a Source of Glutamate Flavors, in *Monosodium Glutamate—A Symposium,* 1948, Quartermaster Food and Container Institute for the Armed Forces

which is similar to these hydrolysates, is produced wholly or partially by enzymic hydrolysis. This results in the formation of ammonia from acid amides; soy sauce contains ammonium complexes of amino acids, including ammonium glutamate.

The flavor of glutamate is difficult to describe. It has sometimes been suggested that glutamate has a meaty or chickeny taste, but it is now generally agreed that glutamate flavor is unique and has no similarity to meat. Pure sodium glutamate is detectable in concentrations as low as 0.03%; at 0.05% the taste is very strong and does not increase at higher concentrations. The taste has been described as a mixture of the four tastes (Crocker 1948). At about 2 threshold values of glutamate concentration, it could be well matched by a solution containing 0.6 threshold of sweet, 0.7 of salty, 0.3 of sour, and 0.9 of bitter. In addition, glutamate is said to cause a tingling feeling and a marked persistency of taste sensation. This feeling is present in the whole of the mouth and provides a feeling of satisfaction or fullness. Apparently glutamate stimulates our tactile sense as well as our taste receptors. The presence of salt is required to produce the glutamate effect. Glutamate taste is most effective in the pH range of 6–8 and decreases at lower pH values. Sugar content also affects glutamate taste. The taste in a complex food, therefore, depends on a complex interaction of sweet, sour, and salty, as well as the added glutamate.

Monosodium glutamate improves the flavor of many food products and is therefore widely used in processed foods. Products benefiting from the addition of glutamate include meat and poultry, soups, vegetables, and seafood.

For many years glutamate was the only known flavor enhancer, but recently a number of compounds that act similarly have been discovered. The 5′-nucleotides, especially 5′-inosinate and 5′-guanylate, have enhancement properties and also show a synergistic effect in the presence of glutamate. This synergistic effect has been demonstrated by determining the threshold levels of the compounds alone and in mixtures. The data in Table 7.13 are quoted from Kuninaka (1966). The 5′-nucleotides were discovered many years ago in Japan as components of dried bonito (a kind of fish). However, they were not produced commercially and used as flavor enhancers until recently, when technical problems in their production were solved. The general structure of the nucleotides with flavor activity is presented in Fig. 7.22. There are three types of inosinic acid, 2′-, 3′-, and 5′-isomers; only the 5′-isomer has flavor activity. Both riboside and 5′-phosphomonoester linkages are required for flavor activity, which is also the case for the OH group at the 6-position of the ring. Replacing the OH group with other groups, such as an amino group, sharply reduces flavor activity but this is not true for the group at the 2-position. Hydrogen at the 2-position corresponds with inosinate and an amino group with guanylate; both have comparable flavor activity, and the effect of the two compounds is additive.

Table 7.13 Threshold levels of flavor enhancers alone and in mixtures in aqueous solution

Solvent	Threshold level (%)		
	Disodium 5′-inosinate	Disodium 5′-guanylate	Monosodium L-glutamate
Water	0.012	0.0035	0.03
0.1% glutamate	0.0001	0.00003	–
0.01% inosinate	–	–	0.002

Source: From A. Kuninaka, Recent Studies of 5′-Nucleotides as New Flavor Enhancers, in *Flavor Chemistry*, I. Hornstein, ed., 1966, American Chemical Society

The synergistic effect of umami substances is exceptional. The subjective taste intensity of a blend of monosodium glutamate and disodium 5′-inosinate was found to be 16 times stronger than that of the glutamate by itself at the same total concentration (Yamaguchi 1979).

5′-nucleotides can be produced by degradation of ribonucleic acid. The problem is that most enzymes split the molecule at the 3′-phosphodiester linkages, resulting in nucleotides without flavor activity. Suitable enzymes were found in strains of *Penicillium* and *Streptomyces*. With the aid of these enzymes, the 5′-nucleotides can be manufactured industrially from yeast ribonucleic acid. Another process produces the nucleoside inosine by fermentation, followed by chemical phosphorylation to 5′-inosinic acid (Kuninaka 1966).

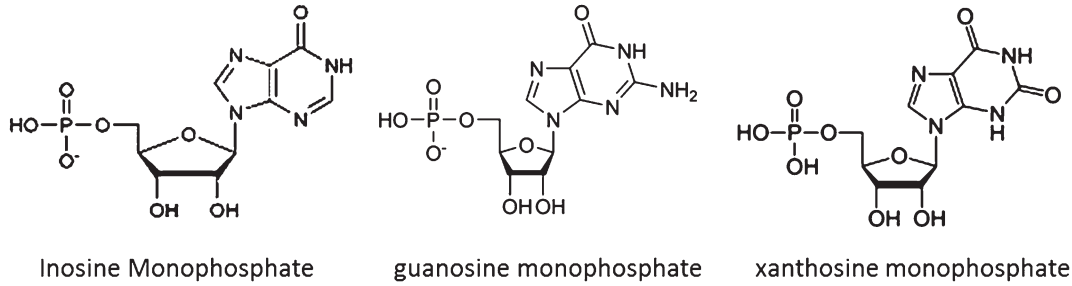


Fig. 7.22 Structure of nucleotides with flavor activity

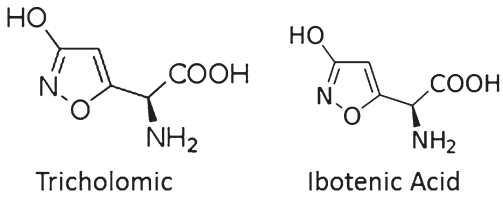


Fig. 7.23 Tricholomic and ibotenic acid

The search for other flavor enhancers has brought to light two new amino acids, tricholomic acid and ibotenic acid, obtained from fungi (Fig. 7.23). These amino acids have flavor activities similar to that of monosodium glutamate. Apparently, the flavor enhancers can be divided into two groups; the first consists of 5′-inosinate and 5′-guanylate with the same kind of activity and an additive relationship. The other group consists of glutamate, tricholomic, and ibotenic acid, which are additive in action. Between the members of the two groups, the activity is synergistic.

A different type of flavor enhancer is maltol, which has the ability to enhance sweetness produced by sugars. Maltol is formed during roasting of malt, coffee, cacao, and grains. During the baking process, maltol is formed in the crust of bread. It is also found in many dairy products that have been heated, as a product of decomposition of the casein-lactose system. Maltol (Fig. 7.24) is formed from di-, tri-, and tetrasaccharides including isomaltose, maltotretraose, and panose but not from maltotriose. Formation of maltol is brought about by high temperatures and is catalyzed by metals such as iron, nickel, and calcium.

Maltol has antioxidant properties. It has been found to prolong storage life of coffee and roasted cereal products. Maltol is used as a flavor enhancer in chocolate and candies, ice cream, baked products, instant coffee and tea, liqueurs, and flavorings. It is used in concentrations of 50–250 ppm and is commercially produced by a fermentation process.

Odor

The olfactory mechanism is both more complex and more sensitive than the process of gustation. Olfaction, the sense of smell is a primal sense for all humans and animals. Olfaction helps both vertebrates and other organisms with olfactory receptors to identify food, mates, and predators. It also can provide sensual pleasure (the odor of flowers and perfume) or threats from spoiled food or chemical dangers. Thus, olfaction is one of our principal means to communicate with our environment. There are thousands of odors, and the sensitivity of the smell organ is about 10,000 times greater than that of the taste organ.

Odorants are volatile chemical compounds that are carried by inhaled air to the Regio olfactoria (olfactory region) which are located in the roof of the two nasal cavities of the human nose (Fig. 7.25). The molecular properties of an odorant determine the sensory properties of the compound. The odorant must be at least partially water solubility, have a sufficiently high vapor pressure, low polarity, some ability to dissolve in fat (lipophilicity), and surface activity

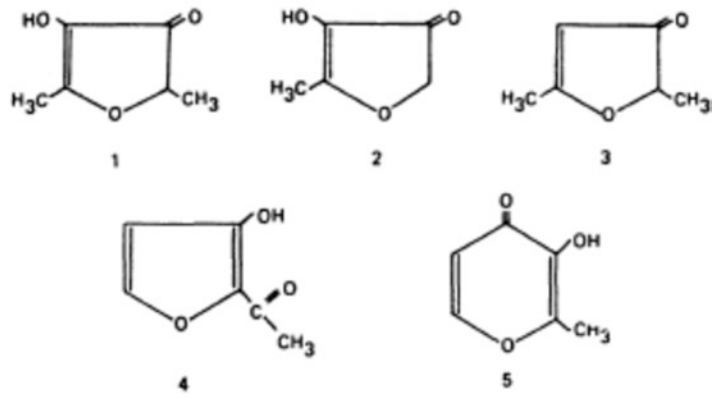


Fig. 7.24 Some furanones (1,2,3), isomaltol (4), and maltol (5)

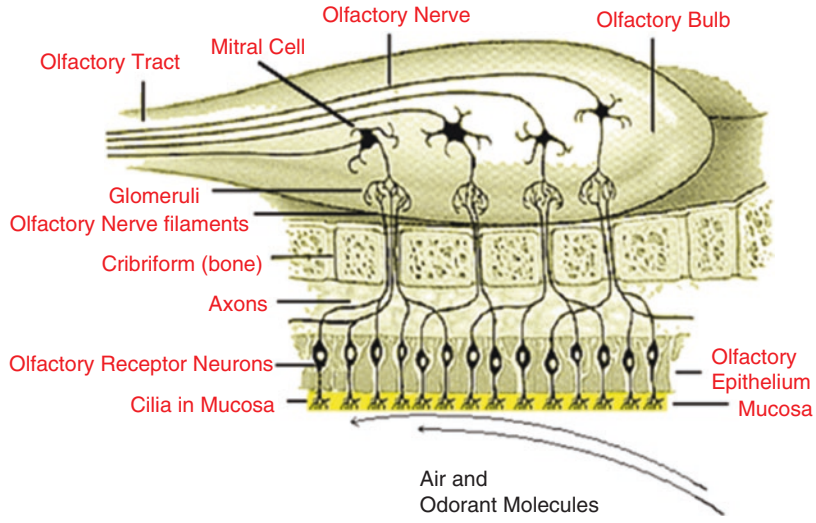


Fig. 7.25 Structure of olifactory cells and olifactory bulb (Leffingwell 2001). The diagrams of the "Olfactory Region" and the "Olfactory Bulb" are adapted from a drawing that appears at http://www.sciencenet.org.uk/database/Social/Senses/s00122b.html

(Leffingwell 2001; Greenman and Benkara Mostefa Saad 2009).

The olfactory sense is able to distinguish among numerous chemical compounds at very low concentrations (Ohloff 1994). The olfactory region in the two nasal passages of humans is a small area (about 2.5 cm^2) containing approximately 50 million primary sensory receptor cells. The olfactory region consists of cilia projecting out of the olfactory epithelium into a layer of mucous which is about 60 μ thick. This mucous layer is a lipid-rich secretion that bathes the surface of the receptors at the epithelium surface. The mucous layer is produced by the Bowman's glands which reside in the olfactory epithelium. The mucous lipids assist in transporting the odorant molecules as only volatile materials that are soluble in the mucous can interact with the olfactory receptors and produce the signals that our brain interprets as odor. Each olfactory receptor neuron has 8–20 cilia that are whip-like extensions 30–200 μ in length. The olfactory cilia are the sites where molecular reception with the odorant occurs and sensory transduction (i.e., transmission) starts (Leffingwell 2001).

The base olfactory epithelium consists partially of basal cells located in the lowest cellular layer of the olfactory epithelium just above the mucous layer. These basal cells undergo mitotic cell division to form olfactory receptor neurons. Olfactory receptor neurons turnover approximately every 40 days. The olfactory receptor neurons extend through the epithelium to contact odorants in the atmosphere. Within the

epithelium, the neuronal cells form axons that are bundled in groups of 10–100 and these bundles penetrate the bone, ending in the olfactory bulb of the brain. In the olfactory bulb of the brain, the neurons converge and terminate with post-synaptic cells to form synaptic structures called glomeruli. The glomeruli are connected in groups that converge into mitral cells. In rabbits, there are 26,000 receptor neurons that converge onto 200 glomeruli, which then converge at 25:1 onto each mitral cell. The total convergence is estimated to be about 1000:1. From the mitral cells, the message is sent directly to the higher levels of the central nervous system in the corticomedial amygdala portion of the brain (via the olfactory nerve tract) where the signaling process is decoded and olfactory interpretation and response occurs (Leffingwell 2001).

The olfactory epithelium contains another sensory system in the form of "trigeminal nerve" receptors. Some chemicals are trigeminal stimulants that produce sensations described as hot, cold, tingling or irritating. For example, leavo-menthol or (−)-menthol produces the trigeminal feeling of cold at moderate concentrations and "hot" at high concentrations in the nasal cavity. Similarly, camphor which possesses markedly more aroma than menthol, also produces the "cold" sensation via interaction with trigeminal receptors. Other commonly encountered trigeminal stimulants include the chemicals allyl isothiocyanate (mustard, mustard oil), capsiacin (hot chile powder, mace spray) and Diallyl sulfide (onion) (Leffingwell 2001). See Fig. 7.26.

Most odorous compounds are soluble in a variety of solvents, but it appears that solubility is less important than type of molecular arrangement, which confers both solubility and chemical reactivity (Moncrieff 1951). Some degree of water solubility does appear to contribute.

There are two common ways to establish relationships between a sample odor and a chemical. One is to find statistical associations between the data obtained from sensory analysis and GC-MS analysis for the specific sample or volatile compounds. The other is to use trained panelists sniffing at GC-MS ports to detect and identify volatile compounds that are also identified by sensors and computer programs (GC-MS Olfactormetry). Moreover, direct relationships to identify an odor compound can be done by comparing the sample odor to a number of volatile compounds that may have a similar odor description, which may require knowledges of odorous volatile compounds. Instrumental measurements are useful when detecting and identifying specific compounds. GC-MS may be used in combination with the sensory aromatic profile analysis to identify the volatile compounds present in the sample. More specifically, GC-MS sniff ports may be used to identify volatile compounds that have an odor detectable by the human nose. In this way, compounds identified by GC-MS can be related to the sensory aromatic results. Drawing correlations is frequently complicated since perceived intensity and aroma concentration are rarely linear. Frequently the detection or recognition threasholds are rapidly reached and

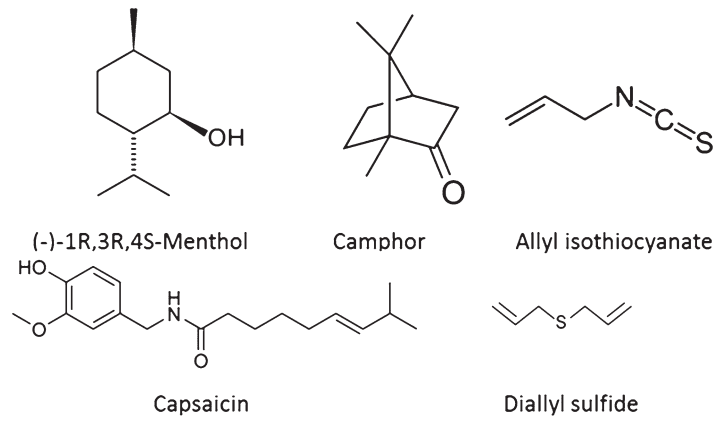


Fig. 7.26 Common food compounds that are trigeminal stimulants

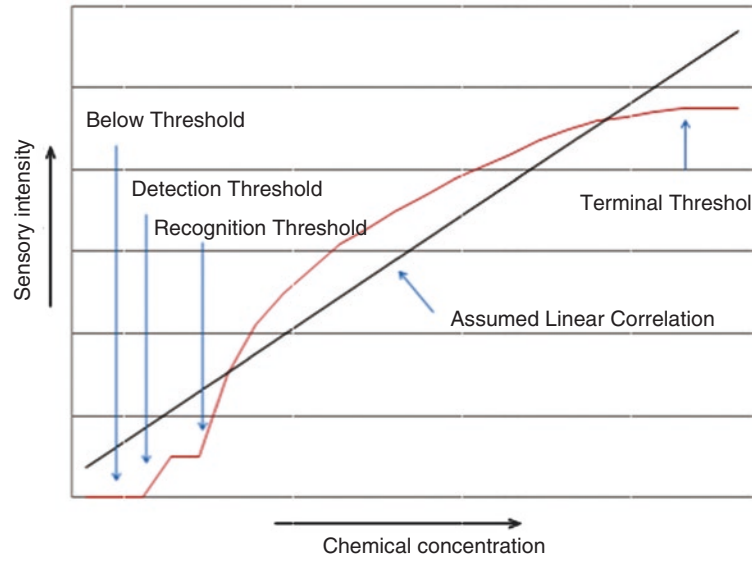


Fig. 7.27 Schematic diagram of potential relationships between chemical concentration and sensory intensity (from Chambers and Koppel 2013)

at higher levels the perceived intensity levels off reaching a terminal threshold as shown in Fig. 7.27 (Chambers and Koppel 2013).

The number of volatile compounds occurring in foods is very high. Maarse (1991) has given the following numbers for some foods: beef (boiled, cooked)—486; beer—562; butter—257; coffee—790; grape—466; orange—203; tea—541; tomato—387; and wine (white)—644. Not all of these substances may be essential in determining the odor of a product. Usually, the relative amounts of a limited number of these volatile compounds are important in establishing the characteristic odor and flavor of a food product.

The sensitivity of the human olfactory organ is inferior to that of many animals. Dogs and rats can detect odorous compounds at threshold concentrations 100 times lower than man. When air is breathed in, only a small part of it is likely to flow over the olfactory epithelium in the upper nasal cavity. When a smell is perceived, sniffing may increase the amount reaching the olfactory tissue. When foods are eaten, the passage of breath during exhalation reaches the nasal cavity from the back. Döving (1967) has quoted the threshold concentrations of odorous substances listed in Table 7.14. Apparently, it is possible to change odor thresholds by a factor of 100 or more by stimulating the sympathetic nervous system so that more odors can reach the olfactory tissue. What is remarkable about the olfactory mechanism is not only that thousands of odors can be recognized, but that it is possible to store the information in the brain for retrieval after long periods of time. The ability to smell is affected by several conditions, such as colds, menstrual cycle, and drugs such as penicillin. Odors are usually the result of the presence of mixtures of several, sometimes many, different

Table 7.14 Odor threshold concentrations of odorous substances perceived during normal inspiration

Compound	Threshold concentration (molecules/cc)
Allyl mercaptan	6×10^{7}
Sec. butyl mercaptan	1×10^{8}
Isopropyl mercaptan	1×10^{8}
Isobutyl mercaptan	4×10^{8}
Tert. butyl mercaptan	6×10^{8}
Thiophenol	8×10^{8}
Ethyl mercaptan	1×10^{9}
1,3-Xylen-4-ol	2×10^{12}
μ-Xylene	2×10^{12}
Acetone	6×10^{13}

Source: From K.B. Döving, Problems in the Physiology of Olfaction, in *Symposium on Foods: The Chemistry and Physiology of Flavors,* H.W. Schultz et al., eds., 1967, AVI Publishing

odorous compounds. The combined effect creates an impression that may be very different from that of the individual components. Many food flavors, natural as well as artificial, are of this compound nature.

Odor and Molecular Structure

M. Stoll wrote in 1957: "The whole subject of the relation between molecular structure and odor is very perplexing, as there is no doubt that there exist as many relationships of structure and odor as there are structures of odorous substances." In 1971 (referring to Stoll 1957), Teranishi wrote: "The relation between molecular structure and odor was perplexing then. It is now." We can observe a number of similarities between the chemical structure of compounds and their odors. However, the field of food flavors, as is the field of perfumery, is still very much an art, albeit one greatly supported by scientists' advancing ability to classify structures and identify the effect of certain molecular configurations. The odor potency of various compounds ranges widely. Table 7.15 indicates a range of about eight orders of magnitude (Teranishi 1971). This indicates that volatile flavor compounds may be present in greatly differing quantities, from traces to relatively large amounts.

Table 7.15 Odor thresholds of compounds covering a wide range of intensity

Odorant	Threshold (μg/L of water)
Ethanol	100,000
Butyric acid	240
Nootkatone	170
Humulene	160
Myrcene	15
n-Amyl acetate	5
n-Decanal	0.0
α- and β-Sinensal	0.05
Methyl mercaptan	0.02
β-Ionone	0.007
2-methoxy-3- isobutylpyra- zine	0.002

Source: From R. Teranishi, Odor and Molecular Structure, in *Gustation and Olfaction*, G. Ohloff and A.F. Thomas, eds., 1971, Academic Press

The musks are a common illustration of compounds with different structures that all give similar odors. These may include tricyclic compounds, macrocyclic ketones and lactones, steroids, nitrocyclohexanes, indanes, tetrahydronaphthalenes, and acetophenoses. Small changes in the structure of these molecules may significantly change in potency but will not affect quality, since all are musky. There are also some compounds that have similar structures and very different odors, such as nootkatone and related compounds (Teranishi 1971). Nootkatone is a flavor compound from grapefruit oil. This compound and 1,10-dihydronootkatone have a grapefruity flavor (Fig. 7.28). Several other related compounds have a woody flavor. The odor character of stereoisomers may be quite different. The case of menthol has already been described. Only menthol isomers have peppermint aroma. The iso-, neo-, and neoisomenthols have an unpleasant musty flavor. Naves (1957) describes the difference between the *cis*- and *trans*- forms of 3-hexenol (CH_2OH–CH_2–CH = CH–CH_2CH_3). The *cis*-isomer has a fresh green odor, whereas the *trans*-isomer has a scent reminiscent of chrysanthemum. The 2-*trans*-6-*cis* nonadienal smells of cucumber and is quite different from the smell of the 2-*trans*-6-*trans* isomer (nonadienal, CHO–CH = CH–$(CH_2)_2$–CH = CH–CH_2-CH_3). Lengthening of the carbon chain may affect odorous properties. The odor of saturated acids changes remarkably as chain length increases. The lower fatty acids, especially butyric, have very intense and unpleasant flavors, because an increased chain length changes flavor character (Table 7.16) and lessens intensity. The fatty acids with 16 or 18 carbon atoms have only a faint flavor.

Another example is given by Kulka (1967). Gamma-nonalactone has a strong coconut-like flavor; γ-undecalactone has a peach aroma. As the chain length is increased by one more carbon atom, the flavor character becomes peach-musk. The lactones are compounds of widely differing structure and odor quality and are found as components of many food flavors. Gamma- and δ-lactones with 10–16 carbon atoms have been reported (Jurriens and Oelej 1965) as flavor components of butter, contributing to the butter flavor

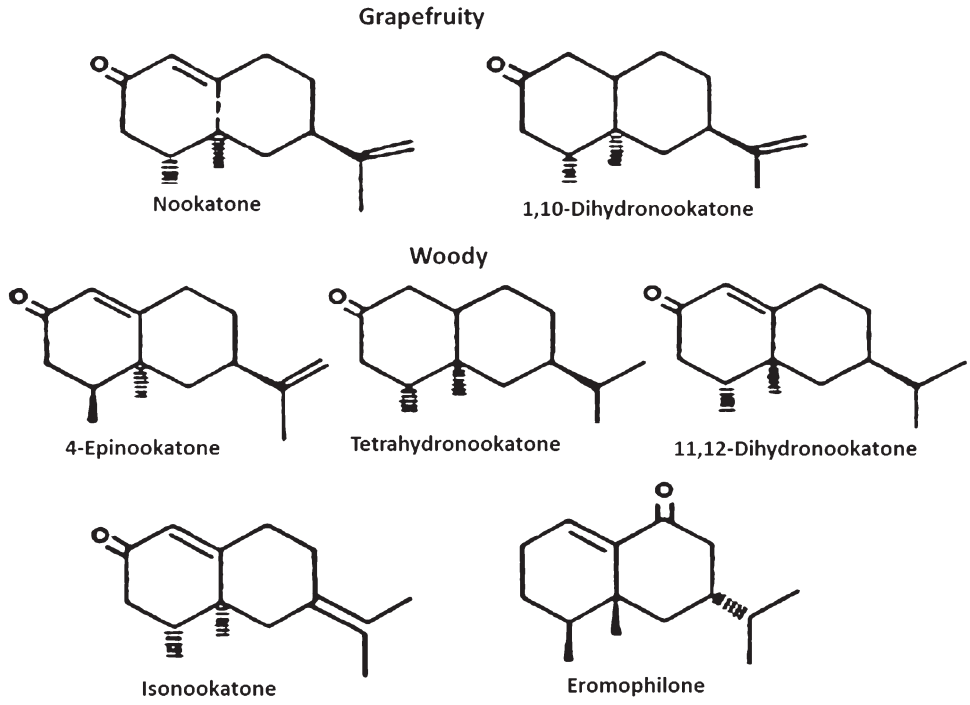


Fig. 7.28 Odor character of nootkatone and related compounds

Table 7.16 Flavor character of some N-carboxylic acids

Acid	Flavor character
Formic	Acid, pungent
Acetic	Acid, vinegary, pungent
Propionic	Acid, pungent, rancid, cheesy
Butyric	Acid, rancid
Hexanoic	Sweaty, goaty
Octanoic	Rancid
Decanoic	Waxy
Lauric	Tallowy
Myristic	Soapy, cardboard
Palmitic	Soapy

in concentrations of only parts per million. The flavor character and chemical structure of some γ-lactones as reported by Teranishi (1971) are shown in Fig. 7.29. One of these, the γ-lactone with a total chain length of 10 carbons, has peach flavor. The α-hydroxy-β-methyl-γ-carboxy-$\Delta^{\alpha-\beta}$-γ-hex-eno-lactone occurs in protein hydrolysate and has very strong odor and flavor of beef bouillon. Gold and Wilson (1963) found that the volatile flavor compounds of celery contain a number of phthalides (phthalides are lactones of phthalic acid, lactones are internal esters of hydroxy acids). These include the following:

- 3-isobutyliden-3a,4-dihydrophthalide (Fig. 7.30).
- 3-isovalidene-3a,4-dihydrophthalide.
- 3-isobutylidene phthalide.
- 3-isovalidene phthalide.

These compounds exhibit celery-like odors at levels of 0.1 ppm in water. Pyrazines have been identified as the compounds giving the characteristic intense odor of green peppers (Seifert et al. 1970). A number of pyrazine derivatives were tested and, within this single class of compounds, odor potencies showed a range of eight orders of magnitude equal to that of the widely varying compounds listed in Table 7.15. The compounds examined by Seifert et al. (1970) are listed in Table 7.17. 2-methoxy-3-isobutylpyrazine appears to be the compound responsible for the green pepper odor. Removal of the methoxy- or alkyl-groups reduces the odor potency by 10^5–

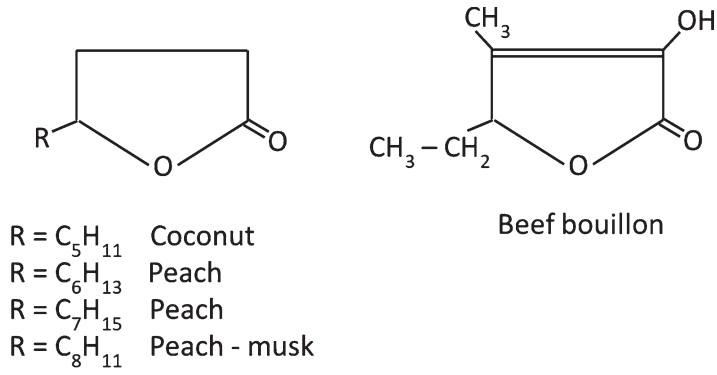


Fig. 7.29 Flavor character of some lactones. *Source*: From R. Teranishi, Odor and Molecular Structure, in *Gustation and Olfaction,* G. Ohloff and A.F. Thomas, eds., 1971, Academic Press

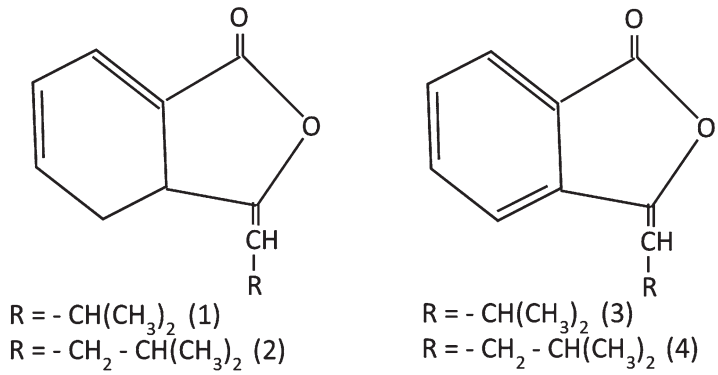


Fig. 7.30 Phthalides of celery volatiles

10^6 times, as is the case with 2-methoxypyrazine, 2-iosbutylpyrazine, and 2,5-dimethylpyrazine. Thus, small changes in molecular structure may greatly affect flavor potency. The odors of isobutyl, propyl, and hexyl methoxypyrazines are similar to that of green peppers. The isopropyl compound is moderately similar to peppers and its odor is somewhat similar to raw potato. The ethyl compound is even more similar to raw potato and less to pepper. In fact, this compound can be isolated from potatoes. The methyl compound has an odor like roasted peanuts. The structure of some of the pyrazines is shown in Fig. 7.31. Pyrazines have been identified as flavor components in a number of foods that are normally heated during processing. Rizzi (1967) demonstrated the presence of seven alkyl-substituted pyrazines in chocolate aroma. These were isolated by steam distillation, separated by gas-liquid chromatography, and identified by mass spectrometry. The components are methyl pyrazine; 2,3-dimethylpyrazine; 2-ethyl-5-methyl-pyrazine; trimethylpyrazine; 2,5-dimethyl-3-ethylpyrazine; 2,6-dimethyl-3-ethylpyrazine; and tetramethylpyrazine. Other researchers (Flament et al. 1967; Marion et al. 1967) have isolated these and other pyrazines from the aroma components of cocoa. Pyrazines are also aroma constituents of coffee. Goldman et al. (1967) isolated and identified 24 pyrazines and pyridines and revealed the presence of possibly 10 more. Bondarovich et al. (1967) isolated and identified a large number of pyrazines from coffee aroma and drew attention to the importance of pyrazines and dihydropyrazines to the flavor of roasted or otherwise cooked foods. These authors also drew attention to the instability of the dihydropyrazines. This instability not only makes their detection and isolation difficult, but may help explain why flavors such as that of

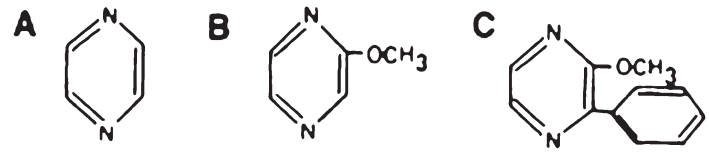


Fig. 7.31 (**a**) Pyrazine, (**b**) 2-methoxypyrazine, and (**c**) 2-methoxy-3-hexylpyrazine

Table 7.17 Odor threshold of pyrazine and derivatives

Compound	Odor threshold (parts per 10^{12} parts of water)
2-methoxy-3-hexylpyrazine	1
2-methoxy-3-isobutylpyrazine	2
2-methoxy-3-propylpyrazine	6
2-methoxy-3-isopropylpyrazine	2
2-methoxy-3-ethylpyrazine	400
2-methoxy-3-methylpyrazine	4000
2-methoxypyrazine	700,000
2-isobutylpyrazine	400,000
2–5-dimethylpyrazine	1,800,000
Pyrazine	175,000,000

Source: From R.M. Seifert et al., Synthesis of Some 2-Methoxy-3-Alkylpyrazines with Strong Bell Pepper–Like Odors, *J. Agric. Food Chem.,* Vol. 18, pp. 246–249, 1970, American Chemical Society

Table 7.18 Primary odors for humans and compounds eliciting these odors

Primary odor	Odor compounds
Camphoraceous	Borneol, *tert*-butyl alcohol *d*-camphor, cineol, pentamethyl ethyl alcohol
Pungent	Allyl alcohol, cyanogen, formaldehyde, formic acid, methylisothiocyanate
Ethereal	Acetylene, carbon tetrachloride, chloroform, ethylene dichloride, propyl alcohol
Floral	Benzyl acetate, geraniol, α-ionone, phenylethyl alcohol, terpineol
Pepperminty	*tert*-butylcarbinol, cyclohexanone, menthone, piperitol, 1,1,3-trimethyl-cyclo-5-hexanone
Musky	Androstan-3α-ol (strong), cyclohexadecanone, ethylene cebacate, 17- methylandrostan-3α-ol, pentadecanolactone
Putrid	Amylmercaptan, cadaverine, hydrogen sulfide, indole (when concentrated, floral when dilute), skatole

Source: From J.E. Amoore et al., The Stereochemical Theory of Odor, *Sci. Am.,* Vol. 210, No. 2, pp. 42–49, 1964

roasted coffee rapidly change with time. Another roasted product from which pyrazines have been isolated is peanuts. Mason et al. (1966) found methylpyrazine; 2,5-dimethylpyrazine; trimethylpyrazine; methylethylpyrazine; and dimethylethylpyrazine in the flavor of roasted peanuts. The pyrazines appear to be present in unprocessed as well as in heated foods. The thresholds of some pyrazines in water are found in Table 7.17.

Another group of compounds that have been related to the aroma of heated foods is the furanones. Teranishi (1971) summarized the findings on several of the furanones (see Fig. 7.24). The 4-hydroxy-2, 5-dimethyl-3-dihydrofuranone (1) has a caramel or burnt pineapple odor. The 4-hydroxy-5-methyl-3-dihydrofuranone (2) has a roasted chicory root odor. Both compounds may contribute to beef broth flavor. The 2,5-dimethyl-3-dihydrofuranone (3) has the odor of freshly baked bread. Isomaltol (4) and maltol (5) are products of the caramelization and pyrolysis of carbohydrates.

Amoore (Amoore et al. 1964; Amoore 1967) compared the various odor qualities that have been used to characterize odors and concluded that seven primary odors would suffice to cover them all: camphoraceous, pungent, ethereal, floral, pepperminty, musky, and putrid. Table 7.18 lists some of the chemical compounds that can be used to demonstrate these primary odors. The theory is based on the assumption that all odorous compounds have a distinctive molecular shape and size that fit into a socket on the receptor site. This would be similar to the "lock-and-key" concept of enzyme action.

The suggestion that odorous character is related to vibrational specificity of odor molecules has led to the vibrational theory of olfaction

(Wright 1957). Vibrational energy levels can be derived from the infrared or Raman spectra. The spectral area of greatest interest is that below 700 cm^{-1}, which is related to vibrations of chains and flexing or twisting of bonds between groups of atoms in the molecule. Wright and others have demonstrated that correlations exist between spectral properties and odor quality in a number of cases, but inconsistencies in other cases have yet to be explained.

Obviously, none of the many theories of olfaction proposed so far have been entirely satisfactory. It might be better to speak of hypotheses rather than of theories. Most of these theories deal with the explanation of odor quality and do not account for the quantitative aspects of the mechanism of olfaction. The classification of odor and the correlation of chemical structure and odor remain difficult to resolve.

Description of Food and Beverage Flavors

The flavor impression of a food is influenced by compounds that affect both taste and odor. The analysis and identification of many volatile flavor compounds in a large variety of food products have been assisted by the development of powerful analytical techniques. Gas-liquid chromatography was widely used in the early 1950s when commercial instruments became available. Introduction of the flame ionization detector increased sensitivity by a factor of 100 and, together with mass spectrometers, gave a method for rapid identification of many components in complex mixtures. These methods have been described by Teranishi et al. (1971). As a result, a great deal of information on volatile flavor components has been obtained in recent years for a variety of food products. The combination of gas chromatography and mass spectrometry can provide identification and quantitation of flavor compounds. However, when the flavor consists of many compounds, sometimes several hundred, it is impossible to evaluate a flavor from this information alone. It is then possible to use pattern recognition techniques to further describe the flavor. The pattern recognition method involves the application of computer analysis of complex mixtures of compounds. Computer multivariate analysis has been used for the detection of adulteration of orange juice and Spanish sherries (Maarse et al. 1987).

Flavors are often described by using the human senses on the basis of widely recognized taste and smell sensations. A proposed wine aroma description system is shown in Fig. 7.32 (Noble et al. 1987). Such systems attempt to provide an orderly and reliable basis for comparison of flavor descriptions by different tasters.

The aroma is divided into first-, second-, and third-tier terms, with the first-tier terms in the center. Examination of the descriptors in the aroma wheel shows that they can be divided into two types, flavors and off-flavors. Thus, it would be useful to divide the flavor wheel into two tables—one for flavors and one for off-flavors, as shown in Tables 7.19 and 7.20.

The difficulty in relating chemical composition and structure to the aroma of a food that contains a multitude of flavor compounds is evident from the work of Meyboom and Jongenotter (1981). They studied the flavor of straight-chain, unsaturated aldehydes as a function of double-bond position and geometry. Some of their results are presented in Table 7.21. Flavors of unsaturated aldehydes of different chain length and geometry may vary from bitter almond to lemon and cucumber when tasted separately.

A method of flavor description, developed by researchers at A.D. Little Inc. (Sjöström 1972), has been named the flavor profile method. The flavor profile method uses the recognition, description, and comparison of aroma and flavor by a trained panel of four to six people. Through training, the panel members are made familiar with the terminology used in describing flavor qualities. In addition to describing flavor quality, intensity values are assigned to each of the quality aspects. The intensity scale is threshold, slight, moderate, and strong, and these are represented by the symbols)(, 1, 2, and 3, respectively.

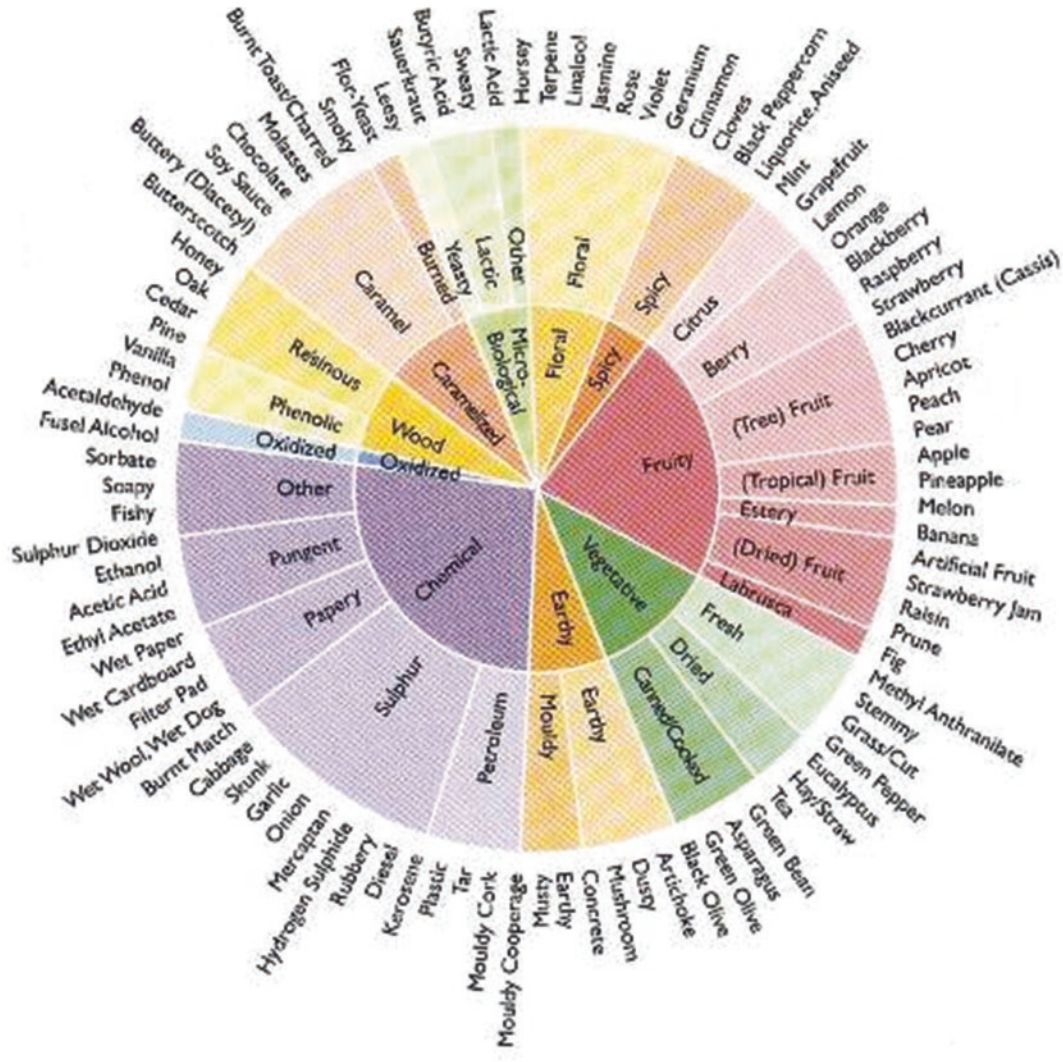


Fig. 7.32 Modified wine aroma wheel for the description of wine aroma. *Source*: From A.C. Noble et al., Modification of a Standardized System of Wine Aroma Terminology, *Am. J. Enol. Vitic*., Vol. 38, pp. 143–146, 1987, American Society of Enology and Viticulture

With the exception of threshold value, the units are ranges and can be more precisely defined by the use of reference standards. In the panel work, the evaluation of aroma is conducted first because odor notes can be overpowered when the food is eaten. This is followed by flavor analysis, called "flavor by mouth," a specialists' description of what a consumer would experience eating the food. Flavor analysis includes such factors as taste, aroma, feeling, and aftertaste. A sample flavor profile of margarine is given in Table 7.22.

Table 7.19 Aroma description of wine as listed in the aroma wheel, listing only the flavor contribution

First tier	Second tier	Third tier	First tier	Second tier	Third tier
Floral	Floral	Geranium		Canned/	Green beans
		Violet		cooked	Asparagus
		Rose			Green olive
		Orange blossom			Black olive
		Linalool			Artichoke
Spicy	Spicy	Licorice anise Black pepper Cloves		Dried	Hay/straw Tea Tobacco
Fruity	Citrus	Grapefruit Lemon	Nutty	Nutty	Walnut Hazelnut
	Berry	Blackberry			Almond
		Raspberry	Caramelized	Caramelized	Honey
		Strawberry			Butterscotch
		Black currant			Diacetyl (butter)
	Tree fruit	Cherry Apricot Peach			Soy sauce Chocolate Molasses
		Apple	Woody	Phenolic	Phenolic
	Tropical fruit	Pineapple			Vanilla
		Melon		Resinous	Cedar
		Banana			Oak
	Dried fruit	Strawberry jam Raisin Prune Fig		Burned	Smoky Burnt toast/charred Coffee
	Other	Artificial fruit Methyl anthranilate			
Vegetative	Fresh	Stemmy Grass, cut green Bell pepper Eucalyptus Mint			

Astringency

The sensation of astringency is considered to be related more to touch than to taste. Astringency causes a drying and puckering over the whole surface of the mouth and tongue. This sensation is caused by interaction of astringent compounds with proteins and glycoproteins in the mouth. Astringent compounds are present in fruits and beverages derived from fruit (such as juice, wine, and cider), in tea and cocoa, and in beverages matured in oak casks. Astringency is caused by tannins, either those present in the food or extracted from the wood of oak barrels. The astringent reaction involves a bonding to proteins in the mouth, followed by a physiological response. The astringent reaction has been found to occur between salivary proteins that are rich in proline (Luck et al. 1994). These proline-rich proteins (PRPs) have a high affinity for polyphenols. The effect of the structure of PRP is twofold: (1) proline causes the protein to have an open and flexible structure, and (2) the proline residue itself plays an important role in recognizing the polyphenols involved in the complex formation. The complex formation between PRP and polyphenol has been represented by Luck et al. (1994) in pictorial form (Fig. 7.33). The reaction is mediated by hydrophobic effects and hydrogen bonding on protein sites close to prolyl

Table 7.20 Aroma description of wine as listed in the aroma wheel, listing only the off-flavors

First tier	Second tier	Third tier
Earthy	Moldy	Moldy cork Musty (mildew)
	Earthy	Mushroom Dusty
Chemical	Petroleum	Diesel Kerosene Plastic Tar
	Sulfur	Wet wool, wet dog Sulfur dioxide Burnt match Cabbage Skunk Garlic Mercaptan Hydrogen sulfide Rubbery
	Papery	Wet cardboard Filterpad
	Pungent	Sulfur dioxide Ethanol Acetic acid Ethyl acetate
	Other	Fusel alcohol Sorbate soapy Fishy
Pungent	Cool	Menthol
	Hot	Alcohol
Oxidized	Oxidized	Acetaldehyde
Microbiological	Yeasty	Leesy Flor yeast
	Lactic	Lactic acid Sweaty Butyric acid Sauerkraut
	Other	Mousey Horsey

residues in the PRP. The resulting cross-linking, aggregation, and precipitation of the PRP causes the sensation of astringency.

Some anthocyanins are both bitter and astringent. Bitter compounds such as quinine and caffeine compete with the tannins in complexing with buccal proteins and thereby lower the astringent response. Astringency is caused by higher molecular weight tannins, whereas the lower molecular weight tannins up to tetrameres are associated with bitterness (Macheix et al. 1990).

Table 7.21 Flavor description of unsaturated aldehydes dissolved in paraffin oil

Aldehyde	Flavor description
trans-3-hexenal	Green, odor of pine tree needles
cis-3-hexenal	Green beans, tomato green
trans-2-heptenal	Bitter almonds
cis-6-heptenal	Green, melon
trans-2-octenal	Nutty
trans-5-octenal	Cucumber
cis-5-octenal	Cucumber
trans-2-nonenal	Starch, glue
trans-7-nonenal	Melon

Source: From P.W. Meyboom and G.A. Jongenotter, Flavor Perceptibility of Straight Chain, Unsaturated Aldehydes as a Function of Double Bond Position and Geometry, *J. Am. Oil Chem. Soc.,* Vol. 58, pp. 680–682, 1981

Table 7.22 Flavor profile of margarine

Aroma		Flavor by mouth	
Amplitude	2	Amplitude	2½
Sweet cream	½	Sweet cream	1½
Oil	)(	Oil	½
Sour	½	Salt	1½
Vanillin sweet	)(	Butter mouthfeel	2
		Sour	1

Note:)(= threshold; 1 = slight; 2 = moderate; 3 = strong.
Source: Reprinted with permission from L.B. Sjöström, *The Flavor Profile*, © 1972, A.D. Little, Inc.

Flavor and Off-Flavor

It is impossible to deal with the subject of flavor without considering off-flavors. In many cases, the same chemical compounds are involved in both flavors and off-flavors. The only distinction appears to be whether a flavor is judged to be pleasant or unpleasant. This amounts to a personal judgment, although many unpleasant flavors (or off-flavors) are universally found to be unpleasant. A distinction is sometimes made between off-flavors—defined as unpleasant odors or flavors imparted to food through internal deteriorative change—and taints—defined as unpleasant odors or flavors imparted to food through external sources (Saxby 1996). Off-flavors in animal products, meat and milk, may be caused by transfer of substances from feed. Off-flavors in otherwise sound foods can be caused by heat, oxidation, light, or enzymic

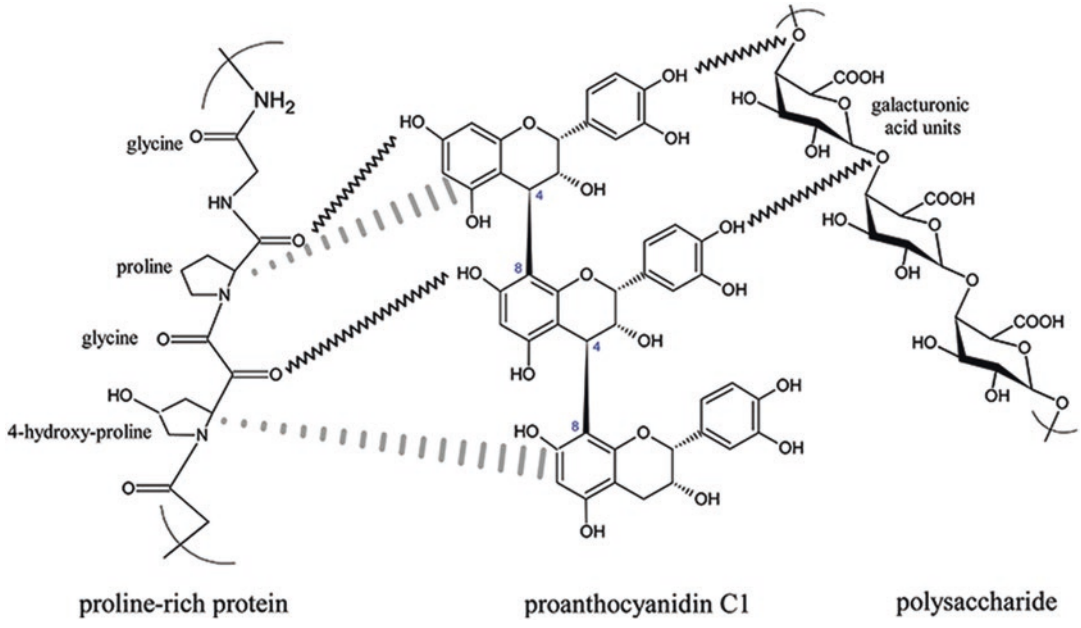


Fig. 7.33 Complex formation between proline-rich proteins and polyphenols L. Federico Casassa (2017). Flavonoid Phenolics in Red Winemaking, Phenolic Compounds - Natural Sources, Importance and Applications, Prof. Marcos Soto-Hernández (Ed.), InTech, DOI: https://doi.org/10.5772/67452. Available from: https://www.intechopen.com/books/phenolic-compounds-natural-sources-importance-and-applications/flavonoid-phenolic

action. The perception of taste and flavor can be defined for a given group of people by the International Standards Organization (ISO) 5492 standard (ISO 1992) as follows: The odor or taste threshold is the lowest concentration of a compound detectable by a certain proportion (usually 50%) of a given group of people. A graphic representation of this relationship has been given by Saxby (1996). The graph in Fig. 7.34 relates the percentage of people within a given group to the ability to detect a substance at varying concentrations. Of the population, 50% can detect the compound at the concentration of one unit. At a concentration of the compound 10 times greater than the mean threshold, about 10% of the population is still not able to detect it. At the other end of the spectrum, 5% of the population can still detect the compound at a concentration 10 times less than the mean threshold. These findings have important consequences for the presence of compounds causing off-flavors. Even very low levels of a chemical that produces off-flavors may cause a significant number of people to complain.

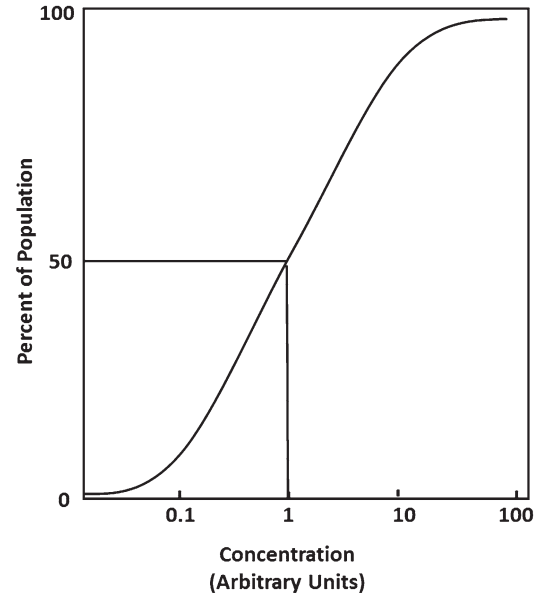


Fig. 7.34 Variation of taste threshold within a given population. *Adapted* from M.J. Saxby, *Food Taints and Off-Flavors,* p. 43, © 1996, Aspen Publishers, Inc.

Certain flavor compounds may appear quite pleasant in one case and extremely unpleasant in another. Many examples of this can be cited. One of the well-known cases is that of short-chain free fatty acids in certain dairy products. Many cheese flavors contain volatile fatty acids as flavor contributors (Day 1967). Yet, the same fatty acids in very low concentrations in milk and other dairy products cause a very unpleasant, rancid off-flavor (Patton 1964). Forss (1969) has drawn attention to the compound non-2-enal. During studies of dairy product off-flavor, this compound was isolated as a component of the oxidation off-flavor and was found to have an odor reminiscent of cucumbers. The same compound was isolated from cucumbers, and the cucumber-like flavor was assigned to the molecular structure of a 2-*trans*-enal with 9 or 10 carbon atoms. Further unsaturation and conjugation to give a 2,4-dienal produces flavors reminiscent of cardboard or linoleum. Lactones were isolated by Keeney and Patton (1956) and Tharp and Patton (1960) and were considered to be the cause of stale off-flavors in certain dairy products. The same lactones, including δ-decalactone and δ-dodecalactone, were subsequently recognized as contributors to the pleasant aroma of butter (Day 1966). Dimethylsulfide is a component of the agreeable aroma of meat and fish but has also been found to cause an off-flavor in canned salmon (Tarr 1966). Acetaldehyde occurs naturally in many foods, especially fruits, and is reported to be essential for imparting the taste of freshness (Byrne and Sherman 1984). The same compound is responsible for a very unpleasant oxidized flavor in wine. Sinki (1988) has discussed the problems involved in creating a universally acceptable taste, and has stated that most individual flavor chemicals are either repugnant or painful outside their proper formulations. This complex interaction between flavor chemicals, and between flavors and the individual, makes the creation of a flavorful product both a science and an art, according to Sinki. The subject of pleasantness and unpleasantness of flavors is the basis of a chapter in *Odour Description and Odour Classification* by Harper et al. (1968) and is the main subject of Moncrieff's *Odour Preferences* (1966).

Flavor of Some Foods

As indicated previously, the two main factors affecting flavor are taste and odor. In a general way, food flavors can be divided into two groups. The first consists of foods whose flavor cannot be attributed to one or a few outstanding flavor notes; their flavor is the result of the complex interaction of a variety of taste and odor components. Examples include bread, meat, and cheese. The second group consists of foods in which the flavor can be related to one or a few easily recognized components (contributory flavor compounds). Examples include certain fruits, vegetables, and spices. Another way of differentiating food flavors is by considering one group in which the flavor compounds are naturally present and another group in which the flavor compounds are produced by processing methods.

Bread

The flavor of white bread is formed mainly from the fermentation and baking processes. Freshly baked bread has a delightful aroma that is rapidly lost on cooling and storage. It has been suggested that this loss of flavor is the result of disappearance of volatile flavor components. However, it is well known that the aroma may be at least partially regenerated by simply heating the bread. Schoch (1965) suggested that volatile flavor compounds may become locked in by the linear fraction of wheat starch. The change in texture upon aging may be a contributory factor in the loss of flavor. During fermentation, a number of alcohols are formed, including ethanol, *n*-propanol, isoamyl and amyl alcohol, isobutyl alcohol, and β-phenol alcohol. The importance of the alcohols to bread flavor is a matter of controversy. Much of the alcohols are lost to the oven air during baking. A large number of organic acids are also formed (Johnson et al. 1966). These include many of the odd and even carbon number saturated aliphatic acids, from formic to capric, as well as lactic, succinic, pyruvic, hydrocinnamic, benzilic, itaconic, and levulinic acid. A large number of carbonyl compounds have been

identified in bread, and these are believed to be important flavor components. Johnson et al. (1966) list the carbonyl compounds isolated by various workers from bread; this list includes 14 aldehydes and 6 ketones. In white bread made with glucose, the prevalent carbonyl compound is hydroxymethylfurfural (Linko et al. 1962). The formation of the crust and browning during baking appear to be primary contributors to bread flavor. The browning is mainly the result of a Maillard-type browning reaction rather than caramelization. This accounts for the presence of the carbonyl compounds, especially furfural, hydroxymethylfurfural, and other aldehydes. In the Maillard reaction, the amino acids are transformed into aldehydes with one less carbon atom. Specific aldehydes can thus be formed in bread crust if the necessary amino acids are present. The formation of aldehydes in bread crust is accompanied by a lowering of the amino acid content compared to that in the crumb. Johnson et al. (1966) have listed the aldehydes that can be formed from amino acids in bread crust as a result of the Strecker degradation (Table 7.23).

Grosch and Schieberle (1991) reported the aroma of wheat bread to include ethanol, 2-methylpropanal, 3-methylbutanal, 2,3-butanedione, and 3-methylbutanol. These compounds contribute significantly to bread aroma, whereas other compounds are of minor importance.

Table 7.23 Aldehydes that can be formed from amino acids in bread crust as a result of the strecker degradation

Amino acid	Aldehyde
Alanine	Acetaldehyde
Glycine	Formaldehyde
Isoleucine	2-Methylbutanal
Leucine	Isovaleraldehyde
Methionine	Methional
Phenylalanine	Phenylacetaldehyde
Threonine	2-Hydroxypropanal
Serine	Glyoxal

Source: From J.A. Johnson et al., Chemistry of Bread Flavor, in *Flavor Chemistry,* 1. Hornstein, ed., 1966, American Chemical Society

Meat

Meat is another food in which the flavor is developed by heating from precursors present in the meat; this occurs in a Maillard-type browning reaction. The overall flavor impression is the result of the presence of a large number of nonvolatile compounds and the volatiles produced during heating. The contribution of nonvolatile compounds in meat flavor has been summarized by Solms (1971). Meat extracts contain a large number of amino acids, peptides, nucleotides, acids, and sugars. The presence of relatively large amounts of inosine-5′-monophosphate has been the reason for considering this compound as a basic flavor component. In combination with other compounds, this nucleotide would be responsible for the meaty taste. Living muscle contains adenosine-5′-triphosphate; this is converted after slaughter into adenosine-5′-monophosphate, which is deaminated to form inosine-5′-monophosphate (Jones 1969). The volatile compounds produced on heating can be accounted for by reactions involving amino acids and sugars present in meat extract. Lean beef, pork, and lamb are surprisingly similar in flavor; this reflects the similarity in composition of extracts in terms of amino acid and sugar components. The fats of these different species may account for some of the normal differences in flavor. In the volatile fractions of meat aroma, hydrogen sulfide and methyl mercaptan have been found; these may be important contributors to meat flavor. Other volatiles that have been isolated include a variety of carbonyls such as acetaldehyde, propionaldehyde, 2-methylpropanal, 3-methylbutanal, acetone, 2-butanone, *n*-hexanal, and 3-methyl-2-butanone (Moody 1983).

Fish

Fish contains sugars and amino acids that may be involved in Maillard-type reactions during heat processing (canning). Proline is a prominent amino acid in fish and may contribute to sweetness.

The sugars ribose, glucose, and glucose-6-phosphate are flavor contributors, as is 5′-inosinic acid, which contributes a meaty flavor note. Volatile sulfur compounds contribute to the flavor of fish; hydrogen sulfide, methylmercaptan, and dimethylsulfide may contribute to the aroma of fish. Tarr (1966) described an off-flavor problem in canned salmon that is related to dimethylsulfide. The salmon was found to feed on zooplankton containing large amounts of dimethyl-2-carboxyethyl sulfonium chloride. This compound became part of the liver and flesh of the salmon and in canning degraded to dimethylsulfide according to the following equation:

$$\left(CH_3\right)_2 - SH - CH_2 - CH_2 - COOH \rightarrow \left(CH_3\right)_2 S + CH_3 - CH_2 - COOH$$

The flavor of cooked, fresh fish is caused by the presence of sugars, including glucose and fructose, giving a sweet impression as well as a umami component arising from the synergism between inosine monophosphate and free amino acids. The fresh flavor of fish is rapidly lost by bacterial spoilage. In fresh fish, a small amount of free ammonia, which has a pH level of below seven, exists in protonated form. As spoilage increases, the pH rises and ammonia is released. The main source of ammonia is trimethylamine, produced as a degradation product of trimethyl-amineoxide.

The taste-producing properties of hypoxanthine and histidine in fish have been described by Konosu (1979). 5′-inosinate accumulates in fish muscle as a postmortem degradation product of ATP. The inosinate slowly degrades into hypoxanthine, which has a strong bitter taste. Some kinds of fish, such as tuna and mackerel, contain very high levels of free histidine, which has been postulated to contribute to the flavor of these fish.

Milk

The flavor of normal fresh milk is probably produced by the cow's metabolism and is comprised of free fatty acids, carbonyl compounds, alkanols, and sulfur compounds. Free fatty acids may result from the action of milk lipase or bacterial lipase. Other decomposition products of lipids may be produced by the action of heat. In addition to lipids, proteins and lactose may be precursors of flavor compounds in milk (Badings 1991). Sulfur compounds that can be formed by heat from β-lactoglobulin include dimethyl sulfide, hydrogen sulfide, dimethyl disulfide, and methanethiol. Some of these sulfur compounds are also produced from methionine when milk is exposed to light. Heterocyclic compounds are produced by nonenzymatic browning reactions. Bitter peptides can be formed by milk or bacterial proteinases.

The basic taste of milk is very bland, slightly sweet, and salty. Processing conditions influence flavor profiles. The extent of heat treatment determines the type of flavor produced. Low heat treatment produces traces of hydrogen sulfide. Ultra-high temperature treatment results in a slight fruity, ketone-like flavor. Sterilization results in strong ketone-like and caramelization/sterilization flavors. Sterilization flavors of milk are caused by the presence of 2-alkanones and heterocyclic compounds resulting from the Maillard reaction. Because of the bland flavor of milk, it is relatively easy for off-flavors to take over.

Cheese

The flavor of cheese largely results from the fermentation process that is common to most varieties of cheese. The microorganisms used as cultures in the manufacture of cheese act on many of the milk components and produce a large variety of metabolites. Depending on the type of culture used and the duration of the ripening process, the cheese may vary in flavor from mild to extremely powerful. Casein, the main protein in cheese, is hydrolyzed in a pattem and at a rate that is characteristic for each type of cheese. Proteolytic enzymes produce a range of peptides of specific composition that are related to the specificity of the enzymes present. Under certain conditions bitter peptides may be

formed, which produce an off-flavor. Continued hydrolysis yields amino acids. The range of peptides and amino acids provides a "brothy" taste background to the aroma of cheese. Some of these compounds may function as flavor enhancers. Breakdown of the lipids is essential for the production of cheese aroma since cheese made from skim milk never develops the full aroma of normal cheese. The lipases elaborated by the culture organisms hydrolyze the triglycerides to form fatty acids and partial glycerides. The particular flavor of some Italian cheeses can be enhanced by adding enzymes during the cheese-making process that cause preferential hydrolysis of short-chain fatty acids. Apparently, a variety of minor components are important in producing the characteristic flavor of cheese. Carbonyls, esters, and sulfur compounds are included in this group. The relative importance of many of these constituents is still uncertain. Sulfur compounds found in cheese include hydrogen sulfide, dimethylsulfide, methional, and methyl mercaptan. All of these compounds are derived from sulfur-containing amino acids. The flavor of blue cheese is mainly the result of the presence of a number of methyl ketones with odd carbon numbers ranging in chain length from 3 to 15 carbons (Day 1967). The most important of these are 2-heptanone and 2-nonanone. The methyl ketones are formed by β-oxidation of fatty acids by the spores of *P. roqueforti.*

Fruits

The flavor of many fruits appears to be a combination of a delicate balance of sweet and sour taste and the odor of a number of volatile compounds. The characteristic flavor of citrus products is largely due to essential oils contained in the peel. The essential oil of citrus fruits contains a group of terpenes and sesquiterpenes and a group of oxygenated compounds. Only the latter are important as contributors to the citrus flavor. The volatile oil of orange juice was found to be 91.6 mg per kg, of which 88.4 was hydrocarbons (Kefford 1959). The volatile water-soluble constituents of orange juice consist mainly of acetaldehyde, ethanol, methanol, and acetic acid. The hydrocarbons include mainly D-limonene, β-myrcene, and a compound of composition $C_{15}H_{24}$. The esters include isovalerate, methyl alphaethyl-*n*-caproate, citronellyl acetate, and terpinyl acetate. In the group of carbonyls, the following compounds were identified: *n*-hexanal, *n*-octanal, *n*-decanal, and citronella; and in the group of alcohols, linalool, α-terpineol, *n*-hexane-1-ol, *n*-octan-1-ol, *n*-decan-1-ol, and 3-hexen-1-ol were identified. The flavor deterioration of canned orange juice during storage results in stale off-flavors. This is due to reactions of the nonvolatile water-soluble constituents. As in the case of citrus fruits, no single compound is completely responsible for any single fruit aroma. However, some organoleptically important compounds characteristic for particular fruits have been found. These include amyl esters in banana aroma, citral in lemon, and lactones in peaches. The major flavor component of Bartlett pears was identified by Jennings and Sevenants (1964) as ethyl *trans*-2-*cis*-4-decadienoate.

Vegetables

Vegetables contain an extensive array of volatile flavor compounds, either in original form or produced by enzyme action from precursors. Maarse (1991) has reviewed these in detail. Onion and garlic have distinctive and pungent aromas that result mostly from the presence of sulfur-containing compounds. A large number of flavor compounds in vegetables are formed after cooking or frying. In raw onions, an important compound is thiopropanal *s*-oxide—the lachrymatory factor. The distinctive odor of freshly cut onions involves two main compounds, propyl methane-thiosulfonate and propyl propanethiosulfonate. Raw garlic contains virtually exclusively sulfur compounds: four thiols, three sulfides, seven disulfides, three trisulfides, and six dialkylthio-sulfinates.

Tea

The flavor of black tea is the result of a number of compounds formed during the processing of green tea leaves. The processing involves wither-

ing, fermentation, and firing. Bokuchava and Skobeleva (1969) indicate that the formation of the aroma occurs mainly during firing. Aromatic compounds isolated and identified from black tea include acrolein, *n*-butyric aldehyde, ethanol, *n*-butanol, isobutanol, hexanal, pentanal, 2-hexanol, 3-hexen-1-ol, benzaldehyde, linalool, terpeneol, methylsalicylate, benzyl alcohol, β-phenylethanol, isobutyric aldehyde, geraniol, and acetophenone. The flavor substances of tea can be divided into the following four fractions: a carbonyl-free neutral fraction including a number of alcohols, a carbonyl fraction, a carboxylic acid fraction, and a phenolic fraction. A compilation (Maarse 1991) identifies a total of 467 flavor constituents in tea. The distinctive flavor of tea is due to its content of lactones, aldehydes, alcohols, acids, and pyridines.

Coffee

The flavor of coffee is developed during the roasting of the green coffee bean. Gas-liquid chromatography can be used to demonstrate (Fig. 7.35) the development of volatile constituents in increasing amounts as intensity of roasting increases (Gianturco 1967). The total number of volatile compounds that have been isolated is in the hundreds, and many of these have been identified. To determine the flavor contribution of each of these is a Herculean task. Many compounds result from the pyrolytic decomposition

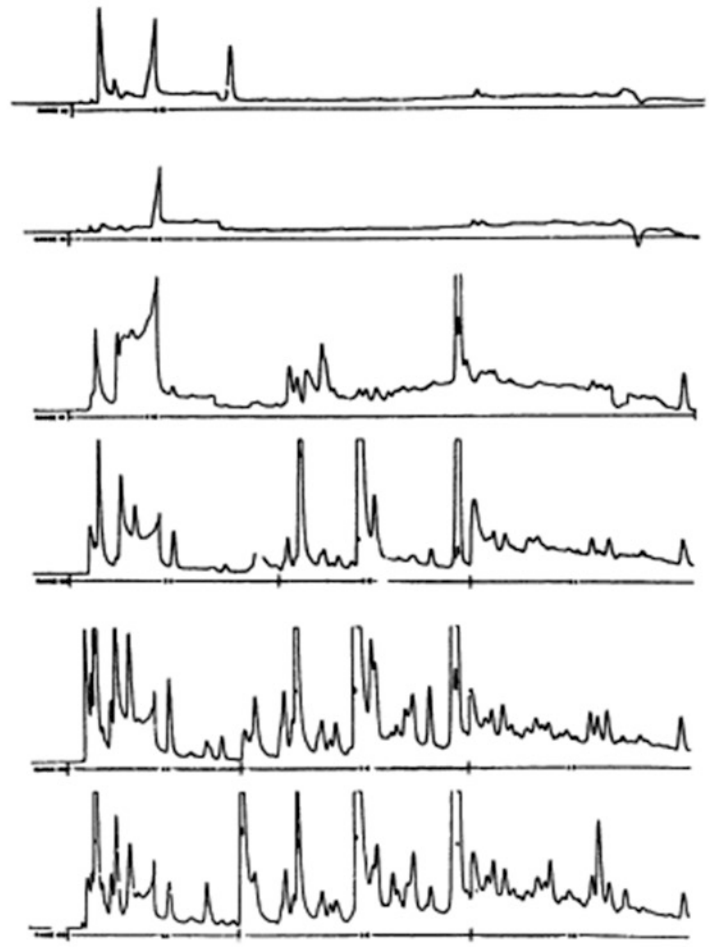


Fig. 7.35 Development of volatile constituents during roasting of coffee. From top to bottom: green coffee after 2, 6, 8, 11, and 15 min of roasting. The gas chromatograms show increasing concentrations of volatile compounds. *Source:* From M.A. Gianturco, Coffee Flavor, in *Symposium on Foods: The Chemistry and Physiology of Flavors*, H.W. Schultz et al., eds., 1967, AVI Publishing Co.

of carbohydrates into units of 2, 3, 4, or 5 carbons. Other compounds of carbohydrate origin are 16 furanic compounds, cyclic diketones, and maltol. Roasting of the proteins of the coffee bean can yield low molecular weight products such as amino acids, ammonia, amines, hydrogen sulfide, methyl mercaptan, dimethylsulfide, and dimethyl disulfide. A series of furanic and pyrrolic compounds identified include the following: furan, furfural, acetylfuran, 5-methylfuran, 5-methylfurfural, 5-methyl-2-acetylfuran and pyrrole, 2-pyrrolaldehyde, 2-acetylpyrrole, *N*-methylpyrrole, *N*-methyl-2-pyrrolaldehyde, and *N*-methyl-2-acetylpyrrole. Differences in the aroma of different coffees can be related to quantitative differences in some of the compounds isolated by gas chromatography, and ratios and amounts of these compounds may be different. Pyrazines, furanes, pyrroles, and thiophen derivatives are particularly abundant in coffee aroma. Furfuryl-methyl-sulfide and its homologs are important contributors to the aroma of coffee. The structures of some of the important aroma contributors are presented in Fig. 7.36. The compounds identified in coffee aroma are listed and differentiated on the basis of functional groups in Table 7.24. It is, of course, impossible to compare the aroma of different coffees on the basis of one or a few of the flavor constituents. Computer-generated histograms can be used for comparisons after selection of important regions of gas-liquid chromatograms by using mathematical treatments. Biggers et al. (1969) differentiated the beverage quality of two varieties of coffee (arabica and robusta) on the basis of contributions of flavor compounds.

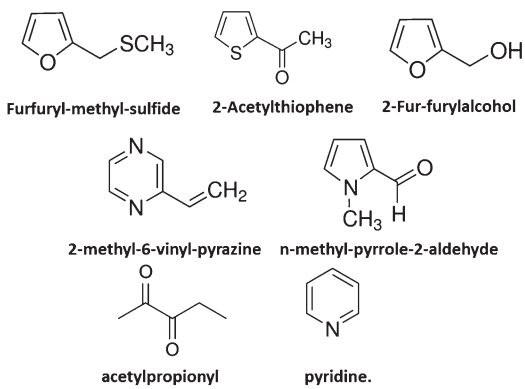


Fig. 7.36 Structure of some important constituents of the aroma of coffee

Recent studies have identified 655 compounds in the flavor of coffee, the principal ones being furans, pyrazines, pyrroles, and ketones (Maarse 1991). The distinctiveness of coffee flavor is related to the fact that it contains a large percentage of thiophenes, furans, pyrroles, as well as oxazoles, thiazoles, and phenols.

Alcoholic Beverages

In distilled beverages, one of the major flavor compounds is acetaldehyde. Acetaldehyde represents about 90% of the total aldehydes present in beverages like whiskey, cognac, and rum. Together with other short-chain aliphatic aldehydes, it produces a pungent odor and sharp flavor, which is masked by other flavor components in cognac, fruit brandies, rum, and whiskey. In vodka the presence of acetaldehyde may result in an off-flavor. Propanol and 2-methylpropanol, as well as unsaturated aldehydes, are also present in distilled beverages. The aldehydes are very reactive and can form acetals by reacting with ethanol. This reaction results in a smoother flavor profile. Another important flavor compound in distilled beverages is the diketone, 2,3-butanedione (diacetyl), which is a product of fermentation. Depending on fermentation and distillation conditions, the level of diacetyl varies widely in different beverages.

Fusel alcohols, which are present in most distilled beverages, influence flavor. They are formed during fermentation from amino acids through decarboxylation and deamination, and include 1-propanol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol, and 2-phenylethanol.

Distilled beverages also contain fatty acids—from acetic acid (which is one of the major fatty acids) to long-chain unsaturated fatty acids.

Maturation in oak barrels has a major effect on flavor of distilled beverages. Maturing fresh distillates in oak barrels can transform a raw-tasting

Table 7.24 Volatile compounds in roasted coffee aroma

Compound Type	None	—OH	—O—		Functional Group			—S—	Other
					C=O	—CO–CO—			
Aliphatic	17	19	—	13	30	10	16	9	25
Isocyclic	3	1	—	—	6	6	—	—	—
Benzenic	20	—	6	4	5	1	5	2	16
Furanic	15	1	4	3	13	4	11	7	3
Thiophenic	6	1	—	2	6	2	3	20	—
Pyrrolic	10	—	—	8	5	1	—	—	—
Pyrazinic	27	—	—	—	—	—	—	—	—
Other	5	2	8	—	7	—	1	5	13
Total number	103	24	18	30	72	24	36	43	57

product into a mellow, well-rounded beverage. The reactions that take place during maturation involve reactions between components of the distillate and reactions between distillate components and compounds present in the oak wood. The alcoholic solution in the barrel extracts lignin from the oak to form an alcohol-soluble ethanol-lignin. Alcoholysis converts this to coniferic alcohol and then by oxidation to coniferaldehyde. Similarly, sinapic alcohol is converted to sinapaldehyde. These aldehydes then produce syringaldehyde and vanillin. The latter compound is important in the flavor of cognac and whiskey. A similar process occurs in the aging of wines in oak barrels to produce the distinctive smoothness of oak-aged wines.

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Texture

8

Harry Levine and John W. Finley

Introduction

Texture is one of the key attributes of foods, which is used to define product quality and acceptability. Food texture can be defined by the way in which the various constituents and structural elements are arranged and combined into a micro- and macrostructure, and by the external manifestations of this structure, in terms of flow and deformation. Texture measurements are used throughout the food value chain to monitor and control quality, from harvest to assessing the impacts of postharvest handling and processing on shelf life and consumer acceptance. Postharvest handling and processing conditions such as storage temperature can have a significant influence on, e.g., the textural properties of meat (Farag et al. 2009), apple quality and acceptability (Konopacka and Plocharski 2004), and storage and ripening of cut tomatoes (Lana et al. 2005). Addition of new ingredients, replacement of existing ones, or changes in processing conditions can result in both desirable and/or undesirable changes in texture. For example, added whey proteins and carbohydrate polymers alter food microstructure (Foegeding et al. 2010), added inulin and fructo-oligosaccharides alter bread texture (Morris and Morris 2012), predrying alters texture and oil uptake in potato chips (Pedreschia and Moyano 2005), frying conditions alter crispness of crackers (Saeleaw and Schleining 2011), and fat replacement alters biscuit quality (Sudha et al. 2007). The texture characteristics of foods can be evaluated by descriptive sensory (subjective) or instrumental (objective) analyses. Sensory analysis can be time-consuming and expensive; consequently, many empirical mechanical tests have been developed, the results of which correlate with the sensory analysis of food texture.

Food texture can be defined as "all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory receptors" (Lawless and Heymann 1998). Most of our foods are complex physico-chemical structures, and, as a result, the physical properties cover a wide range—from fluid, Newtonian materials to the most complex disperse systems with semi-solid character. There is a direct relationship between the chemical composition of a food, its physical structure, and the resulting textural properties. Chemical composition determines the basic physical structure, and this in turn influences the texture of the food, whether measured mechanically or by sensory evaluation. These interrelationships, with examples of testing methods, are illustrated in Fig. 8.1. Food texture can be evaluated by a range of mechanical tests (instrumental methods) or by sensory analysis. In the latter case, we use the human sense organs as analytical tools. A proper

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_8

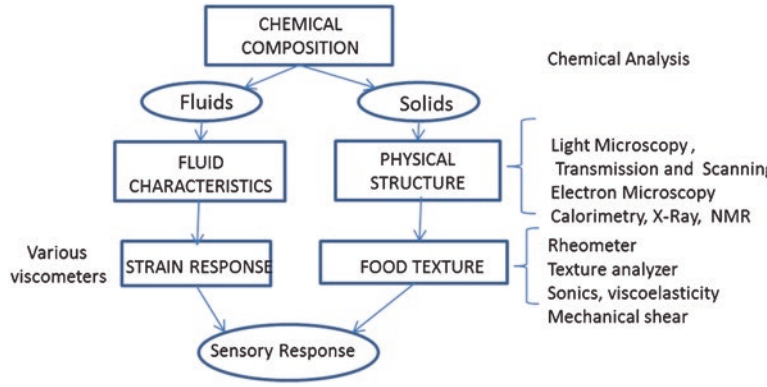


Fig. 8.1 The interrelationships of chemical composition, physical structure, and the texture characteristics of a food. Each area corresponds to a range of applicable measurement methods. Such test results are then related to those from the sensory evaluation of the food

understanding of textural properties often requires study of the physical structure. This is most often accomplished by light and/or electron microscopy, as well as by several other physical methods. X-ray diffraction analysis provides information about crystalline structure, differential scanning calorimetry provides information about melting, solidification, and other phase or state transitions, and particle size analysis and sedimentation methods provide information about particle size distribution and particle shape.

A discussion of food texture can be divided into two parts: fluids and solids. Chemical composition and chemical interactions ultimately drive the characteristics of both solids and fluids. Different testing techniques are employed to obtain objective data to help explain the sensory responses to foods. Figure 8.1 illustrates the different approaches used to define physical parameters.

In the study of food texture, attention is given to two interdependent areas: the flow and deformation properties and the macro- and microstructure. The study of food texture is carried out for three important reasons:

1. to evaluate the resistance of products against mechanical action, such as in mechanical harvesting of fruits and vegetables;
2. to determine the flow properties of products during processing, handling, and storage;
3. to understand the mechanical behavior of a food when consumed.

Most foods are complex systems, in which many textural characteristics are experienced simultaneously when we eat. Examples include sandwich cookies (with a viscoelastic filling and a brittle base cake), vegetables with cream sauce, and macaroni and cheese. Such multi-texture systems present great difficulty in developing suitable mechanical testing methods. Generally, the different phases must be considered separately. As a result, it is difficult in many cases to relate results obtained by instrumental measurement techniques to the types of responses obtained by sensory panel tests.

Fluids

Fluid dynamics are best described as the flow and deformation of a fluid under stress. Viscosity (η) is a measure of the resistance to flow of a fluid under stress. The higher the viscosity of a fluid, the generally greater is the resistance to flow. Fluids can be grouped into Newtonian and non-Newtonian fluids. In 1687, Sir Isaac Newton developed his three laws of motion:

1. A body continues in its state of rest or uniform motion in a straight line, unless it is acted upon by another force.
2. The rate of change in momentum is proportional to the applied force and in the direction of the applied force.
3. For every action, there is an equal and opposite reaction.

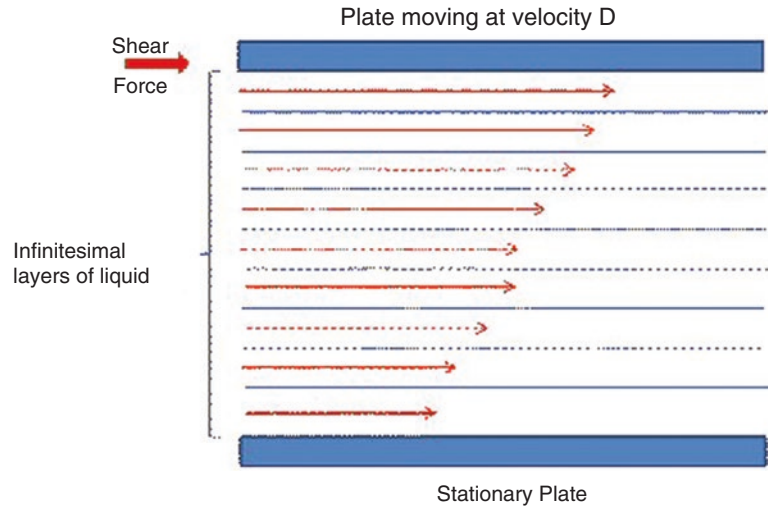


Fig. 8.2 The effect of shear force exerted by a moving plate on the flow of a fluid at increasing distances from the surface where the force is applied

The application with relevance to fluids is illustrated in Fig. 8.2. This model illustrates the shear force exerted by a moving plate, against the infinitesimal layers of liquid between the moving plate at velocity v and a stationary plate. It can be seen that, the further from the moving plate, the less the liquid moves with the force.

Consider a liquid contained between two parallel plates, each of area A cm^2 (Fig. 8.2). The plates are h cm apart, and a force of P dynes is applied horizontally to the upper plate. This shearing stress causes it to move, with respect to the lower plate, with a velocity of v cm s^{-1}. The shearing stress τ acts throughout the liquid contained between the plates, and can be defined as the shearing force P divided by the area A, or P/A dynes/cm^2. The deformation can be expressed as the mean rate of shear $\dot{\gamma}$ or the velocity gradient, and is equal to the velocity difference divided by the distance between the plates, $\dot{\gamma} = v / h$, expressed in units of s^{-1}.

In Newtonian flow, it is assumed that, at a given temperature, and for all such materials, viscosity is independent of shear rate. In such systems, twice the force would cause the fluid to move twice as fast. Shear is the movement of the fluid relative to the parallel surface, shear stress is the force per unit area to cause flow, and shear rate is the difference in flow between two layers of liquid, as illustrated by the arrows in Fig. 8.2.

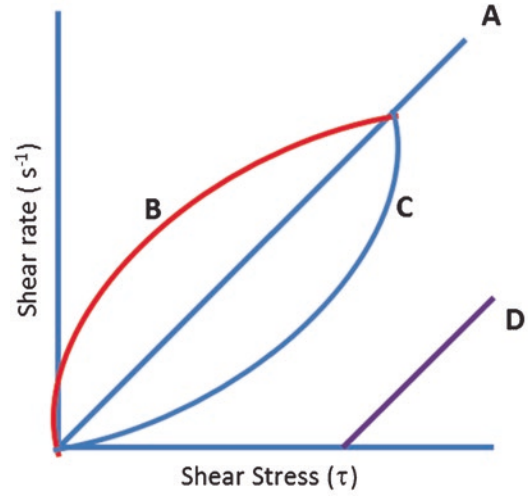


Fig. 8.3 Shear stress-shear rate diagrams: (*A*) Newtonian liquid, viscous flow; (*B*) dilatant flow; (*C*) pseudoplastic flow; (*D*) plastic flow

The relationship between shear stress and shear rate can be used to define the flow properties of materials. In the simplest case, the shear stress is directly proportional to the mean shear rate, $\tau = \eta\dot{\gamma}$ (Fig. 8.3). The proportionality constant η is called the viscosity coefficient, or dynamic viscosity, or simply the viscosity of the liquid. The metric unit of viscosity is the dyne.s cm^{-2}, or Poise (P). The commonly used unit is 100 times smaller than P, and called centiPoise (cP).

Table 8.1 Viscosity coefficients of some foods

Product	Temperature (°C)	Viscosity (cP)
Water	0	1.79
Water	20	1.00
Beer	4	1.1
Milk, whole	18	2.0
Milk, whole	49	10
Cream (30% fat)	16	14
Cream (50% fat)	32	55
Soybean oil	24	60
Soybean oil	80	12
Sucrose solution (60%)	21	60.2
Olive oil	30	84.0
Cottonseed oil	16	91.0
Mayonnaise	21	20,000
Molasses	21	1400–13,000

In the SI system, η is expressed in N.s/m^2, or Pa.s. Therefore, 1 Pa.s = 10 P = 1000 cP. Some instruments measure kinematic viscosity, which is equal to dynamic viscosity × density and is expressed in units of Stokes. The viscosity of water at room temperature is about 1.0 cP. Table 8.1 lists the absolute viscosities for a range of fluid food materials, expressed in centipoise.

Materials that exhibit a direct proportionality between shear stress and shear rate are called Newtonian fluids. These include water and aqueous solutions, simple organic liquids, and dilute suspensions and emulsions. In contrast, most foods are non-Newtonian in character, and their shear stress–shear rate curves are either not straight lines, or do not go through the origin, or both. This introduces a considerable difficulty, because their flow behavior cannot be expressed by a single value, as is the case for Newtonian liquids.

In non-Newtonian fluids, the relationship between force and shear is not constant. When the shear rate is varied, the shear stress does not respond in a linear fashion. The viscosity of such liquids will change as the shear rate is varied. Such phenomena can be seen in heterogeneous liquid and solid dispersions, such as colloidal suspensions, emulsions, and liquid suspensions. Generally, there are three classes of non-Newtonian systems that must be considered: (1) plastic flow, (2) pseudoplastic flow, and (3) dilatant flow.

Plastic flow occurs when a preparation undergoes flocculation or aggregation of particles in a suspension. These are referred to as Bingham bodies. These bodies do not begin to flow, until a shear force or stress corresponding to a yield value is exceeded. The yield value is the force that must be applied to convert the system to a Newtonian fluid, as seen for line D in Fig. 8.3. Examples of such materials are tooth paste in a tube, chili paste, or tomato paste. The ratio of shear stress to shear rate in such materials is not a constant value, so the value is designated as *apparent viscosity.* To be useful, a reported value for the apparent viscosity of a non-Newtonian material should be given together with the value of shear rate or shear stress used in the determination. The relationship between shear stress and shear rate for non-Newtonian materials, such as the dilatant and pseudoplastic bodies in Fig. 8.3, can be represented by a power law as follows:

$$\tau = A\dot{\gamma}^n$$

where A and n are constants. A is the consistency index or apparent viscosity, and n is the flow behavior index. The exponent is $n = 1$ for Newtonian liquids; for dilatant materials, it is greater than 1; and for pseudoplastic materials, it is less than 1. In its logarithmic form,

$$\log \tau = \log A + n \log \dot{\gamma}$$

A plot of log τ versus log $\dot{\gamma}$ will yield a straight line with a slope of n.

For non-Newtonian materials that have a yield stress, the Casson or Hershel-Bulkley models can be used. The Casson model is represented by the equation,

$$\sqrt{\tau} = \sqrt{\tau_0} + A\sqrt{\dot{\gamma}}$$

where τ_0 = yield stress.

This model has been found useful for several food products, especially chocolate (Kleinert 1976).

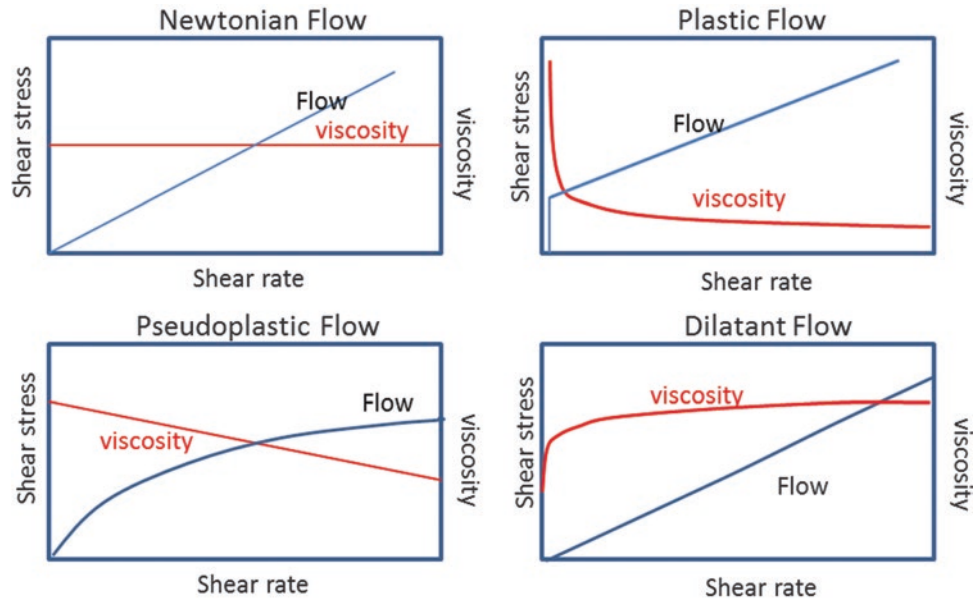


Fig. 8.4 The interaction of shear stress, flow, and viscosity for the four different types of fluids

The Hershel-Bulkley model describes a material with a yield stress and a linear relationship between log shear stress and log shear rate:

$$\tau = \tau_0 + A\dot{\gamma}^n$$

The value of n indicates how close the plot of shear stress vs. shear rate is to being a straight line. Fig. 8.4 represents the four types of flow, illustrating the responses of different types of fluids to shear stress and how the flow and viscosity are affected.

Texture of Solids

The terms for the textural properties of foods have a long history. Many of the terms are accepted, but are often poorly defined descriptive terms. The following are some examples of such terms:

- *Consistency* denotes those aspects of texture that relate to flow and deformation. It can be said to encompass all of the rheological properties of a product.
- *Hardness* has been defined as resistance to deformation.
- *Firmness* is essentially identical to hardness, but is occasionally used to describe the property of a substance able to resist deformation under its own weight.
- *Brittleness* is the property of fracturing before significant flow has occurred.
- *Stickiness* is a surface property related to the adhesion between material and adjoining surface. When the two surfaces are of identical material, we use the term *cohesion.*

A variety of other words and expressions are used to describe textural characteristics, such as body, crisp, greasy, brittle, tender, juicy, mealy, flaky, crunchy, and so forth. Many of these terms have been discussed by Szczesniak (1963) and Sherman (1969); most have no objective physical meaning and cannot be expressed in units of measurement that are universally applicable. Kokini (1985) has attempted to relate some of these ill-defined terms to the physical properties involved in their evaluation. Through the years, many types of instruments have been developed

for measuring certain aspects of food texture. More recently, instruments have been developed that are more widely applicable and are based on sound physical and engineering principles.

Texture Profile

Texture is an important aspect of food quality, sometimes even more important than flavor and color. Szczesniak and Kleyn (1963) conducted a consumer-awareness study of texture and found that texture significantly influences people's image of food. Texture was most important in bland foods and foods that are crunchy or crisp. The characteristics most often referred to were hardness, cohesiveness, and moisture content. Several attempts have been made to develop a classification system for textural characteristics. Szczesniak (1963) divided textural characteristics into three main classes, as follows:

1. mechanical characteristics;
2. geometrical characteristics;
3. other characteristics, related mainly to moisture and fat content.

Mechanical characteristics include five basic parameters.

1. *Hardness*—the force necessary to attain a given deformation.
2. *Cohesiveness*—the strength of the internal bonds making up the body of the product.
3. *Viscosity*—the rate of flow per unit force.
4. *Elasticity*—the rate at which a deformed material reverts to its undeformed condition, after the deforming force is removed.
5. *Adhesiveness*—the work necessary to overcome the attractive forces between the surface of a food and the surfaces of other materials with which the food comes in contact (e.g., tongue, teeth, and palate).

In addition, there are in this class the three following secondary parameters:

1. *Brittleness*—the force with which the material fractures. This is related to hardness and cohesiveness. In brittle materials, cohesiveness is low, and hardness can be either low or high. Brittle materials often create sound effects when masticated (e.g., toast, carrots, celery);
2. *Chewiness*—the energy required to masticate a solid food product to a state ready for swallowing. It is related to hardness, cohesiveness, and elasticity;
3. *Gumminess*—the energy required to disintegrate a semi-solid food to a state ready for swallowing. It is related to hardness and cohesiveness.

Table 8.2 Classification of textural characteristics of foods

Mechanical characteristics		
Primary parameters	Secondary parameters	Popular terms
Hardness		Soft → firm → hard
Cohesiveness	Brittleness	Crumbly → crunchy → brittle
	Chewiness	Tender → chewy → tough
	Gumminess	Short → mealy → pasty → gummy
Viscosity		Thin → viscous
Elasticity		Plastic → elastic
Adhesiveness		Sticky → tacky → gooey
Geometrical characteristics		
Class	*Examples*	
Particle size and shape	Gritty, grainy, coarse, etc.	
Particle shape and orientation	Fibrous, cellular, crystalline, etc.	
Other characteristics		
Primary parameters	*Secondary parameters*	*Popular terms*
Moisture content		Dry → moist → wet → watery
Fat content	Oiliness	Oily
	Greasiness	Greasy

Source: from Szczesniak (1963)

Geometrical characteristics include two general groups: those related to size and shape of the particles, and those related to shape and orientation. Terms for geometrical characteristics include smooth, cellular, fibrous, and so on. The group of other characteristics in this system is related to moisture and fat content, and includes qualities such as moist, oily, and greasy. A summary of this system is given in Table 8.2.

Based on the Szczesniak system of textural characteristics, Brandt et al. (1963) developed a method for profiling texture, so that a sensory evaluation could be given that would assess the entire texture of a food. This texture profile method was based on the earlier development of the flavor profile (Cairncross and Sjöström 1950).

The Szczesniak system was critically examined by Sherman (1969), who proposed some modifications. In this improved system, no distinction is drawn among analytical, geometrical, and mechanical attributes. Instead, the only criterion is whether a characteristic is a fundamental property or is derived by a combination of two or more attributes in unknown proportions. The Sherman system contains three groups of characteristics (Fig. 8.5). The primary category includes analytical characteristics from which all other attributes are derived. The basic rheological parameters—elasticity, viscosity, and adhesion—form the secondary category. The remaining attributes form the tertiary category, since they are a complex mixture of these secondary parameters. This system is interesting, because it attempts to relate sensory responses to mechanical strain-time tests. Sensory panel responses, associated with masticatory tertiary characteristics of the Sherman texture profile for solid, semi-solid, and liquid foods, are given in Fig. 8.6.

Measurement of Texture

The objective measurement of texture belongs in the area of rheology, which is the science of flow and deformation of matter. However, determining the rheological properties of a food does not necessarily mean that the complete texture of the product is determined. Nevertheless, knowledge of some of the rheological properties of a food may give important clues as to its acceptability and may be important in determining the nature and design of processing methods and equipment.

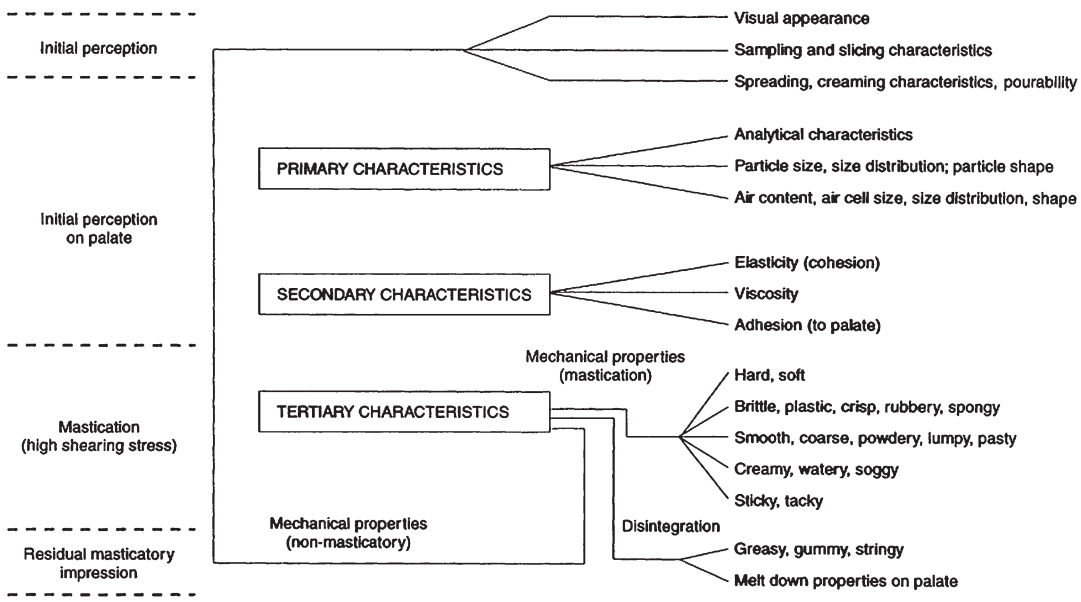


Fig. 8.5 The modified texture profile (Sherman 1969)

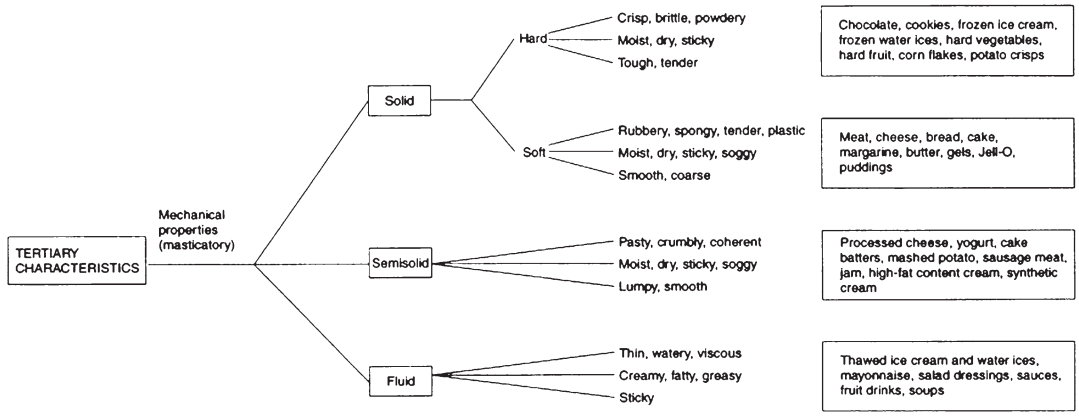


Fig. 8.6 Panel responses associated with masticatory tertiary characteristics of the modified texture profile

Food rheology is mainly concerned with forces and deformations. In addition, time is an important factor; many rheological phenomena are time-dependent. Temperature is another important variable. Many products show important changes in rheological behavior as a result of changes in temperature. In addition to flow and deformation of cohesive bodies, food rheology includes such phenomena as the breakup or rupture of solid materials and surface phenomena such as stickiness (adhesion).

Deformation may be of one or both of two types, irreversible deformation, called flow, and reversible deformation, called elasticity. The energy used in irreversible deformation is dissipated as heat, and the body is permanently deformed. The energy used in reversible deformation is recovered upon release of the deforming stress, when the body regains its original shape.

Force and Stress

When a force acts externally on a body, several different cases may be distinguished: tension, compression, and shear. Bending involves tension and compression, torque involves shear, and hydrostatic compression involves all three. All other cases may involve one of these three factors or a combination of them. In addition, the weight or inertia of a body may constitute a force leading to deformation. Generally, however, the externally applied forces are of much greater magnitude, and the effect of weight is usually neglected. The forces acting on a body can be expressed in grams or in pounds. Stress is the intensity factor of force and is expressed as force per unit area; it is similar to pressure. There are several types of stress: compressive stress (with the stress components directed at right angles toward the plane on which they act); tensile stress (in which the stress components are directed away from the plane on which they act); and shearing stress (in which the stress components act tangentially to the plane on which they act). A uniaxial stress is usually designated by the symbol σ, a shearing stress by τ. Shear stress is expressed in dynes/cm^2, when using the metric system of measurement; in the SI system, it is expressed in N/m^2 or Pascals (Pa).

Deformation and Strain

When the dimensions of a body change, we speak of deformation. Deformation can be linear, as in a tensile test, when a body of original length L is subjected to a tensile stress. The linear deformation ΔL can then be expressed as strain $\varepsilon = \Delta L/L$. Strain can be expressed as a ratio or percent; inches per inch or centimeters per centimeter. In addition to linear deformations, there are other

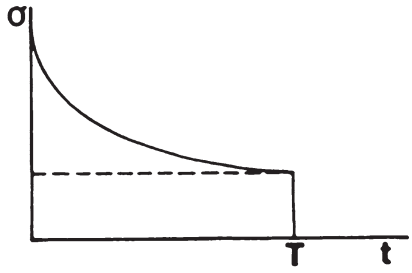


Fig. 8.7 Relaxation curve (relationship of stress to time under constant strain)

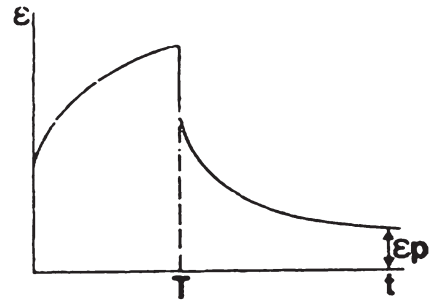


Fig. 8.8 Creep curve (relationship of strain to time under constant stress)

types of deformation, such as in a hydrostatic test, where there will be a volumetric strain $\Delta V/V$.

For certain materials, the deformation resulting from an applied force can be very large; this indicates the material is a liquid. In such cases, we deal with rate of deformation, or shear rate: $d\gamma/dt$ or $\dot{\gamma}$. This is the velocity difference per unit thickness of the liquid; $\dot{\gamma}$ is expressed in units of s^{-1}.

Principles of Measurement

For Newtonian fluids, it is sufficient to measure the ratio of shear stress to shear rate, from which the viscosity can be calculated. This can be done in a viscometer, which can be one of various types, including capillary, rotational, falling-ball, and so on. For non-Newtonian materials, such as the dilatant, pseudoplastic, and plastic bodies shown in Fig. 8.4, the problem is more difficult. With non-Newtonian materials, several methods of measurement involve the ratio of shear stress to shear rate, the relationship of stress to time under constant strain (relaxation), and the relationship of strain to time under constant stress (creep). In relaxation measurements, a material is subjected to a sudden deformation ε_o, which is held constant. In many materials, the stress will decay with time, according to the curve of Fig. 8.7. The point at which the stress has decayed to σ/e, or 36.7% of the original value of σ_o, is called the relaxation time T. When the strain is removed at time T, the stress returns to zero. In a creep experiment, a material is subjected to the instantaneous application of a constant load or stress, and the strain is measured as a function of time. The resulting creep curve has the shape indicated in Fig. 8.8. At time zero, the applied load results in a strain ε_o, which increases with time. When the load is removed at time T, the strain immediately decreases, as indicated by the vertical straight portion of the curve at T; the strain continues to decrease thereafter with time. In many materials, the value of ε never reaches zero, and we know, therefore, that a permanent deformation ε_p has resulted. The ratio of strain to applied stress in a creep experiment is a function of time and is called the creep compliance (J). Creep experiments are sometimes plotted as graphs relating J to time.

Different Types of Bodies

The Elastic Body

For certain solid bodies, the relationship between stress and strain is represented by a straight line through the origin (Fig. 8.9), up to the so-called limit of elasticity, according to the law of Hooke, $\sigma = E\varepsilon$. The proportionality factor E for uniaxial stress is called *modulus of elasticity*, or Young's modulus. For a shear stress, the modulus is G, or Coulomb modulus. Note that a modulus is the ratio of stress to strain, $E = \sigma/\varepsilon$. The behavior of a Hookean body is further exemplified by the stress-time and strain-time curves of Fig. 8.10. When a Hookean body is subjected to a constant strain ε_o, the stress σ will remain constant with time, and will return to zero, when the strain is removed at time T. The strain ε will follow the same pattern, when a constant stress is applied, and then released at time T.

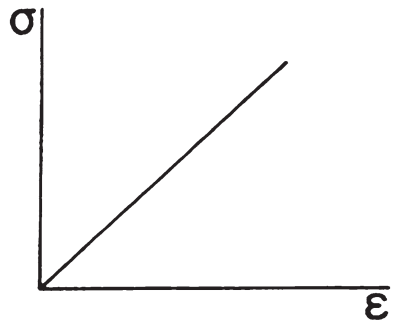


Fig. 8.9 Stress-strain curve for a perfectly elastic body

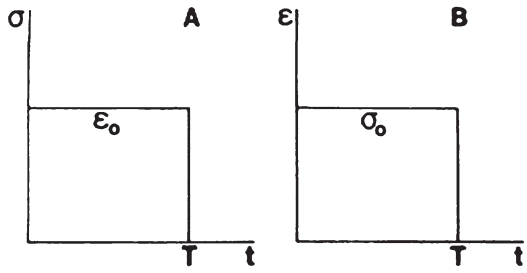


Fig. 8.10 (**a**) Stress-time and (**b**) strain-time curves for a Hookean body

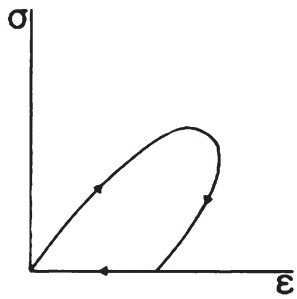


Fig. 8.11 Stress-strain curve for a retarded elastic body

The Retarded Elastic Body

In bodies showing retarded elasticity, the deformation is a function of time as well as stress. Such a stress-strain curve is shown in Fig. 8.11. The upward part of the curve represents increasing values of stress; when the stress is reduced, the corresponding strains are greater on the downward part of the curve. When the stress reaches 0, the strain has a finite value, which will

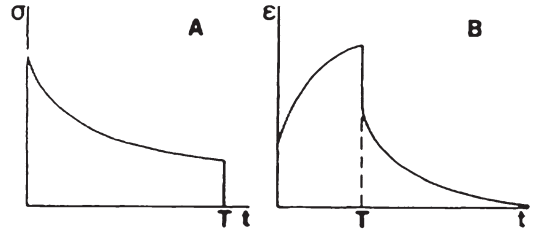


Fig. 8.12 (**a**) Stress-time and (**b**) strain-time curves for a retarded elastic body

slowly return to zero. There is no permanent deformation. The corresponding relaxation (stress-time) and creep (strain-time) curves for this type of body are given in Fig. 8.12.

The Viscous Body

A viscous or Newtonian liquid is one showing a direct proportionality between shear stress and shear rate, as indicated by curve A in Fig. 8.3.

The Viscoelastic Body

Certain bodies combine the properties of both viscous and elastic materials. The elastic component can be partially retarded elasticity. Viscoelastic bodies may flow slowly and nonreversibly under the influence of a small stress. Under larger stresses, the elastic component becomes apparent. The relaxation curve for viscoelastic materials has the shape indicated in Fig. 8.13a. The curve has the tendency to approach the time axis. The creep curve indicates that the strain increases for as long as the stress is applied (Fig. 8.13b). The magnitude of the permanent deformation of the body increases with the applied stress and with the length of time of application.

Mechanical models can be used to visualize the behavior of different bodies. Thus, a spring denotes a Hookean body, and a dash-pot denotes a purely viscous body or Newtonian fluid. These elements can be combined in a variety of ways to represent the rheological behavior of complex substances. Two basic viscoelastic models are the Voigt-Kelvin and the Maxwell bodies. The Voigt-

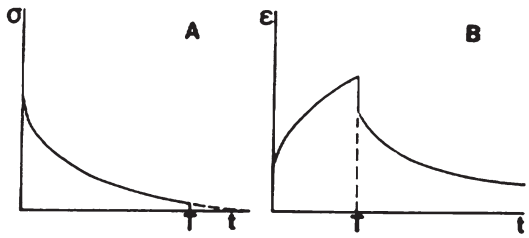


Fig. 8.13 (**a**) Stress-time and (**b**) strain-time curves for a viscoelastic body

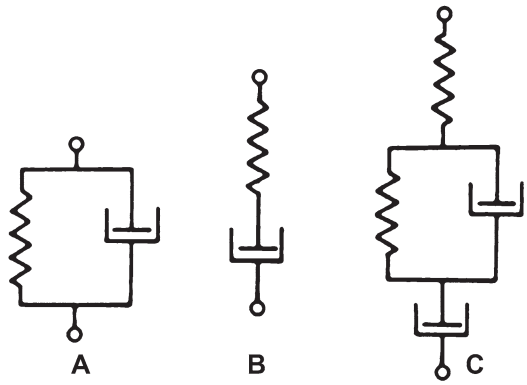


Fig. 8.14 (**a**) Voigt-Kelvin, (**b**) Maxwell, and (**c**) Burgers models

Kelvin model employs a spring and dashpot in parallel; the Maxwell model employs a spring and dashpot in series (Fig. 8.14). In the Voigt-Kelvin body, the stress is the sum of two components, where one is proportional to the strain and the other to the rate of shear. Because the elements are in parallel, they must move together. In the Maxwell model, the deformation is composed of two parts—one purely viscous, the other purely elastic. Although both the Voigt-Kelvin and Maxwell bodies represent viscoelasticity, they react differently in relaxation and creep experiments. When a constant load is applied in a creep test to a Voigt-Kelvin model, a final steady-state deformation is obtained, because the compressed spring element resists further movement. The Maxwell model will give continuing flow under these conditions, because the viscous element is not limited by the spring element. When the load is removed, the Voigt-Kelvin model recovers completely, but not instantaneously. The Maxwell body does not recover completely, but, rather, instantly. The Voigt-Kelvin body, therefore, shows no stress relaxation, but the Maxwell body does. A variety of models can be constructed to represent the rheological behavior of viscoelastic materials. By placing a number of Kelvin models in series, a so-called generalized Kelvin model is obtained. Similarly, a generalized Maxwell model is obtained by placing a number of Maxwell models in parallel. The combination of a Kelvin and a Maxwell model in series (Fig. 8.14c) is called a Burgers model.

For ideal viscoelastic materials, the initial elastic deformation at the time the load is applied should equal the instantaneous elastic deformation when the load is removed (Fig. 8.15). For most food products, this is not the case. As is shown by the example of butter in Fig. 8.15, the initial deformation is greater than the elastic recovery at time *t*. This may result from the fact that such foods are plastic as well as viscoelastic, which means they have a yield value. Therefore, the initial deformation consists of both an instantaneous elastic deformation and a permanent deformation (viscous flow component). It has also been found (deMan et al. 1985) that the magnitude of the instantaneous elastic recovery in fat products is time-dependent and decreases as the time of application of the load increases. It appears that the fat crystal network gradually collapses, as the load remains on the sample.

The Plastic Body

A plastic material is defined as one that does not undergo a permanent deformation, until a certain yield stress has been exceeded. A perfectly plastic body showing no elasticity would have the stress-strain behavior depicted in Fig. 8.16. Under the influence of a small stress, no deformation occurs; when the stress is increased, the material will suddenly start to flow at applied stress σ_o (the yield stress). The material will then continue to flow at the same stress, until this is removed; the material retains its total deformation. In reality, few bodies are perfectly plastic; rather, they are plasto-elastic or plasto-viscoelastic. The mechanical model used to represent a plastic body, also called a

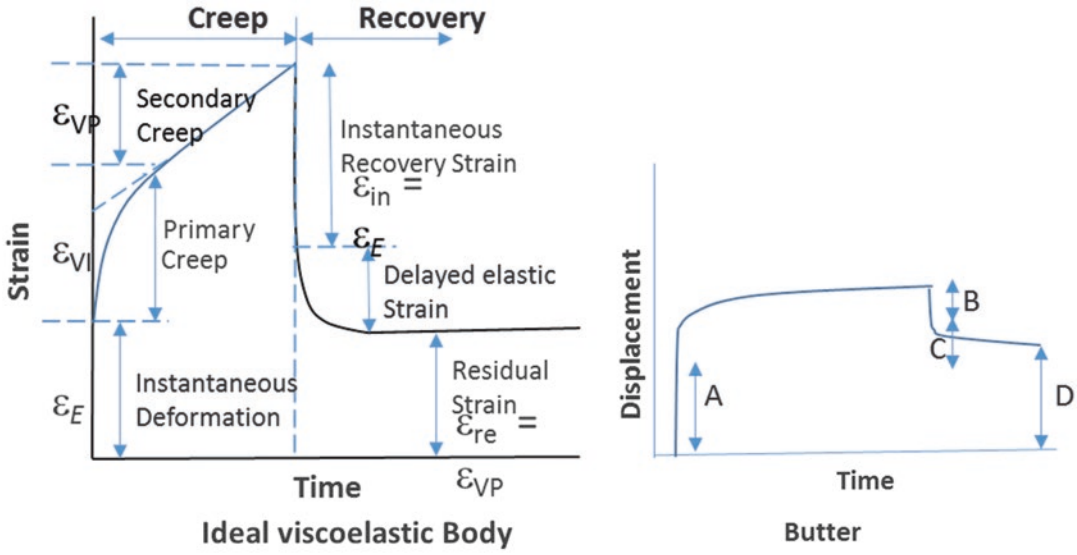


Fig. 8.15 Creep curve for an ideal viscoelastic body, and creep curve for butter

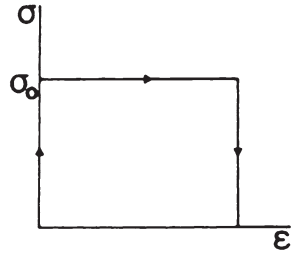


Fig. 8.16 Stress-strain curve for an ideal plastic body

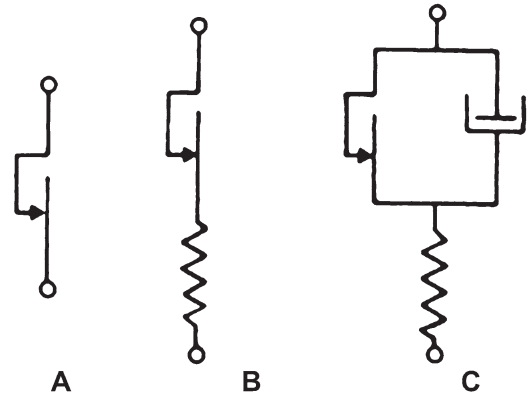


Fig. 8.17 Mechanical models for a plastic body: (**a**) St. Venant body, (**b**) plasto-elastic body, and (**c**) plasto-viscoelastic or Bingham body

St. Venant body, is a friction element. The model is analogous to a block of solid material that rests on a flat horizontal surface. The block will not move, when a force is applied to it, until the force exceeds the friction existing between block and surface. The models for ideal plastic and plasto-elastic bodies are shown in Fig. 8.17a, b.

A more common body is the plasto-viscoelastic, or Bingham body. Its mechanical model is shown in Fig. 8.17c. When a stress below the yield stress is applied, the Bingham body reacts as an elastic body. At stress values beyond the yield stress, there are two components, one of which is constant and is represented by the friction element, and the other, which is proportional to the shear rate and represents the viscous flow element. In a creep experiment with stress not exceeding the yield stress, the creep curve would be similar to the one for a Hookean body (Fig. 8.10b). When the shear stress is greater than the yield stress, the strain increases with time, similar to the behavior of a Maxwell body (Fig. 8.18). Upon removal of the stress at time *T,* the strain decreases instantaneously and remains constant thereafter. The decrease represents the elastic component; the plastic deformation is permanent. The relationship

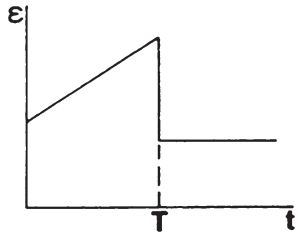


Fig. 8.18 Creep curve for a Bingham body subjected to a stress greater than the yield stress

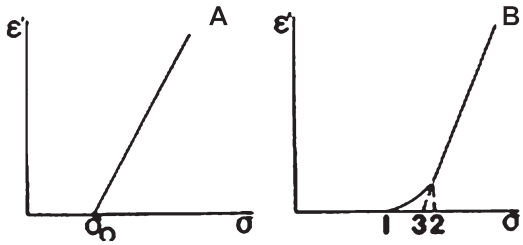


Fig. 8.19 Shear rate-shear stress diagrams for Bingham bodies: (**a**) ideal case, and (**b**) practical case. The yield values are as follows: lower yield value (1), upper yield value (2), and Bingham yield value (3)

of shear rate to shear stress for a Bingham body would have the form shown in Fig. 8.19a. When flow occurs, the relationship between shear stress and shear rate is given by

$$\sigma - \sigma_o = UD$$

where
σ_o = yield stress
U = proportionality constant
D = mean rate of shear

The constant U can be termed plastic viscosity, and its reciprocal $1/U$ is referred to as mobility. In reality, plastic materials are more likely to have a curve similar to the one in Fig. 8.19b. The yield stress or yield value can be taken at three different points: the lower yield value, at the point where the curve starts on the stress axis; the upper yield value, where the curve becomes straight; and the Bingham yield value, which is found by extrapolating the straight portion of the curve to the stress axis.

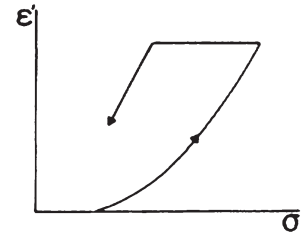


Fig. 8.20 Shear stress–shear rate diagram for a thixotropic body. *Source:* from deMan and Wood (1959)

The Thixotropic Body

Thixotropy can be defined as an isothermal, reversible, sol-gel transformation, and is a behavior common to many foods. Thixotropy is an effect brought about by mechanical action, and it results in a lowered apparent viscosity. When the body is allowed sufficient time, the apparent viscosity will return to its original value. Such behavior would result in a shear stress-shear rate diagram, as shown in Fig. 8.20. Increasing shear rate results in increased shear stress, up to a maximum; after the maximum is reached, decreasing shear rates will result in substantially lower shear stress.

Dynamic Behavior

Viscoelastic materials are often characterized by their dynamic behavior. Because viscoelastic materials are prone to structural breakdown when subjected to large strains, it is useful to analyze them by small amplitude sinusoidal strain. The relationship between stress and strain under these conditions can be evaluated from Fig. 8.21 (Bell 1989). The applied stress is alternating at a selected frequency and is expressed in cycles s^{-1}, or ω in radians s^{-1}. The response of a purely elastic material will show a stress and strain response that is in phase, with the phase angle $\delta = 0°$. A purely viscous material will show the stress being out of phase by 90°, and a viscoelastic material will show intermediate behavior, with δ between 0° and 90°. The viscoelastic dynamic response is composed of an in-phase component ($\sin \omega t$) and an out-of-phase component ($\cos \omega t$). The energy

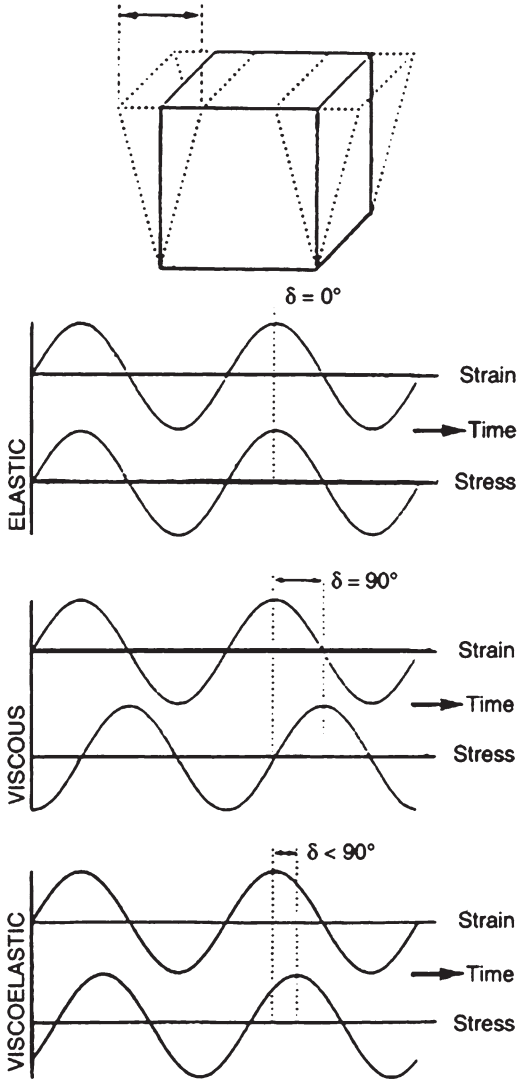


Fig. 8.21 Dynamic (oscillation) measurement of viscoelastic materials. As an oscillating strain is applied, the resulting stress values are recorded; δ is the phase angle, and its value indicates whether the material is viscous, elastic, or viscoelastic. *Source*: reprinted from Bell (1989)

used for the viscous component is lost as heat; that used for the elastic component is retained as stored energy. This results in two moduli, the storage modulus (G′) and the loss modulus (G″). The ratio of the two moduli is known as tan δ and is given by tan δ = G″/G′.

Rheology Applications in Foods

Rheology has multiple applications in consideration of food acceptability, food processing, and food handling (Barbosa-Canovas et al. 1996). Foods are complex materials often composed of mixtures of solids and fluids (Finney 1972). Rheology measures the flow and deformation of substances in the transient region between solids and fluids. Rheology defines the relationship between the stress applied to a given material and the resulting deformation and/or flow. Rheological instruments measure the force and deformation of materials as a function of time.

Fundamental rheological methods account for the magnitude and direction of forces and deformations, placing restrictions on acceptance of sample shapes and compositions. Fundamental tests have the advantage of being based on known concepts and equations of physics. Empirical methods are applied when sample composition or geometry is too complex to account for forces and deformations. Empirical methods correlate with a particular property of interest, whereas fundamental tests determine fundamental physical properties. Rheology assesses the responses of materials to applied forces and deformations. Basic concepts of stress (force per area) and strain (deformation per length) are key to all rheological evaluations. Stress (τ) is always a measurement of force per unit of surface area and is expressed in units of Pascals (Pa). The direction of the force with respect to the impacted surface area determines the type of stress. Normal stress occurs when the force is directly perpendicular to a surface, and can be achieved during tension or compression. Shear stress occurs when the forces act parallel to a surface (Tabilo-Munizaga and Barbosa-Canovas 2005). Many of the rheological properties of complex biological materials are time-dependent, and Mohsenin (1970) has suggested that many foods can be regarded as viscoelastic materials. Many foods are disperse systems of interacting non-spherical particles and show thixotropic behavior. Such particles may interact to form a three-dimensional network that imparts rigidity to a system. The interaction may

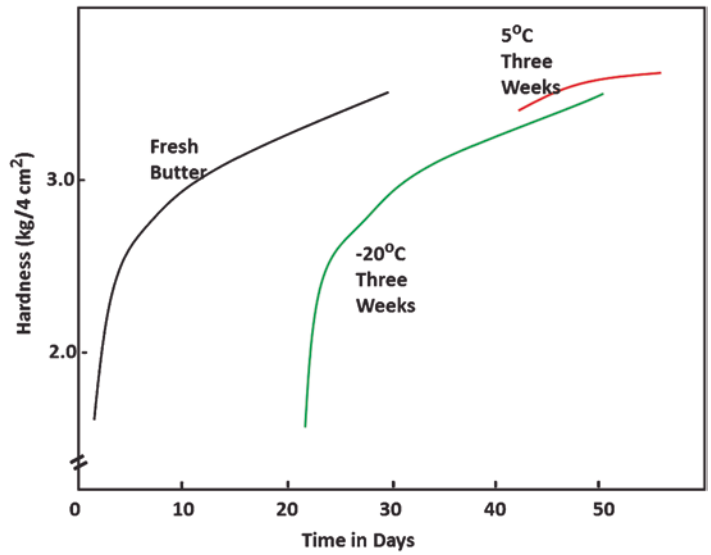


Fig. 8.22 Thixotropic hardness change in butter: (**a**) freshly worked butter left undisturbed for 4 weeks at 5 °C; (**b**) the same butter stored at −20 °C for 3 weeks, then left at 5 °C; (**c**) the same butter left at 5 °C for 3 weeks, then frozen for 3 weeks, and again placed at 5 °C. *Source:* from deMan and Wood (1959)

be the result of ionic forces in aqueous systems, or of hydrophobic or van der Waals interactions in systems that contain fat crystals in liquid oil (e.g., butter, margarine, and shortening). Mechanical action, such as agitation, kneading, or working, results in disruption of the network structure and a corresponding loss in hardness. When such a system is then left undisturbed, the bonds between particles will reform, and hardness will increase with time, until maximum hardness is reached. The nature of thixotropy was demonstrated for butter by deMan and Wood (1959). Hardness of freshly worked butter was determined over a period of 3 weeks (Fig. 8.22). The same butter was frozen and then removed from frozen storage after 3 weeks. No thixotropic change had occurred with the frozen sample. The freezing had completely immobilized the crystal particles. Thixotropy is important in many food products; great care must be exercised, so that measurements are not influenced by thixotropic changes.

The viscosity of Newtonian liquids can be measured simply, by one-point determinations with viscometers, such as rotational, capillary, or falling-ball viscometers. For non-Newtonian materials, measurement of rheological properties is more difficult, because single-point determinations (i.e., at one single shear stress) will yield no useful information. We can visualize the rate of shear dependence for Newtonian fluids by considering a diagram of two fluids, as shown in Fig. 8.23 (Sherman 1973). The behavior of these fluids is represented by two straight lines parallel to the shear-rate axis. With non-Newtonian fluids, a situation as shown in Fig. 8.24 may arise. The fluids 3 and 4 have curves that intersect. Below this point of intersection, fluid 4 will appear more viscous; beyond the intersection, fluid 3 will appear more viscous. Fluids 5 and 6 do not intersect, and the problem does not arise. In spite of the possibility of such problems, many practical applications of rheological measurements for non-Newtonian fluids are carried out at only one shear rate. Note that results obtained in this way should be interpreted with caution. Shoemaker et al. (1987) have given an overview of the application of rheological techniques for foods.

Probably the most widely used type of viscometer in the food industry is the Brookfield rotational viscometer. An example of this instrument's application to a non-Newtonian food product was given in the work of Saravacos and Moyer (1967) on fruit purees. Viscometer scale

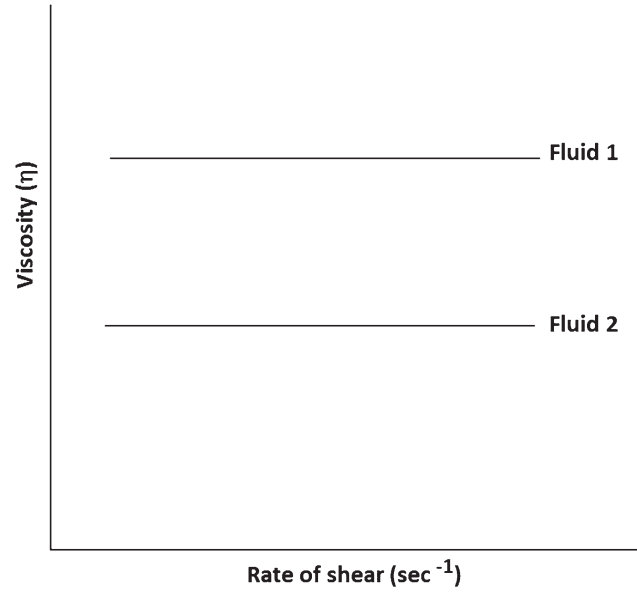


Fig. 8.23 Rate of shear dependence of the viscosity of two Newtonian fluids. *Adopted from* Sherman (1973)

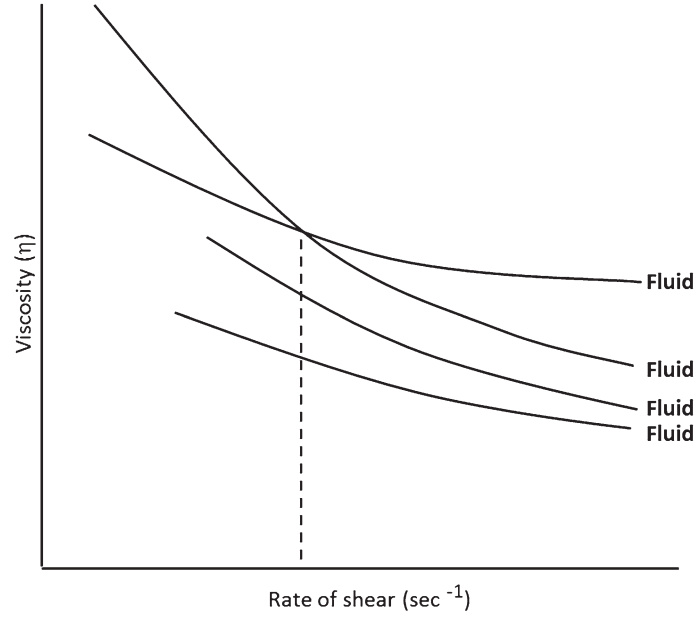


Fig. 8.24 Rate of shear dependence of the apparent viscosity of several non-Newtonian fluids. *Adopted from* Sherman (1973)

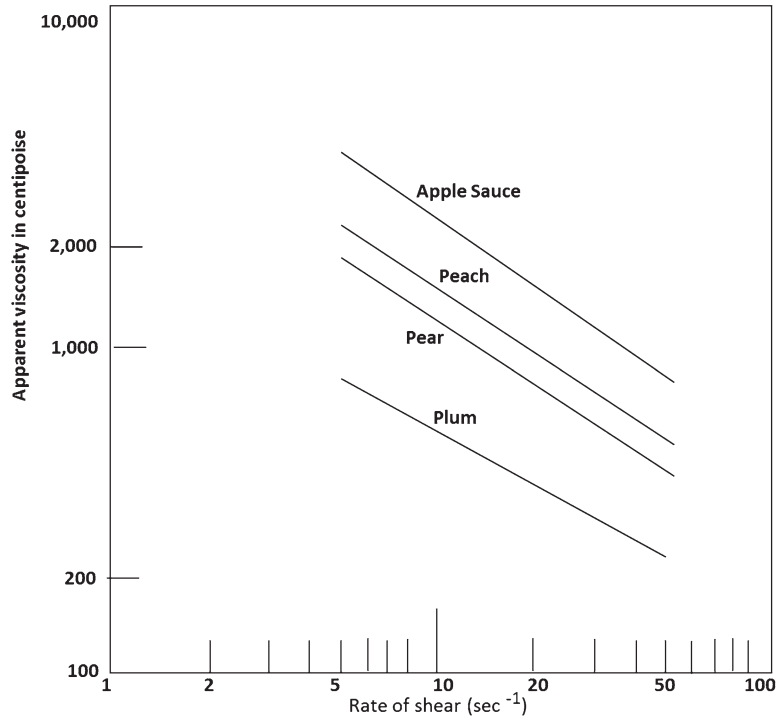


Fig. 8.25 Apparent viscosities of fruit purees determined at 86 °C. *Modified* from Saravacos and Moyer (1967)

readings were plotted against rotational speed on a logarithmic scale, and the slope of the straight line obtained was taken as the exponent n in the following equation for pseudoplastic materials:

$$\tau = K\dot{\gamma}^n$$

where
τ = shear stress (dyne/cm^2)
K = constant
$\dot{\gamma}$ = shear rate (s^{-1})

The instrument readings were converted into shear-stress values by using an oil of known viscosity. The shear rate at a given rotational speed N was calculated from

$$\dot{\gamma} = 4\pi\, \mathrm{N}/n$$

When shear stress τ was plotted against shear rate γ on a double-logarithmic scale, the intercept of the straight line on the τ axis at $\gamma = 1$ s^{-1} was taken as the value of the constant K. The apparent viscosity μ_{app} at a given shear rate was then calculated from the equation

$$\mu_{\text{app}} = K\dot{\gamma}^{n-1}$$

Apparent viscosities for fruit purees, determined in this manner, are shown in Fig. 8.25.

Factors have been reported in the literature (Johnston and Brower 1966) for the conversion of Brookfield viscometer scale readings to yield values or viscosities. Saravacos (1968) has also used capillary viscometers for rheological measurements of fruit purees.

For products not sufficiently fluid to be studied with viscometers, a variety of texture-measuring devices is available. These range from simple penetrometers, such as the Magness-Taylor fruit pressure tester, to complex universal testing machines, such as the Instron. All these instruments either apply a known and constant stress and measure deformation, or cause a constant deformation and measure stress. Some of the more sophisticated instruments can do both. In the Instron Universal Testing Machine, the crosshead moves at a speed that can be selected by changing gears. The drive is by rotating screws, and the force measurement is done with load cells. Mohsenin (1970) and coworkers have developed a type of universal testing machine, in which the movement is achieved by air pressure. The Kramer shear press uses a hydraulic system for movement of the crosshead.

Texture-measuring instruments can be classified according to their use of penetration, compression, shear, or flow.

Penetrometers come in a variety of types. One of the most widely utilized is the Precision penetrometer, which is used for measuring the consistency of fats. The procedure and cone dimensions are standardized and described in the Official Methods and Recommended Practices of the American Oil Chemists' Society. According to this method, the results are expressed in mm/10 of penetration depth. Haighton (1959) proposed the following formula for the conversion of penetration depth into yield value:

$$C = K\ W/P^{1.6}$$

where
C = yield value
K = constant dependent on the angle of the cone
p = penetration depth
W = weight of cone

Vasic and deMan (1968) suggested a conversion of the penetration-depth readings into hardness, using the formula

$$H = G/A$$

where
H = ha.rdness
G = total weight of the cone assembly
A = area of impression

The advantage of this conversion is that changes in hardness are more uniform than changes in penetration depth. With the latter, a difference of an equal number of units, at the tip of the cone and higher up on the cone, is not at all comparable.

Many penetrometers use punches of various shapes and sizes as penetrating bodies. Little was known about the relationship between shape and size and penetrating force until Bourne's (1966) work. He postulated that when a punch penetrates a food, both compression and shear occur. Shear, in this case, is defined as the movement of interfaces in opposite directions. Bourne suggested that compression is proportional to the area under the punch and to the compressive strength of the food, and also that the shear force is proportional to the perimeter of the punch and to the shear strength of the food (Fig. 8.26). The following equation was suggested:

$$F = K_c A + K_s P + C$$

where
F = measured force
K_c = compression coefficient of the tested food
K_s = shear coefficient of the tested food
A = area of the punch
P = perimeter of the punch
C = constant

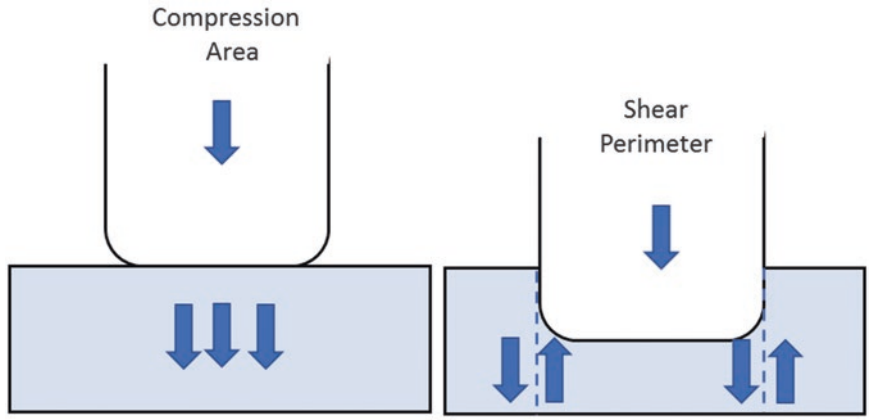


Fig. 8.26 Compression and shear components in penetration tests. *Adopted*: from Bourne (1966)

The relationship between penetration force and the cross-sectional area of a cylindrical punch has been established by Kamel and deMan (1975).

Bourne (1966) showed that, for a variety of foods, the relationships between punch area and force and between punch perimeter and force were represented by straight lines. deMan (1969) later showed that, for certain products such as butter and margarine, the penetrating force was dependent only on area and was not influenced by perimeter. deMan suggested that, in such products, flow is the only factor affecting force readings. It appears that useful conclusions can be drawn, regarding the textural characteristics of a food, by using penetration tests.

A variation on the penetration method is the back-extrusion technique, where the sample is contained in a cylinder, and the penetrating body leaves only a small annular gap for the product to flow. The application of the back-extrusion method to non-Newtonian fluids has been described by Steffe and Osorio (1987).

Many instruments combine shear and compression testing. One of the most widely used is the Kramer shear press. Based on the principle of the shear cell used in the pea tenderometer, the shear press was designed to be a versatile and widely applicable instrument for texture measurement on a variety of products. The shear press is essentially a hydraulically driven piston, to which a standard ten-blade shear cell or a variety of other specialized devices can be attached. Force measurement is achieved either by a direct-reading proving ring or by an electronic recording device. The results obtained with the shear press are influenced by the weight of the sample and the speed of the crosshead. These factors have been exhaustively studied by Szczesniak et al. (1970). The relationship between maximum force value and sample weight was found to be different for different foods. Products fell into three categories: those having a constant force-to-weight ratio (e.g., white bread, sponge cake); those having a continuously decreasing force-to-weight ratio (e.g., raw apples, cooked white beans); and those giving a constant force value, independent of sample weight beyond a certain fill level (e.g., canned beets, canned and frozen peas). This is demonstrated by the curves in Fig. 8.27. Some of the available attachments to the shear press are the succulometer cell, the single-blade meat shear cell, and the compression cell.

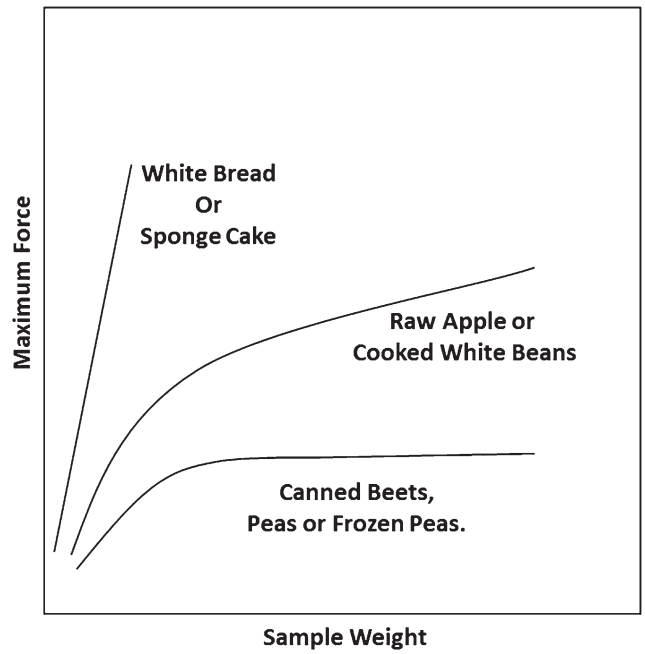


Fig. 8.27 Effect of sample weight on maximum force registered with the shear press and using the ten-blade standard cell: (1) white bread and sponge cake, (2) raw apples and cooked white beans, (3) canned beets and peas and frozen peas. *Adopted* from Szczesniak (1973)

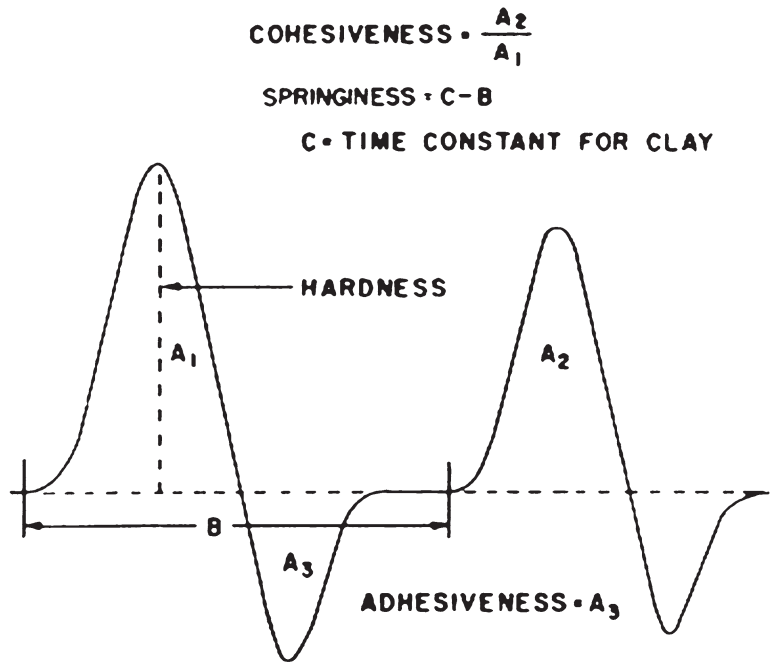


Fig. 8.28 Typical texturometer curve

Based on the Szczesniak classification of textural characteristics, a new instrument was developed in the General Foods research laboratories; it was called the General Foods Texturometer. This device was an improved version of the MIT denture tenderometer (Proctor et al. 1956). From the reciprocating motion of a deforming body on a sample, which was contained in a tray equipped with strain gages, a force record called a texture profile curve (Fig. 8.28) was obtained. From this texturometer curve, a variety of rheological parameters can be obtained. Hardness is measured from the height of the first peak. Cohesiveness is expressed as the ratio of the areas under the second and first peaks. Elasticity is measured as the difference between distance *B*, measured from initial sample contact to sample contact on the second "chew," and the same distance (distance B) measured for a completely inelastic material such as clay. Adhesiveness is measured as the area of the negative peak, A_3, beneath the baseline. In addition, other parameters can be derived from the curve, such as brittleness, chewiness, and gumminess.

Textural Properties of some Foods

Meat Texture

Meat texture is usually described in terms of tenderness or the lack of it—toughness. This obviously is related to the ease with which a piece of meat can be cut with a knife or with the teeth. The oldest and most widely used device for measuring meat tenderness is the Warner-Bratzler shear device (Bratzler 1932). In this device, a cylindrical core of cooked meat is subjected to the shearing action of a steel blade, and the maximum force is indicated by a spring-loaded mechanism. A considerable improvement was provided by the shear apparatus described by Voisey and Hansen (1967). In this apparatus, the shearing force is sensed by a strain-gage transducer, and a complete shear force-time curve is recorded on a strip chart. The Warner-Bratzler shear method has several disadvantages. It is very difficult to obtain uniform meat cores. Cores from different positions in one cut of meat may vary in tenderness, and cooking method may affect tenderness.

Meat tenderness has been measured with the Kramer shear press. This can be done with the ten-blade universal cell or with the single-blade meat-shear attachment. There is no standard procedure for measuring meat tenderness with the shear press; sample size, sample preparation, and shear rate are factors that may affect the results.

A pressure method for measuring meat tenderness has been described by Sperring et al. (1959). A sample of raw meat is contained in a cylinder that has a small hole in its bottom. A hydraulic press forces a plunger into the cylinder, and the pressure required to squeeze the meat through the hole is taken as a measure of tenderness.

A portable rotating-knife tenderometer has been described by Bjorksten et al. (1967). A rotating blunt knife is forced into the meat sample, and a tracing of the area traversed by a recording pen is used as a measure of tenderness.

A meat-grinder technique for measuring meat tenderness was reported by Miyada and Tappel (1956); in this method, power consumption of the meat-grinder motor was used as a measure of meat tenderness. The electronic-recording food grinder described by Voisey and Voisey and deMan (1970) measured the torque exerted on a strain-gage transducer. This apparatus was used successfully for measuring meat tenderness.

Other methods used for meat tenderness evaluation have included measurement of sarcomere length (Howard and Judge 1968) and determination of the amount of connective tissue present.

Stoner et al. (1974) proposed a mechanical model for postmortem striated muscle; it is shown in Fig. 8.29. The model is a combination of the Voigt model with a four-element viscoelastic model. The former includes a contractile element (CE), which is the force generator. The element SE is a spring that is passively elongated by the shortening of the CE, and thus develops an internal force. The parallel elastic component (PE) contributes to the resting tension of the muscle. The combination of elements PE, CE, and SE represents the purely elastic properties of the muscle as the fourth component of a four-element model (of which E_2, η_3, and η_2 are the other three components).

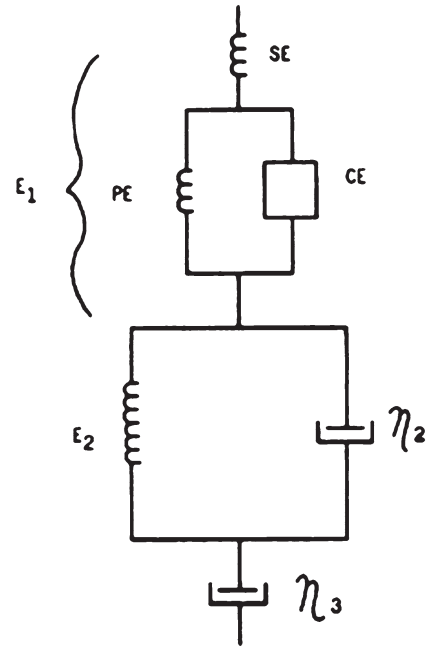


Fig. 8.29 Mechanical model for postmortem striated muscle. *Source:* from C.W. Brabender Instruments, Inc., South Hackensack, New Jersey

Wheat Flour Dough

The rheological properties of wheat flour dough are important in determining the baking quality of wheat flour. For many years, the Farinograph was used to measure the physical properties of wheat flour dough. The Farinograph is a dough mixer hooked up to a dynamometer for recording torque. The instrument can measure the water absorption of a wheat flour. A typical Farinograph curve is presented in Fig. 8.30. The amount of water, added from a buret, required to bring the middle of the curve to 500 units is a measure of the water absorption of a wheat flour dough. The measurement from zero to the point where the top of the curve first intersects the 500-unit line on the chart is called the arrival time; the measurement from zero to maximum consistency is called the peak time; the point where the top of the curve leaves the 500-unit line is called the departure time; and the difference between the departure and arrival times is taken as the stability. The elasticity of a wheat flour dough is measured with the

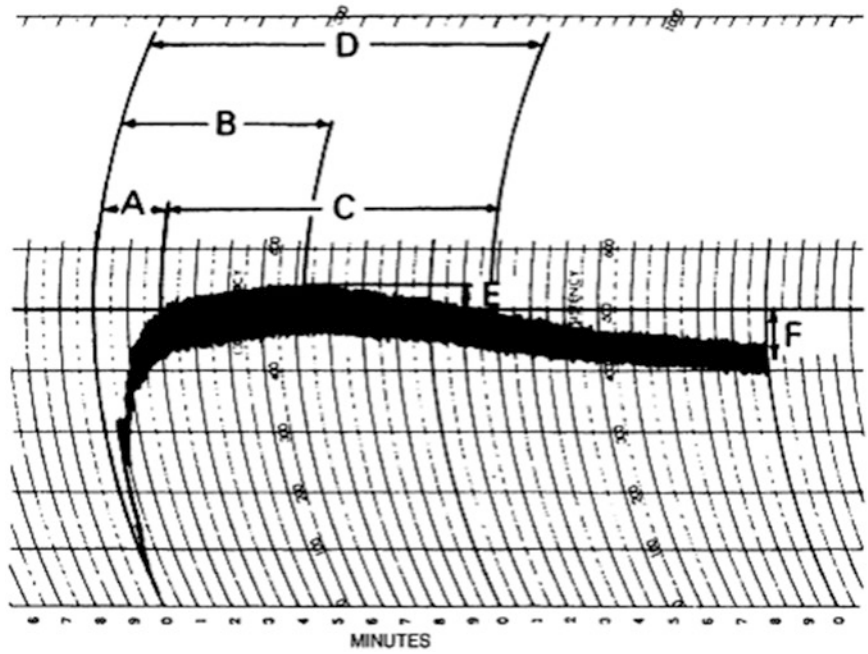


Fig. 8.30 Typical Farinograph curve: (*A*) arrival time, (*B*) peak time, (*C*) stability, (*D*) departure, (*E*) mixing tolerance index, (*F*) 20-min drop. *Source*: from Wehrli and Pomeranz (1969)

Extensigraph, which records the force required to stretch a piece of dough of standard dimensions.

The unique viscoelastic properties of wheat flour dough are the result of the presence of a three-dimensional network of gluten proteins. The network is formed by thiol-disulfide exchange reactions among gluten proteins. Peptide disulfides can interfere in a thiol-disulfide exchange system, by reacting with a protein (PR)-thiol to liberate a peptide (R)-thiol and form a mixed disulfide, as follows:

$$\mathrm{PR-SH+R-SS-R \rightarrow R-H+PR-SS-R}$$

Disulfide bonds between proteins have an energy of 49 kcal/mol and are not broken at room temperature, except as the result of a chemical reaction. The effects of oxidizing agents on the rheological properties of a wheat flour dough may be quantitatively explained in terms of the breaking of disulfide cross-links; their reformation may be explained as due to exchange reactions with sulfhydryl groups (Wehrli and Pomeranz 1969). The baking quality of a wheat flour is strongly influenced by its gluten protein quality and content and the disulfide/sulfhydryl ratio. A schematic diagram of the bonds within and between polypeptide chains in a wheat flour dough is given in Fig. 8.31.

Fats

The consistency of fats is commonly determined with a cone penetrometer, as specified in the Official Methods and Recommended Practices of the American Oil Chemists' Society (Method Cc 16–60). Other methods that have frequently been employed involve extrusion; they include the extrusion attachment to the Kramer shear press (Vasic and deMan 1967), an extrusion rheometer used with the Instron Universal Testing Machine (Scherr and Wittnauer 1967), and the FIRA-NIRD extruder (Prentice 1954).

Other devices used for fat-consistency measurements include wire-cutting instruments (sectilometers), penetration of a probe when a sample

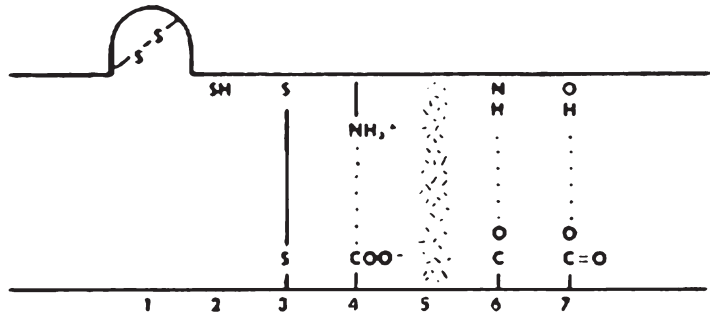


Fig. 8.31 Schematic diagram of bonds within and between polypeptide chains in a wheat flour dough. Solid lines represent covalent bonds; dotted lines represent other bonds: (1) intramolecular disulfide bond, (2) free sulfhydryl group, (3) intermolecular disulfide bond, (4) ionic bond, (5) van der Waals bond, (6) interpeptide hydrogen bond, (7) side-chain hydrogen bond. *Source*: from Bloksma (1972)

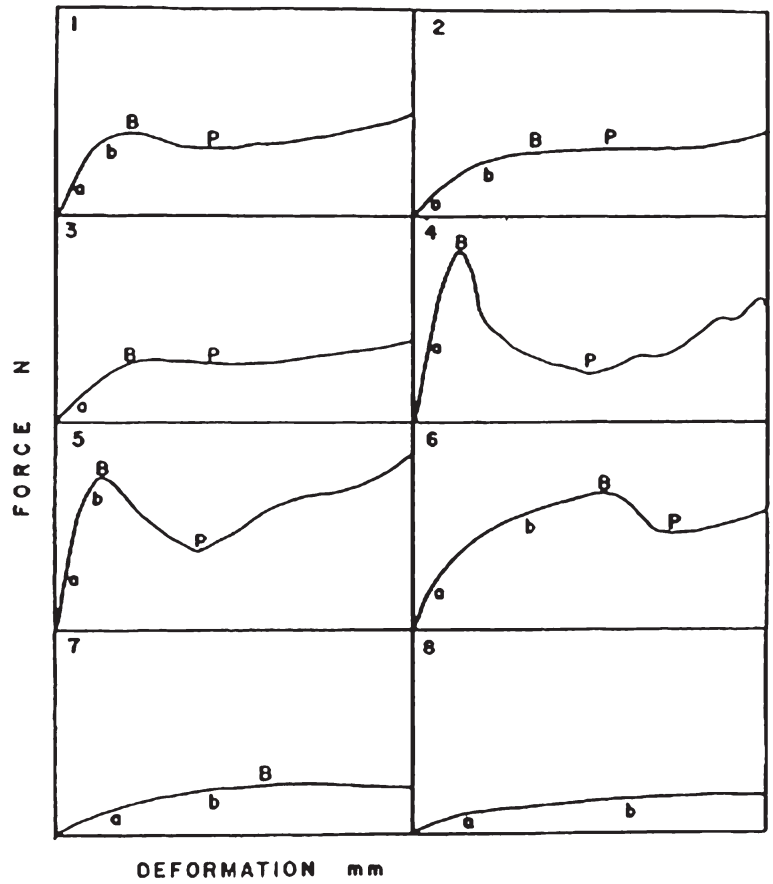


Fig. 8.32 Examples of compression curves for shortenings: (1) and (2) soy-palm; (3) soy-canola-palm; (4) soy only; (5) tallow-lard; (6) lard; (7) palm-vegetable; (8) palm-palm kernel; a = elastic non-recoverable deformation; b = viscous flow; B = breaking force; P = plateau force; distance between B and P is an indication of brittleness

is contained in a small cup, and compression of a cylindrical sample between two parallel plates. The compression method reveals detailed information about plastic fats (deMan et al. 1991), including elasticity, viscous flow, and degree of brittleness. These characteristics are important in shortenings destined for use in cakes and puff pastries. Compression curves for a variety of shortenings are displayed in Fig. 8.32. Temperature treatment of a fat has a profound

effect on its texture. deMan and deMan (1996) studied the effects of crystallization temperature and tempering temperature on the textures of palm oil and hydrogenated fats, using the compression method, and found that lowering the crystallization temperature from 10 to 0 °C resulted in softer textures, especially for palm oil. Increasing the tempering temperature from 25° to 30 °C also resulted in softer textures, especially for hydrogenated fats.

The hardness or consistency of fats is a result of the presence of a three-dimensional network of fat crystals. All fat products, such as margarine, shortening, and butter, are mixtures of solid fat, in crystallized form, and liquid oil. Because the individual glycerides in fats have a wide range of melting points, the ratio of solid-to-liquid fat is highly temperature-dependent. The crystal particles are linked by weak van der Waals forces. These bonds are easily broken by mechanical action during processing, and the consistency may be greatly influenced by such mechanical forces. After a rest period, some of these bonds are reformed, and the reversible sol-gel transformation taking place is called thixotropy. There appear to be two types of bonds in fats—those that are reformed after mechanical action, and those that do not reform. The latter result in a portion of the hardness loss that is irreversible. The nature of these bonds has not been established with certainty, but it is assumed to mainly involve van der Waals forces. The hardness loss of fats, as a result of working, is called work softening and can be expressed as follows:

$$WS = \frac{H_o - H_w}{H_o} \times 100\%$$

where H_o and H_w are the hardness before and after working.

The work softening is influenced not only by the nature of the mechanical treatment, but also by the temperature conditions and the size and quantity of fat crystals.

Tanaka et al. (1971) used a two-element mechanical model (Fig. 8.33) to represent fats as viscoplastic materials. The model consists of a dashpot representing the viscous element, in parallel with a friction element representing the yield value.

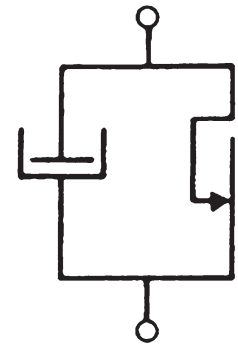


Fig. 8.33 Mechanical model for foods as viscoplastic materials. *Source*: from Tanaka et al. (1971)

The theory of bond formation between the crystal particles in plastic fats needs revision. It has been proposed that a process of "sintering"—the formation of solid bridges between fat crystals—occurs during post-crystallization hardening (Heertje et al. 1987; Johansson and Bergenstahl 1995). The use of the word *sintering* is unfortunate, since it ordinarily describes the fusion of small particles into a solid block; this is not the case, however, because the fat crystals in fats can be suspended in a solvent such as isobutanol (Chawla and deMan 1990), without showing any sign of fusion into larger aggregates. At the solid-fat level found in plastic fats (15–35%), the crystals are tightly packed together and are suggested to exist in a state of entanglement (deMan 1997). Entanglement of crystals is a more realistic description of the structure formation process in fats. The sintering process described above can be considered to be a form of entanglement.

Many interrelated factors influence the texture of plastic fats. Fatty acid and glyceride composition are basic factors in establishing the properties of a fat. These factors, in turn, are related to solid-fat content, crystal size and shape, and polymorphic behavior. Once a crystal network is formed, mechanical treatment and temperature history may influence the texture.

The network systems in plastic fats differ from those in protein or carbohydrate systems. Fat crystals are embedded in liquid oil, and the crystals have no ionized groups. Therefore, the interactive forces in fat crystal networks are weak. The minimum concentration of solid particles in a fat, required to provide a yield value, is in the range of 10–15%.

Fruits and Vegetables

Much of the texture work with fruits and vegetables has been done with the Kramer shear press. The shear press was developed because of the tenderometer's limitations, and has been widely used for measuring the tenderness of peas for processing (Kramer 1961). The shear press has also been used, for example, in the quality method suggested for raw and canned sweet com (Kramer and Cooler 1962). This procedure determines shear force with the standard shear cell, and the amount of juice pressed out with the juice extraction cell. It is possible to relate the quality of corn to these parameters. In addition to the shear press, the Instron Universal Testing Machine and others based on the same principle are popular for use on fruit and vegetable products. A special testing machine has been developed by Mohsenin (1970). This machine uses an air motor for movement of the crosshead, but is otherwise similar to other universal testing machines. A mechanically driven test system has been developed by Voisey (1971). Voisey (1970) has also described a number of test cells that are simpler in design than the standard shear cell of the Kramer shear press.

The texture of fruit and vegetable products is related to the cellular structure of these materials. Reeve and Brown (1968a, b) studied the development of cellular structure in the green bean pod, as it relates to texture and eating quality. Sterling (1968) studied the effect of solutes and pH on the structure and firmness of cooked carrot. Sterling also related histological changes, such as cellular separation and collapse, to product texture. In fruits and vegetables, the relationship between physical structure and physical properties is probably more evident than in many other types of products.

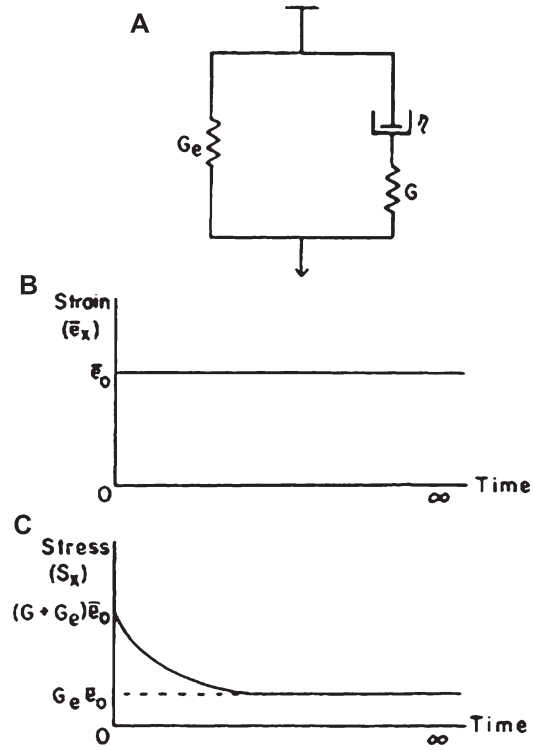


Fig. 8.34 Mechanical model proposed by Morrow and Mohsenin: (**a**) viscoelastic foods, (**b**) the strain-time, and (**c**) stress-time, characteristics of the system

Morrow and Mohsenin (1966) have studied the physical properties of a variety of vegetables; they assumed these products to behave as viscoelastic materials and thus according to the three-element model represented in Fig. 8.34a. Such viscoelastic materials are characterized by the strain-time and stress-time relationships shown in Fig. 8.34b, c, respectively.

Starch

The texture of starch suspensions in water is determined by the source of the starch, the chemical and/or physical modification of the starch granules, and the cooking conditions of the starch (Kruger and Murray 1976). The texture of starch suspensions can be measured by means of a viscoamylograph. The viscosity is recorded, while the temperature of the suspension is raised from 30° to 95 °C, held at 95 °C for 30 min, lowered to

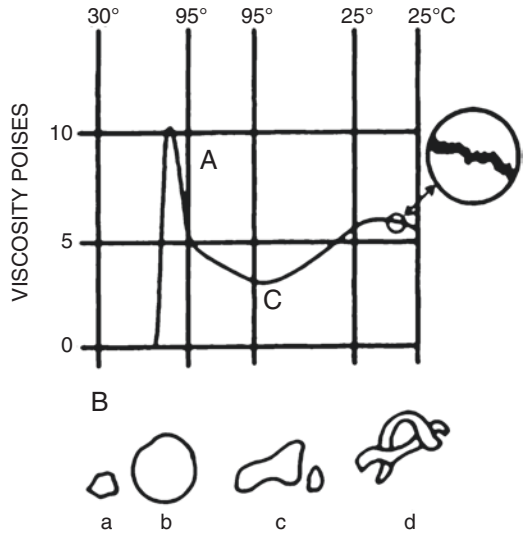


Fig. 8.35 Viscosity and granule appearance in a viscoamylograph test of a 5% suspension of waxy corn starch in water: A = viscosity curve, B = starch granule shape and size, C = magnified portion of curve to indicate cohesiveness; a = unswollen granule, b = swollen granule, c = collapsed granule, d = entwined collapsed granules. *Source*: reprinted from Kruger and Murray (1976)

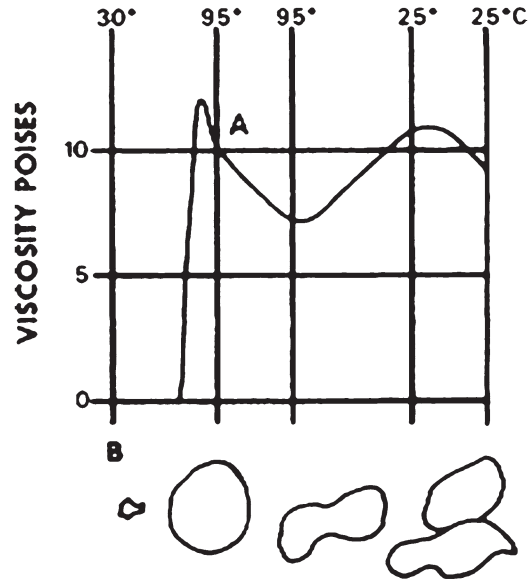


Fig. 8.36 Viscosity and granule appearance in a viscoamylograph test of a 5% aqueous suspension of waxy corn starch with 1 cross-bond per 100,000 glucose units: A = viscosity curve, B = granule appearance. *Source*: reprinted from Kruger and Murray (1976)

25 °C, and held at that temperature for 30 min. The viscosity of a 5% suspension of waxy corn in water is shown in Fig. 8.35. Initially, the viscosity is low, but it increases rapidly at the gelatinization temperature of about 73 °C. As the granules swell, they become more fragile and start to disintegrate, causing the viscosity to drop. When the temperature is lowered to 25 °C, there is another increase in viscosity, caused by the interaction of the broken and deformed granules. This phenomenon is demonstrated by the width and irregularity of the recorded line, which is indicative of the cohesiveness of the starch particles.

Modification of a starch has a profound effect on the texture of its suspensions. Introduction of as little as 1 cross-bond per 100,000 glucose units slows the breakdown of the swollen granules during and after cooking (Fig. 8.36). This results in a higher final viscosity. Increasing the cross-bonding to 1, 3, or 6 cross-bonds per 10,000 glucose units results in no breakdown during the heating cycle (Fig. 8.37). As the cross-bonding increases, the granule is strengthened and does not swell much during heating, but the viscosity is decreased. Most food starches, used at pH values from 4 to 8, have 2 to 3 cross-bonds per 10,000 glucose units.

Waxy corn starch contains only amylopectin; normal corn starch contains both amylopectin and amylose. This results in different viscosity profiles (Fig. 8.38). Normal corn starch shows a lower peak viscosity and less breakdown during heating. After cooling, the viscosity continues to increase, possibly because the amylose interlinks with the amylopectin. On further storage at 25 °C, the slurry sets to a firm gel. Tapioca starch shows behavior intermediate between that of normal corn starch and waxy corn starch (Fig. 8.38). This could be explained by the fact that tapioca amylose molecules are larger than those in normal corn starch.

Starches can be modified with non-ionic or ionic groups. The latter can be made anionic by the introduction of phosphate or succinate groups. Such modified starches have lower gelatinization temperature, higher peak viscosity, and higher final cold viscosity than non-ionically modified starches (Fig. 8.39).

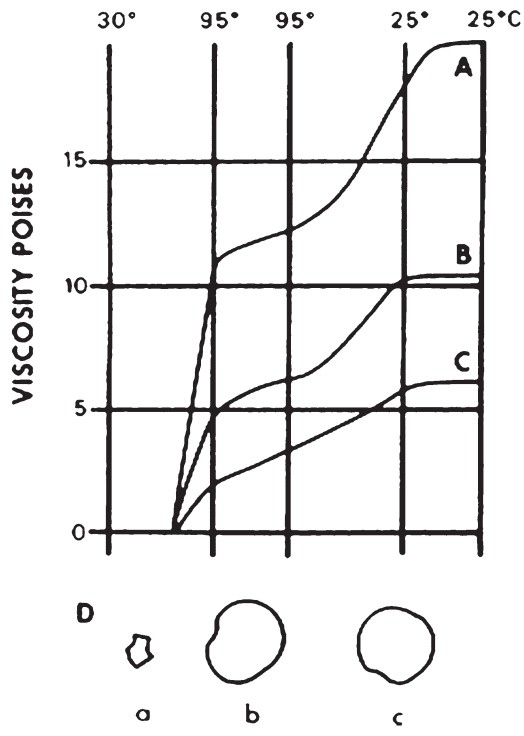


Fig. 8.37 Viscosity and granule appearance in a viscoamylograph test of a 5% aqueous suspension of cross-bonded waxy corn starch: A = 1 cross-bond per 10,000 glucose units, B = 3 cross-bonds, C = 6 cross-bonds, and D = starch granule appearance. *Source*: reprinted from Kruger and Murray (1976)

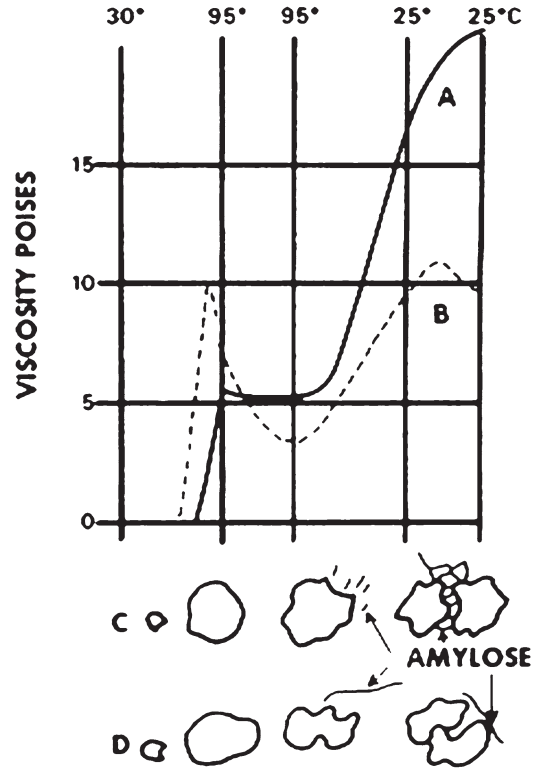


Fig. 8.38 Viscosity and granule appearance in viscoamylograph tests of aqueous suspensions of corn and tapioca starches: A = 6% corn starch, B = 5% tapioca starch, C = corn starch granule appearance, and D = tapioca starch granule appearance. *Source:* reprinted from Kruger and Murray (1976)

Microstructure

With only a few exceptions, food products are non-Newtonian and possess a variety of internal structures. Cellular and fibrous structures are found in fruits and vegetables; fibrous structures are found in meats; and many manufactured foods contain protein, carbohydrate, or fat-crystal networks.

Many such food systems are dispersions that fall within the realm of colloids. Colloids are defined as heterogeneous or dispersed systems that contain at least two phases—a dispersed phase and a continuous phase. Colloids are characterized by their ability to exist in either a sol or a gel form. In the former, the dispersed particles exist as independent entities; in the latter, they associate to form network structures that may entrap large volumes of the continuous phase. The isothermal, reversible sol-gel transformation exhibited by many foods is called thixotropy. Disperse systems can be classified on the basis of particle size. Coarse dispersions have particle sizes greater than 0.5 μm. Their particles can be seen in a light microscope, can be filtered over a paper filter, and will sediment rapidly. Colloidal dispersions have particle sizes in the range of 0.5 μm to 1 nm. Such particles remain in suspension by Brownian movement, and can run through a paper filter, but cannot run through a membrane filter. Particles smaller than these are molecular dispersions or solutions. Depending on the nature of the two phases, disperse systems can be classified into a number of types. A solid dispersed in a

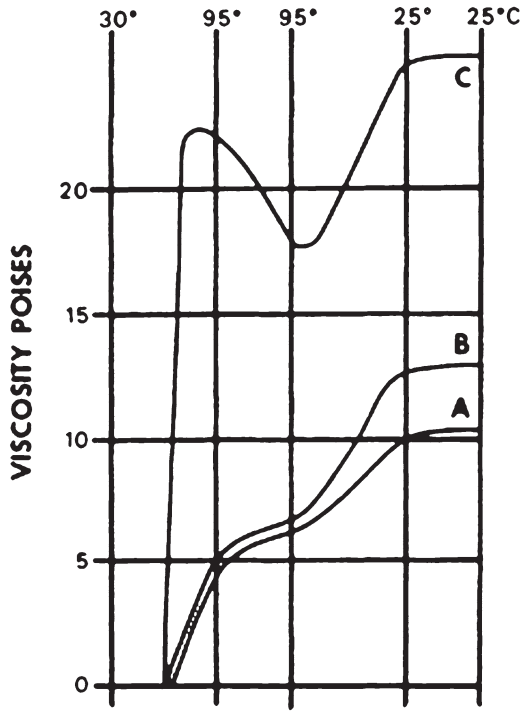


Fig. 8.39 Viscoamylograph viscosity curves for modified waxy corn starches: A = cross-bonded waxy corn starch, B = non-ionically modified cross-bonded waxy corn starch, and C = anionically modified cross-bonded waxy corn starch. *Source*: reprinted from Kruger and Murray (1976)

liquid is called a sol; for example, margarine, which has solid-fat crystals dispersed in liquid oil, is a sol. Dispersions of liquid in liquid are called emulsions; many examples of these are found among foods, such as milk and mayonnaise. Dispersions of gas in liquid are called foams (e.g., whipped cream). In many cases, such dispersions are more complex, and have more than one disperse phase. In fact, many foods have several dispersed phases. For instance, in chocolate, both solid cocoa particles and fat crystals are dispersed phases.

The production of disperse systems is often achieved by dispersion methods, in which the disperse phase is subdivided into small particles by mechanical means. Liquids are emulsified by stirring and homogenization; solids are subdivided by grinding, as, for instance, roller mills are used in chocolate making and colloid mills are used in other food preparations.

An important aspect of the subdivision of a disperse phase is the enormous increase in specific surface area. If a sphere with a radius R = 1 cm is dispersed into particles with radius $r = 10^{-6}$ cm, the area of the interface will increase by a factor of 10^6. The mechanical work, dA, needed to increase the interfacial area is proportional to the area increase, as follows:

$$dA = \sigma dO$$

where O = total interfacial area. The proportionality factor σ is the. surface tension. In the production of emulsions, the surface tension is reduced by using surface-active agents (see Chap. 2).

As particle size is reduced to colloidal dimensions, the particles become subject to Brownian movement. Brownian movement is a result of the random thermal movement of molecules, which impact on colloidal particles to give them a random movement as well (Fig. 8.40). The size of dispersed particles has a profound effect on the properties of dispersions (Schubert 1987). Figure 8.41 shows the qualitative relationships between particle size and system properties. As particle size decreases, fracture resistance increases. The particles become increasingly uniform, which results in a grinding limit, below which particles cannot be further reduced in size. The terminal settling rate, illustrated by a flour particle falling through air, increases rapidly as a function of increasing particle size. According to Schubert (1987), a flour particle of 1 μm in size takes more than 6 h to fall a distance of 1 m in still air. Wetting becomes more difficult as particle size decreases. The specific surface area (the surface per unit volume) increases rapidly with decreasing particle size.

Colloidal systems, because of their large number of dispersed particles, show non-Newtonian flow behavior. For a highly dilute dispersion of spherical particles, the following equation was proposed by Einstein:

$$\eta = \eta_o \left(1 + 2.5\phi\right)$$

where

η_o = viscosity of the continuous phase

phi = ratio of the volumes of the disperse and continuous phases

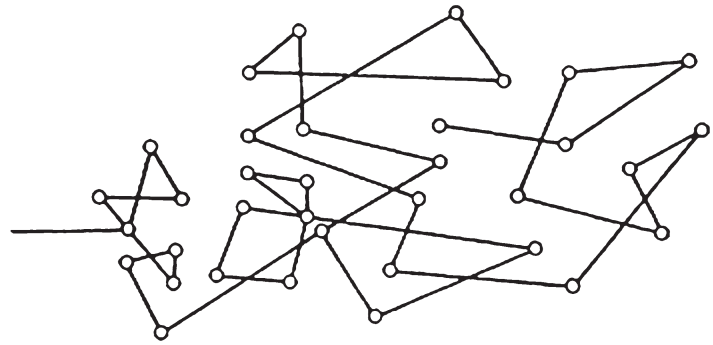
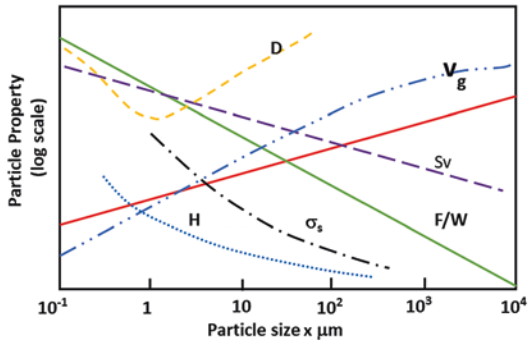


Fig. 8.40 Flat-plane projection of the location of a colloidal particle subject to Brownian movement. *Source*: from Schubert (1987)



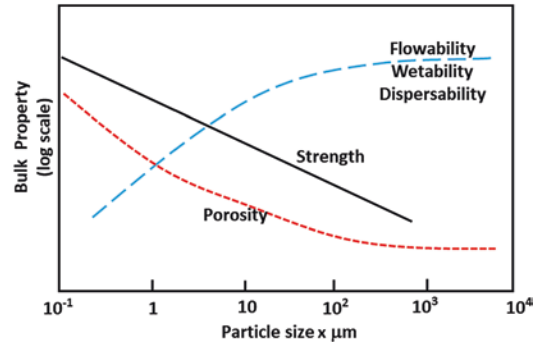


Fig. 8.41 Relationships between particle size and system properties: D = particle deposition in fibrous fillers, F = adhesion force, H = homogeneity of a particle, S_v = surface area per unit volume, W = particle weight, Vg = terminal setting rate, σ_s = particle fracture resistance. *Source:* from Schubert (1987)

In this equation, viscosity is independent of the degree of dispersion. As soon as the ratio of disperse and continuous phases increases to the point where particles start to interact, the flow behavior becomes more complex. The effect of increasing the concentration of the disperse phase on the flow behavior of a disperse system is shown in Fig. 8.42. The disperse phase, as well as the low-solids dispersion (curves 1 and 2), shows Newtonian flow behavior. As the solids content increases, the flow behavior becomes non-Newtonian (curves 3 and 4). Especially with anisotropic particles, interactions between them will result in the formation of three-dimensional network structures. These network structures usually show non-Newtonian flow behavior and viscoelastic properties, and often have a yield value. Network structure formation may occur in emulsions (Fig. 8.43), as well as in particulate systems. The forces between particles, which

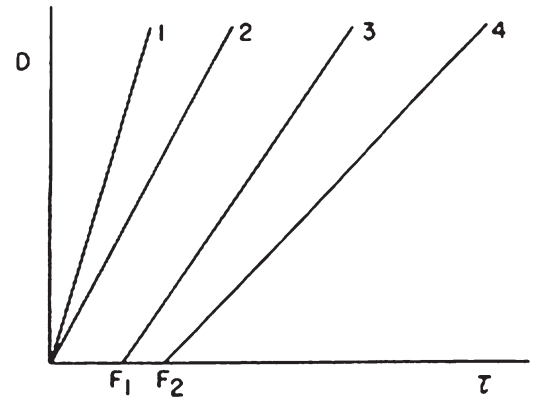


Fig. 8.42 Effect of increasing the concentration of a disperse phase on the flow behavior of a disperse system: 1—continuous phase, 2—low solids content, 3—medium solids content, 4—high solids content

result in the formation of networks, may be van der Waals forces, hydrophobic interactions, or covalent bonds. Network formation may result

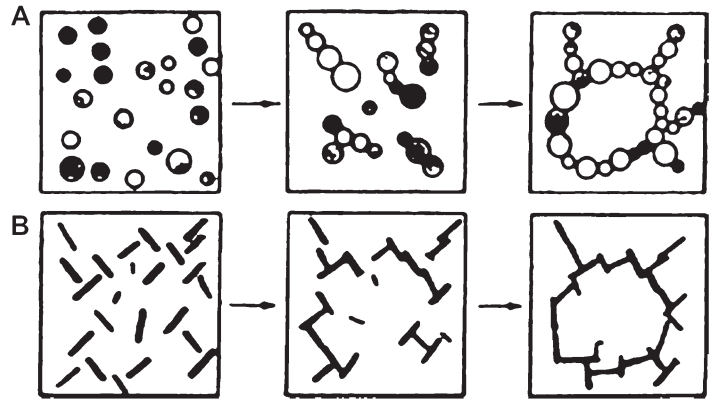


Fig. 8.43 Structure formation in particulate systems: (**a**) flocculation of an emulsion, (**b**) network formation in crystallized fat

Table 8.3 Relationship between critical particle fraction (α_c) and number of bond sites (f)

α_c	f
0.05	21
0.10	11
0.15	8
0.20	6
0.30	4
0.50	3

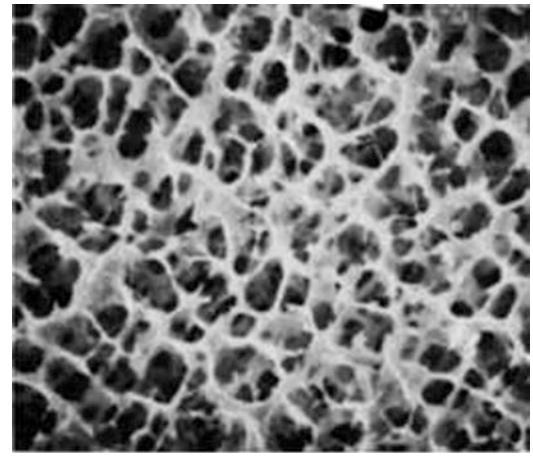


Fig. 8.44 Microstructure of soybean curd (tofu), as seen in a scanning electron microscope

from heating or from chemical reactions that occur spontaneously, involving either components already present in a food or added enzymes or coagulants. The formation of networks requires a minimal fraction of particles to be present, the critical fraction α_c, and the larger the number of sites f, used for bond formation, the sooner a network will form. These two quantities are related as follows:

$$\alpha_c = 1/(f-1)$$

At particle concentrations below 10%, numerous contact points are required to form a network structure (Table 8.3). This means that only certain types of molecules or particles can form networks at such concentrations. As a network is formed, the viscosity increases, until, at a certain point, the system acquires plastic and/or viscoelastic properties. Network formation thus depends on particle concentration, reactive sites on the particles, and particle size and shape. Heertje et al. (1985) investigated structure formation in acidic milk gels and found that the final texture of such products was influenced by many factors, including heat, salt balance, pH, culture, and thickening agents. Structure formation in soy milk, induced by coagulants in the form of calcium or magnesium salts, results in a semi-solid food called tofu, which has a fine internal protein network structure (Fig. 8.44). Hermansson and Larsson (1986) reported on the structure of gluten gels and concluded that such gels consist of a continuous phase of densely packed protein units.

DeMan and Beers (1987) reviewed the factors that influence the formation of three-dimensional fat-crystal networks. The fat-crystal networks in plastic fats are highly thixotropic, and mechanical action on such systems will result in a drastic

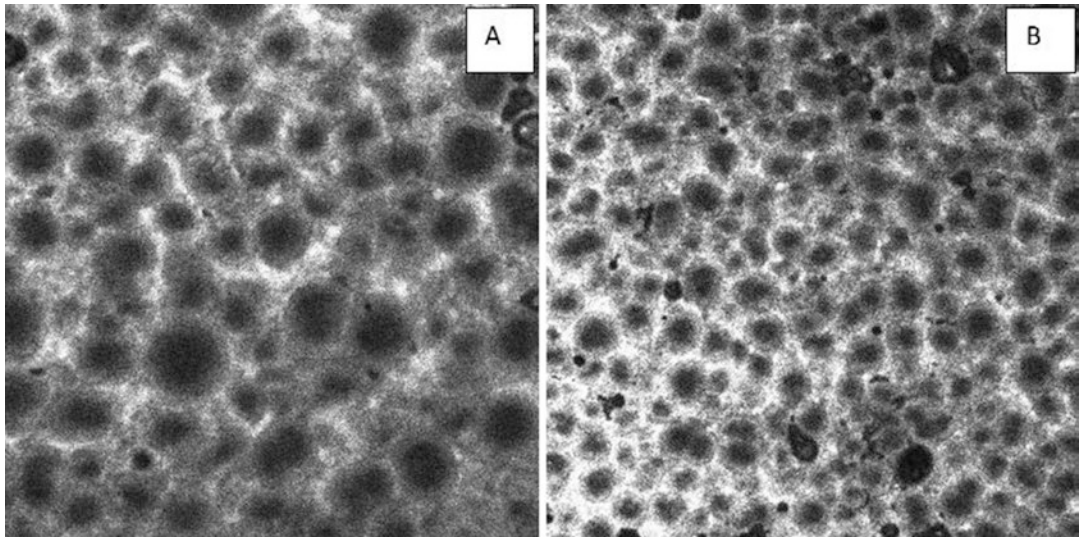


Fig. 8.45 Effect of cooling rate on crystal size for milk fat, after 90 min at 35 °C and 24 h cooling at 10 °C/min: (**a**) slow cooling (0.1 °C/min), and (**b**) fast cooling (5.5 °C/min). http://www.intechopen.com/books/confocal-laser-microscopy-principles-and-applications-in-medicine-biology-and-the-food-sciences/applications-of-confocal-laser-scanning-microscopy-clsm-in-foods

reduction of hardness. Figure 8.45 illustrates the influence of cooling rate on the degree of crystallization in butterfat, which impacts the plasticity of the fat.

A variety of rheological tests can be used to evaluate the nature and properties of different network structures in foods. The strength of bonds in a fat-crystal network can be evaluated by stress relaxation, and by the decrease in elastic recovery in creep tests as a function of loading time (deMan et al. 1985). Van Kleef et al. (1978) reported on a determination of the number of cross-links in a protein gel, from its mechanical and swelling properties. Oakenfull (1984) used shear-modulus measurements to estimate the size and thermodynamic stability of junction zones in non-covalently cross-linked gels.

Dynamic measurements of gels can provide information on the extent of cross-linking (Bell 1989). Systems with a relatively high storage modulus G' show a low value for G"/G', which indicates a highly cross-linked system, such as an agar gel.

Water Activity and Texture

Water activity (a_w) and water content have a profound influence on the textural properties of foods. The three regions of a typical sorption isotherm can be used to classify foods on the basis of their textural properties (Fig. 8.46). Region 3 is the high-moisture area, which includes many soft foods. Foods in the intermediate-moisture area (region 2) appear dry and firm. At the lowest values of a_w (region 1), most products are hard and crisp (Bourne 1987).

Katz and Labuza (1981) examined the relationship between a_w and crispness, in a study of the crispness of popcorn (Fig. 8.47). They found a direct relationship between crispness and a_w.

Many foods contain biopolymers and low-molecular-weight carbohydrates. These materials can be present in a metastable amorphous state that is sensitive to temperature and the state of the water present. The amorphous state can exist in the form of a rubbery liquid structure or a very viscous, glassy solid, as shown in Fig. 8.48

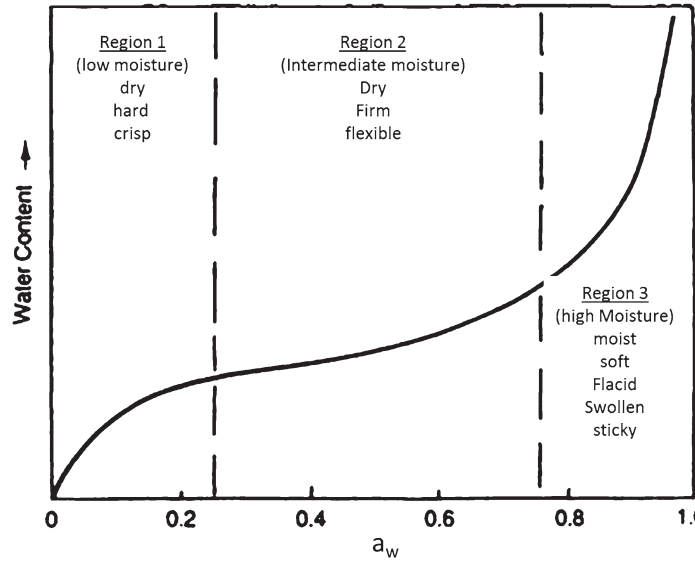


Fig. 8.46 The three regions of a schematic sorption isotherm, as related to the textural properties of food systems. *Source*: reprinted with permission from Bourne (1987)

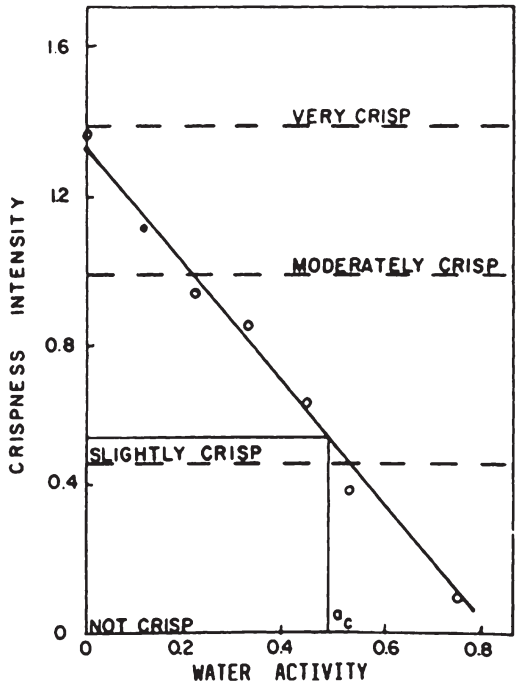


Fig. 8.47 Relationship between the water activity and the crispness of popcorn. *Source*: reprinted with permission from Katz and Labuza (1981)

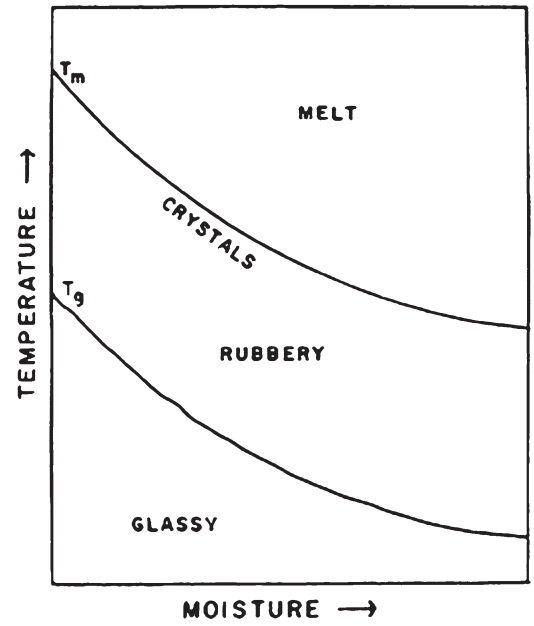


Fig. 8.48 Rubbery liquid and glassy solid states of moisture-containing foods, as affected by temperature: T_m = melting point; T_g = glass transition temperature

(Slade and Levine 1991; Levine and Slade 1992; Roos and Karel 1991). A more detailed analysis of the effect of temperature on textural properties, expressed as modulus, is presented in Fig. 8.49 (Slade and Levine 1991). Below the crystalline melting temperature (T_m), the material enters a state of rubbery liquid flow. As the temperature is lowered further, a leathery state is observed. In the leathery region, the modulus increases sharply, until the glass transition temperature (T_g) is reached, and the material transforms to a glassy solid.

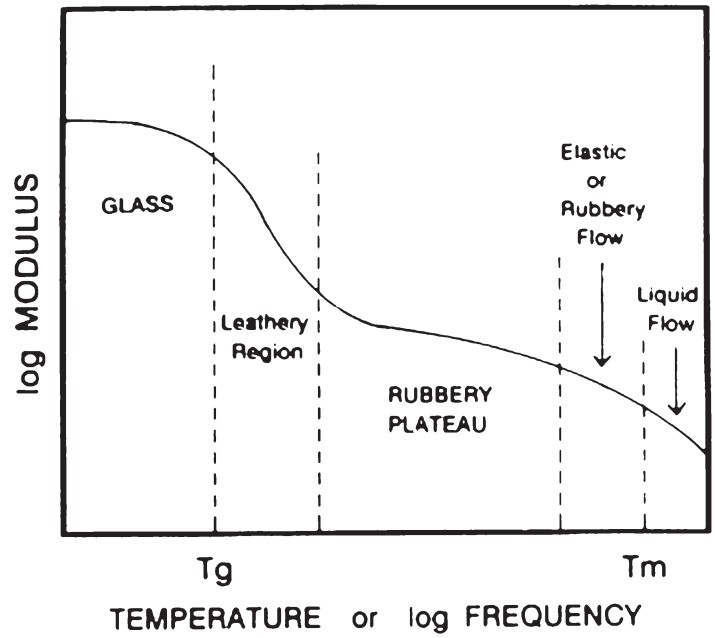


Fig. 8.49 Effect of temperature on texture, expressed in terms of modulus: T_m = crystalline melting temperature, T_g = glass transition temperature. *Source*: from Slade and Levine (1991)

Kapsalis et al. (1970) reported on a study of the textural properties of freeze-dried beef at different points on the moisture-sorption isotherm, over the entire range of water activity. Important changes in textural properties were observed at a_w values of 0.85 and at 0.15–0.30.

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Vitamins

9

John W. Finley and John M. deMan

Introduction

Vitamins are minor components of foods that play an essential role in human nutrition. Many vitamins are unstable under certain conditions of processing and storage (Table 9.1), and their levels in processed foods, therefore, may be considerably reduced. Synthetic vitamins are used extensively to compensate for these losses and to restore vitamin levels in foods. The vitamins are usually divided into two main groups, the water-soluble and the fat-soluble vitamins. The occurrence of the vitamins in the various food groups is related to their water-or fat-solubility. The contribution of foods to vitamins in the diet is varies significantly between food groups and specific products within the food groups. Some vitamins function as part of a coenzyme, without which the enzyme would be ineffective as a biocatalyst. Frequently, such coenzymes are phosphorylated forms of vitamins and play a role in the metabolism of fats, proteins, and carbohydrates. Some vitamins occur in foods as provitamins—compounds that are not vitamins but can be changed by the body into vitamins. Vitamers are members of the same vitamin family.

The contribution of vitamins by foods varies greatly among food groups. Water soluble vitamins are found in fruits and vegetables. Vitamin D is found almost exclusively in animal sources. In Table 9.2 the vitamins found in a range of commonly consumed foods are reported. This data is from the USDA Nutrient data base and more data can be found on raw, and processed foods including various commercial preparations.

Healthy levels of vitamins in the diet refers to the absence of disease based on clinical signs and symptoms of deficiency or excess, and normal function of the individual. The concept of protective nutrient intake for some vitamins refers to an amount which may be protective against a specified health or nutritional risk, for example vitamin C intake of 25 mg with each meal to enhances iron absorption and prevent anemia) (Cook and Reddy 2001). Protective intake levels for a specific vitamin can be expressed either as a daily value or as an amount to be consumed with a meal.

In Fig. 9.1 the acceptable range of intake in the shaded ranges correspond to approaches defining requirements to prevent deficit and excess. The estimated average requirement is the average daily intake required to prevent deficit in half of the population. The recommended nutrient intake is the amount necessary to meet the needs of most (97.5%) of the population, which is defined as the set as the estimated average requirement plus two standard deviations. The tolerable upper intake level is the level at which no evidence of toxicity is observed. In Fig. 9.1 the risk function of deficiency and excess for individuals in a population related to food intake, assuming a Gaussian distribution of requirements to prevent deficit and avoid excess. The upper tolerable nutrient intake

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_9

Table 9.1 Stability of vitamins under different conditions

Vitamin	Vitamer	Unstable to:						Most stable
		UV Light	Heat[a]	O_2	Acid	Base	Metals[b]	
Vitamin A	Retinol	+		+	+		+	Dark, seal
	Retinal			+	+		+	Seal
	Retinoic acid							Good stability
	Dehydroret.			+				Seal
	Ret. esters							Good stability
	β-carotene			+				Seal
Vitamin D	D_2	+	+	+	+		+	Dark, cool, seal
	D_3	+	+	+	+		+	Dark, cool, seal
Vitamin E	Tocopherols		+	+	+	+	+	Cool, neutral pH
	Tocopherol esters				+	+		Good stability
Vitamin K	K	+		+		+	+	Avoid reductants
	MK	+		+		+	+	Avoid reductants
	Menadione	+				+	+	Avoid reductants
Vitamin C	Ascorbic acid			+[b]		+	+	Seal, neutral pH
Thiamine	Disulfide form		+	+	+	+	+	Neutral pH
	Hydrochloride[d]		+	+	+	+	+	Seal, neutral pH
Riboflavin	Riboflavin	+[e]	+			+	+	Dark, pH 1.5–4
Niacin	Nicotinic acid							Good stability
	Nicotinamide							Good stability
Vitamin B_6	Pyridoxal	+	+					Cool
	Pyridoxol (HCl)							Good stability
Biotin	Biotin			+		+		Seal, neutral pH
Pantothenic	Free acid[f]	+		+		+		Cool, neutral pH
Acid	Ca salty[d]		+					Seal, pH 6–7
Folate	FH_4	+	+	+	+[g]		+	Good stability
Vitamin B_{12}	Cn-b_{12}	+			+[h]		+[i]	Good stability

[a]i.e., 100°C
[b]In solution with Fe^{+++} and Cu^{++}
[c]Unstable to reducing agents
[d]Slightly hygroscopic
[e]Especially in alkaline solution
[f]Very hygroscopic
[g]pH < 5
[h]pH < 3
[i]pH > 9
Source: Reprinted with permission from G.F. Combs, *The Vitamins: Fundamental Aspects in Nutrition and Health,* p. 449, © 1992, Academic Press

have been set for some vitamins and other micronutrients and are defined as the maximum intake from food, water and supplements that is unlikely to pose risk of adverse health effects from excess in almost all (97.5%) of healthy individuals in specific age groups. ULs should be based on long-term exposure to all foods, including fortified food products. Most vitamins cause no adverse effects when they are consumed as foods because their absorption and/or excretion are regulated. The special situation of consumption of nutritional supplements which, when added to the nutrient intake from food, may result in a total intake in excess of the upper limit of the nutrient.

Lack of vitamins has long been recognized to result in serious deficiency diseases. It is now also recognized that overdoses of certain vitamins, especially some of the fat-soluble vitamins

which can result in serious toxic effects. For this reason, the addition of vitamins to foods should be carefully controlled.

Our requirements for vitamins changes as a function of age. Table 9.3 illustrates the recommended daily allowance for the water and fat soluble vitamins. Vitamin requirements are listed either in mg/day or in International Units per day (IU). The international units are used in cases where the actual amounts per day are small and more accurately expressed in activity units.

Fat-Soluble Vitamins

Vitamin A (Retinol)

The structural formula of vitamin A is shown in Fig. 9.2. It is an alcohol that occurs in nature predominantly in the form of fatty acid esters. Highest levels of vitamin A are found in certain fish liver oils, such as cod and tuna. Other important sources are mammalian liver, egg yolk, and milk and milk products. The levels of vitamin A and its provitamin carotene in some foods are listed in Table 9.4. The structural formula of Fig. 9.2 shows the unsaturated character of vitamin A. The all-*trans* form is the most active biologically. The 13-*cis* isomer is known as neovitamin A; its biological activity is only about 75% of that of the all-*trans* form. The amount of neo-vitamin A in natural vitamin A preparations is about one-third of the total. The amount is usually much less in synthetic vitamin A. The synthetic vitamin A is made as acetate or palmitate and marketed commercially in the form of oil solutions, stabilized powders, or aqueous emulsions. The compounds are insoluble in water but soluble in fats, oils, and fat solvents.

There are several provitamins A; these belong to the carotenoid pigments. The most important one is β-carotene, and some of the pigments that can be derived from it are of practical importance. These are β-apo-8′-carotenal and β-apo-8′-carotenoic acid ethyl ester (Fig. 9.3). Other provitamins are α- and γ-carotene and cryptoxanthin.

Beta-carotene occurs widely in plant products and has a high vitamin A activity. In theory, one molecule of β-carotene could yield two molecules of vitamin A. The enzyme 15-15′-dioxygenase is able to cleave a β-carotene molecule symmetrically to produce two molecules of vitamin A (Fig. 9.4). This enzyme occurs in intestinal mucosa, but the actual conversion is much less efficient. As shown in Fig. 9.4, there are other reactions that may cause the yield of retinol to be less than two. After cleavage of the β-carotene, the first reaction product is retinal, which is reduced to retinol (Rouseff and Nagy 1994). A general requirement for the conversion of a carotenoid to vitamin A is an unsubstituted β-ionone ring. Citrus fruits are a good source of provitamin A, which results mostly from the presence of β-cryptoxanthin, β-carotene, and α-carotene. Gross (1987) reported a total of 16 carotenoids with provitamin A activity in citrus fruits.

Vitamin A levels are frequently expressed in International Units (IU). One IU equals 0.344 μg of crystalline vitamin A acetate, or 0.300 μg vitamin A alcohol, or 0.600 μg β-carotene. The recommended daily allowance (RDA) of vitamin A of the National Research Council Food and Nutrition Board as seen in Table 9.2. Other sources quote the human requirement at about 1 μg/day. Conditions of rapid growth, pregnancy, or lactation increase the need for vitamin A.

Vitamin A, or retinol, is also known as vitamin A_1. Another form, vitamin A_2, is found in fish liver oils and is 3-dehydroretinol.

The Food and Agriculture Organization and the World Health Organization of the United Nations (FAO/WHO) and the National Academy of Sciences of the United States (1974a) have recommended that vitamin A activity be reported as the equivalent weight of retinol. To calculate total retinol equivalents, it is proposed that food analyses list retinol, carotene, and other provitamin A carotenoids separately. It is also desirable to distinguish between the *cis*- and *trans*- forms of the provitamins in cooked vegetables. By definition, 1 retinol equivalent is equal to 1 μg of retinol, or 6 μg of β-carotene, or 12 μg of other provitamin A carotenoids. The National Academy of Sciences (1974a) states that 1 retinol equivalent is equal to 3.3 IU of retinol or 10 IU of β-carotene.

Table 9.2 Vitamin content of some commonly consumed foods Data from USDA Nutrient Data base

	Water soluble vitamins 100 g food							Fat soluble vitamins 100 g food			
Food	Ascorbic acid (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	B-6 (mg)	Folate (µg)	B-12 (µg)	A (IU)	E (mg)	D (IU)	K (µg)
Vegetables											
Asparagus	5.6	1.143	0.141	0.978	0.091	52	0.00	756	1.13	0	41.6
Pinto beans raw, sprouted	21.7	0.230	0.175	2.280	0.171	118	0.00	2	–	0	–
Green beans, snap raw	12.2	0.082	0.104	0.734	0.141	33	0.00	690	0.41	0	43
Beets, raw	4.9	0.031	0.040	0.334	0.067	109	0.00	33	0.04	0	0.20
Broccoli, raw	89.2	0.071	0.117	0.639	0.175	63	0.00	623	0.78	0	101.6
Carrots, raw	5.9	0.066	0.058	0.983	0.138	19	0.00	16,706	0.66	0	13.2
Corn, sweet yellow	6.8	0.155	0.055	1.770	0.093	42	0.00	187	0.07	0	0.30
Lettuce, iceberg	2.8	0.041	0.025	0.123	0.042	29	0.00	502	0.18	0	24.1
Onions, raw	7.4	0.046	0.027	0.116	0.120	19	0.00	2	0.02	0	0.4
Green peas, raw	40	0.266	0.132	2.090	0.169	65	0.00	765	0.13	0	24.8
Green peppers, raw	80.4	0.057	0.028	0.480	0.224	10	0.00	370	0.37	0	7.4
Potatoes, fresh with skin, raw	19.7	0.081	0.032	1.061	0.298	15	0	2	0.01	0	2.0
Spinach, raw	28.1	0.078	0.189	0.724	0.195	194	0	9377	2.03	0	482.9
Summer squash, all varieties, raw	17.0	0.48	0.142	0.487	0.218	29	0	200	0.12	0	3.0
Fruits											
Apples, raw with skin	4.6	0.017	0.026	0.091	0.041	3	0	54	0.18	0	2.2
Avocados, raw	10	0.067	0.130	1.738	0.277	81	0	146	2.07	0	21.0
Blueberries, raw	9.7	0.037	0.041	0.418	0.052	6	0	3	0.57	0	19.30

Cranberries, raw	14	0.012	0.020	0.101	0.057	1	0	63	1.32	0	5.0
Oranges, Florida, raw	45	0.100	0.040	0.400	0.051	17	0	225	0.18	0	0.0
Pomegranates, raw	10.2	0.067	0.053	0.293	0.075	38	0	0	0.60	0	16.4
Strawberries, raw	58.8	0.024	0.022	0.386	0.047	24	0	12	0.29	0	2.2
Cereals and grains											
Corn flour, whole grain-yellow	0	0.246	0.080	1.90	0.370	25	0	214	0.42	0	0.3
Pasta, dry enriched	0	0.90	0.060	1.70	18	18	0	0	0.11	0	0.1
Rice, brown long grain	0	0.541	0.095	6.494	0.477	23	0	0	0.60	0	0.6
Rice, long grain, unenriched	0	0.224	0.050	5.048	0.452	8	0	0	0.03	0	0.1
Wheat, bread flour unenriched	0	0.080	0.060	1.000	0.037	33	0	2	0.40	0	0.3
Meat, eggs and dairy											
Ground beef, 20% fat uncooked	0	0.43	0.151	4.227	0.323	7	2.14	14	0.17	3	1.8
Beef tenderloin steak	0	0.071	0.441	6.235	0.765	7	4.27	5	0.28	3	1.4
Lamb, whole leg	0	0.141	0.287	4.964	0.359	2.70	2.70	–	–	–	–
Pork, fresh, composite of trimmed retail cuts (leg, loin, shoulder, and spareribs), raw	0.5	0.841	0.254	4.504	0.445	5	–	7	–	–	–
Chicken, broilers or fryers, back, meat and skin, raw	1.6	0.051	0.116	4.935	0.190	6	0.25	251	0.37	–	2.4
Cheese, cheddar	0	0.29	4.28	0.059	0.066	27	1.10	1242	0.71	24	4.2
Milk, whole, 3.25% milkfat Not fortified	0	0.046	0.169	0.089	0.036	5	0.45	46	162	2	0.3
Egg, raw. fresh	0	0.004	0.4390	0.105	0.005	4	0.09	0	0	0	0

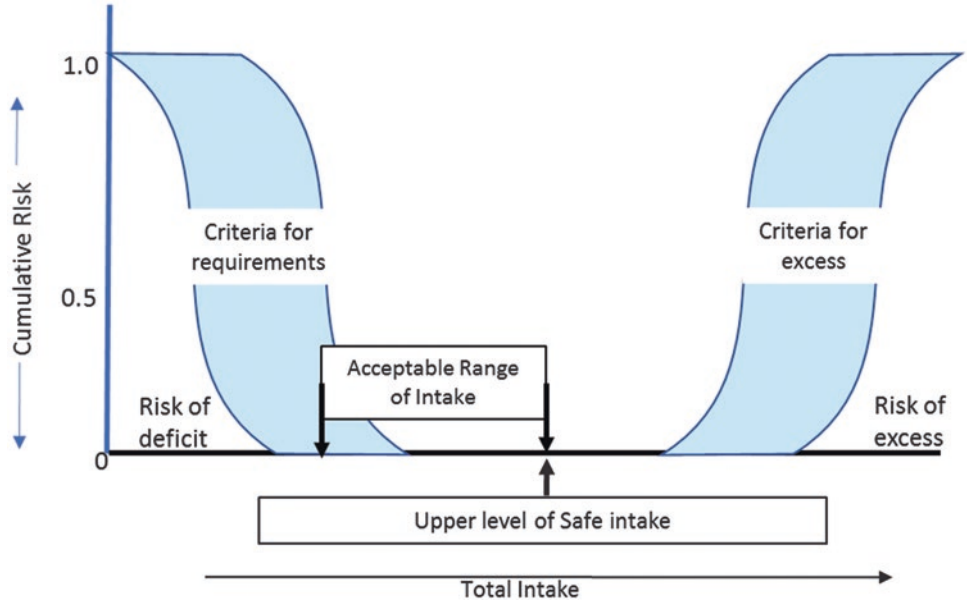


Fig. 9.1 Risk function of deficiency or excess for individuals in a population related to vitamin intake assuming a Gaussian distribution of requirements to prevent deficit and avoid excess

Vitamin A occurs only in animals and not in plants. The A_1 form occurs in all animals and fish, the A_2 form in freshwater fish and not in land animals. The biological value of the A_2 form is only about 40% of that of A_1. Good sources of provitamin A in vegetable products are carrots, sweet potatoes, tomatoes, and broccoli. In milk and milk products, vitamin A and carotene levels are subject to seasonal variations. Hartman and Dryden (1978) report the levels of vitamin A in fluid whole milk in winter at 1083 IU/L and in summer at 1786 IU/L. Butter contains an average of 2.7 μg of carotene and 5.0 μg of vitamin A per g during winter and 6.1 μg of carotene and 7.6 μg of vitamin A per g during summer.

Vitamin A is used to fortify margarine and skim milk. It is added to margarine at a level of 3525 IU per 100 g. Some of the carotenoids (provitamin A) are used as food colors.

Vitamin A is relatively stable to heat in the absence of oxygen (Table 9.5). Because of the highly unsaturated character of the molecule, it is quite susceptible to oxidation—especially under the influence of light, whether sunlight or artificial light. Vitamin A is unstable in the presence of mineral acids but stable in alkali. Vitamin A and the carotenoids have good stability during various food processing operations. Losses may occur at high temperatures in the presence of oxygen. These compounds are also susceptible to oxidation by lipid peroxides, and conditions favoring lipid oxidation also result in vitamin A breakdown. The prooxidant copper is especially harmful, as is iron to a lesser extent. Pasteurization of milk does not result in vitamin A loss, but exposure to light does. It is essential, therefore, that sterilized milk be packaged in light-impervious containers. Possible losses during storage of foods are more affected by duration of storage than by storage temperature. Blanching of fruits and vegetables helps prevent losses during frozen storage.

Vitamin A added to milk is more easily destroyed by light than the native vitamin A. This is not because natural and synthetic vitamin A are different, but because these two types of vitamin A are dispersed differently in the milk (deMan 1981). The form in which vitamin A is added to food products may influence its stability. Vitamin A in beadlet form is more stable than that added as a solution in oil. The beadlets are stabilized by a protective coating. If this coating is damaged by water, the stability of the vitamin is greatly reduced (deMan 1986).

Table 9.3 Relative daily requirements of water and fat soluble vitamins as a function of age (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx)

	Water soluble vitamins							Fat soluble vitamins			
Age	Vitamin C (mg)	Folate (mcg)	Niacin (mg)	Pantothenic acid (mg)	Riboflavin (mg)	B-6 (mg)	B-12 (mcg)	A (IU)	D (IU)	E (IU)	K (mcg)
1–3	15	150	6	2	0.5	0.5	0.9	1000	600	13 (S) 9 (N)	30
4–8	25	200	8	3	0.6	0.6	1.2	1300	600	16 (S) 10(N)	55
9–13	45	300	12	4	0.9	1.0	1.8	2000	600	24 (S) 16(N)	60
14–18	75(m) 65 (f)	400	16 (m) 14 (f)	5	1.3 (m) 1 (f)	1.3 (m) 1.2 (f)	2.4	1000	600	33 (S) 22(N)	75
Adult	90 (m) 75 (f)	400	16 (m) 14 (f)	5	1.3 (m) 1.1 (f)	1.3 (19–50) 1.5 (50+)	2.4	3000 (m) 2300 (f)	600 800 (51+)	33 (S) 22(N)	120 (m) 90 (f)

m male, *f* female, *S* synthetic, *N* natural

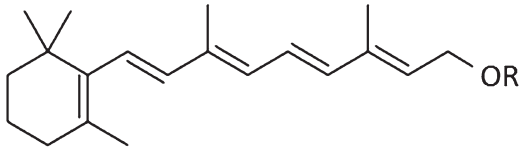


Fig. 9.2 Structural formula of vitamin A. Acetate: R=CO CH_3. Palmitate: R=CO $(CH_2)_{14}$ CH_3

Table 9.4 Vitamin A and carotene content of some foods

Product	Vitamin A (IU/100 g)	Carotene (mg/100 g)
Beef (grilled sirloin)	37	0.04
Butter (May–November)	2363–3452	0.43–0.77
Cheddar cheese	553–1078	0.07–0.71
Eggs (boiled)	165–488	0.01–0.15
Herring (canned)	178	0.07
Milk	110–307	0.01–0.06
Tomato (canned)	0	0.5
Peach	0	0.34
Cabbage	0	0.3
Broccoli (boiled)	0	2.5
Spinach (boiled)	0	6.0

Vitamin D

This vitamin occurs in several forms; the two most important are vitamin D_2, or ergocalciferol, and vitamin D_3, or cholecalciferol. The structural formulas of these compounds are presented in Fig. 9.5. Vitamin D does not occur in plant products. Vitamin D_2 occurs in small amounts in fish liver oils; vitamin D_3 is widely distributed in animal products, but large amounts occur only in fish liver oils. Smaller quantities of vitamin D_3 occur in eggs, milk, butter, and cheese (Table 9.6).

The precursors of vitamins D_2 and D_3 are ergosterol and 7-dehydrocholesterol, respectively. These precursors or provitamins can be converted into the respective D vitamins by irradiation with ultraviolet light. In addition to the two major provitamins, there are several other sterols that can acquire vitamin D activity when irradiated. The provitamins can be converted to vitamin D in the human skin by exposure to sunlight. Because very few foods are good sources of vitamin D, humans have a greater likelihood of vitamin D deficiency than of any other vitamin deficiency. Enrichment of some foods with vitamin D has significantly helped to eradicate rickets, which is a vitamin D deficiency disease. Margarine and milk are the foods commonly used as carrier for added vitamin D.

The unit of activity of vitamin D is the IU, which is equivalent to the activity of 1 mg of a standard preparation issued by the WHO. One IU is also equivalent to the activity of 0.025 μg of pure crystalline vitamin D_2 or D_3. The human requirement amounts to 400–500 IU but increases to 1000 IU during pregnancy and lactation. Adults who are regularly exposed to sunlight are likely to have a sufficient supply of vitamin D. Excessive intakes are toxic.

Vitamin D is extremely stable, and little or no loss is experienced in processing and storage. Vitamin D in milk is not affected by pasteurization, boiling, or sterilization (Hartman and Dryden 1978). Frozen storage of milk or butter also has little or no effect on vitamin D levels, and the same result is obtained during storage of dry milk.

The vitamin D potency of milk can be increased in several ways: by feeding cows substances that are high in vitamin D activity, such as irradiated yeast; by irradiating milk; and by adding vitamin D concentrates. The latter method is now the only commonly used procedure. The practice of irradiating milk to increase the vitamin D potency has been discontinued, undoubtedly because of the deteriorative action of the radiation on other milk components. Vitamin D is added to milk to provide a concentration of 400 IU per quart. Addition of vitamin D to margarine is at a level of 550 IU per 100 g.

Tocopherols (Vitamin E)

The tocopherols are derivatives of tocol, and the occurrence of a number of related substances in animal and vegetable products has been demonstrated. Cottonseed oil was found to contain α-, β-, and γ-tocopherol, and a fourth, δ-tocopherol, was isolated from soybean oil. Several other tocopherols have been found in other products, and Morton (1967) suggests that there are

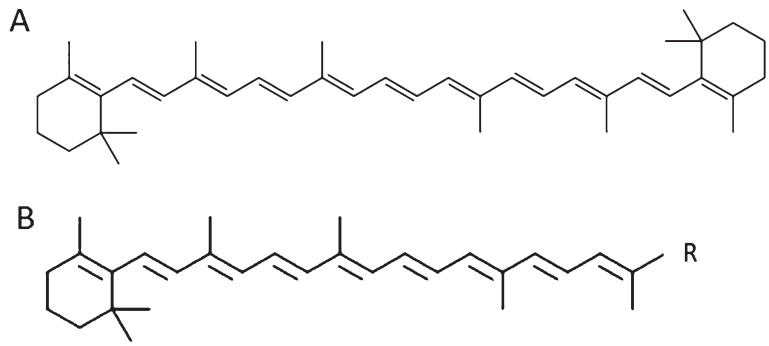


Fig. 9.3 Structural formulas of some provitamins A. (**a**) β-carotene, and (**b**) apocarotenal (R=CHO) and apocarotenoic acid ester (R=$COOC_2H_5$)

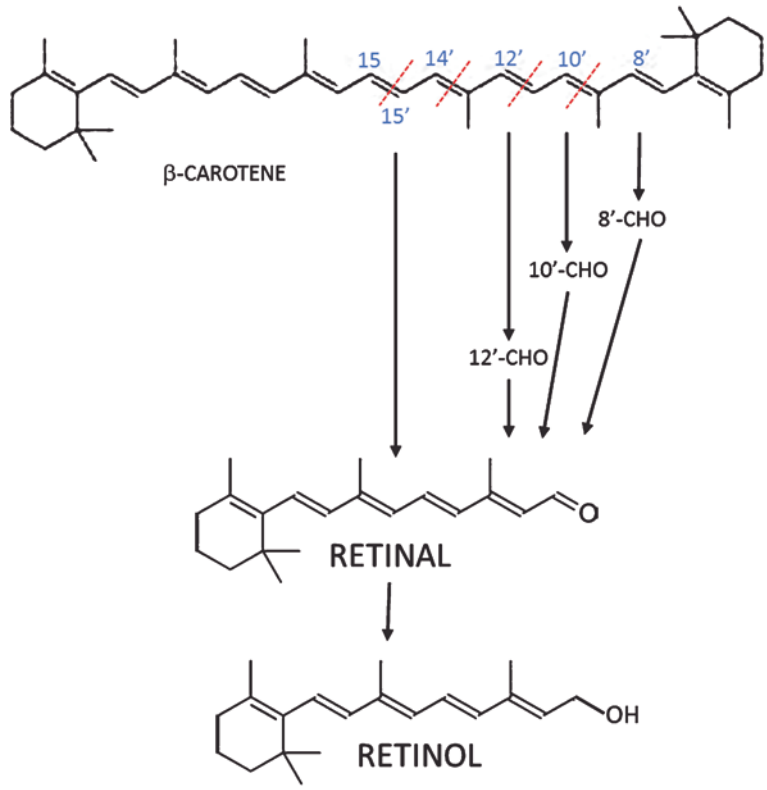


Fig. 9.4 Conversion of beta-carotene to vitamin A

four tocopherols and four tocotrienols. The tocotrienols have three unsaturated isoprenoid groups in the side chain. The structure of tocol is given in Fig. 9.6 and the structures of the tocopherols and tocotrienols in Fig. 9.7. The four tocopherols are characterized by a saturated side chain consisting of three isoprenoid units. The tocotrienols have three double bonds at the 3′, 7′, and 11′ carbons of the isoprenoid side chain (Fig. 9.7). The carbons at locations 4′ and 8′ in the side chains of the tocopherols are asymmetric, as is the number 2 carbon in the chroman ring. The resulting possible isomers are described as having R or S rotation. The natural tocopherols and tocotrienols are predominantly RRR isomers. Stocker and Kearney (2004) have summarized the chemistry of the oxidation of tocopherols as shown in Fig. 9.8.

Table 9.5 Vitamin A and carotene stability in foods

Product	Nutrient content	Storage conditions	Retention (%)
Vitamin A			
Butter	17,000–30,000 IU/Ib	12 months @ 5 °C	66–98
		5 months @ 28 °C	64–68
Margarine	15,000 IU/Ib	6 months @ 5 °C	89–100
		6 months @ 23 °C	83–100
Nonfat dry milk	10,000 IU/Ib	3 months @ 37 °C	94–100
		12 months @ 23 °C	69–89
Fortified ready-to-eat cereal	4000 IU/oz	6 months @ 23 °C	83
Fortified potato chips	700 IU/100 g	2 months @ 23 °C	100
Carotene			
Margarine	3 mg/Ib	6 months @ 5 °C	98
		6 months @ 23 °C	89
Lard	3.3 mg/lb	6 months @ 5 °C	100
		6 months @ 23 °C	100
Dried egg yolk	35.2 mg/100 g	3 months @ 37 °C	94
		12 months @ 23 °C	80
Carbonated beverage	7.6 mg/29 oz	2 months @ 30 °C	94
		2 months @ 23 °C	94
Canned juice drinks	0.6–1.3 mg/8 fl oz	12 months @ 23 °C	85–100

Source: From E. deRitter, Stability Characteristics of Vitamins in Processed Foods, *Food Technol.,* Vol. 30, pp. 48–51, 54, 1976

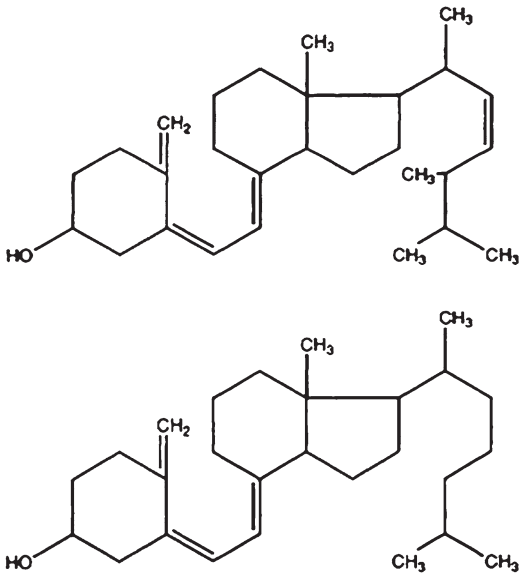


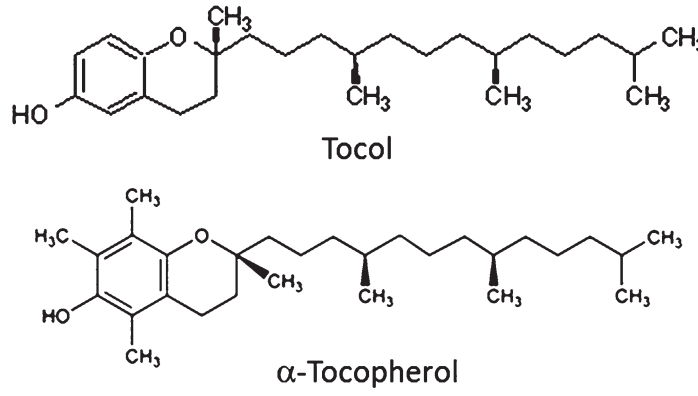
Fig. 9.5 Structural formulas of (**a**) vitamin D_2 and (**b**) vitamin D_3

On oxidation, α-tocopherol can form a meta-stable epoxide that can be irreversibly converted to α-tocopherolquinone. Reduction of the quinone yields a quinol. Tocopherolquinones occur naturally. Oxidation with nitric acid yields the *o*-quinone or tocopherol red, which is not found in nature. Alpha-tocopheronic acid and α-tocopheronolactone are some of the products of metabolism of tocopherol. Much of the biological activity of the tocopherols is related to their antioxidant activity. Because α-tocopherol is the most abundant of the different tocopherols, and because it appears to have the greatest biological activity, the α-tocopherol content of foods is usually considered to be most important.

Table 9.6 Vitamin D content of some foods

Product	Vitamin D (μg/1000 g edible portion)
Liver (beef, pork)	2–5
Eggs	44
Milk	0.9
Butter	2–40
Cheese	12–47
Herring oil	2500

The biological activity of the tocopherols and tocotrienols varies with the number and position of the methyl groups on the chroman ring and by



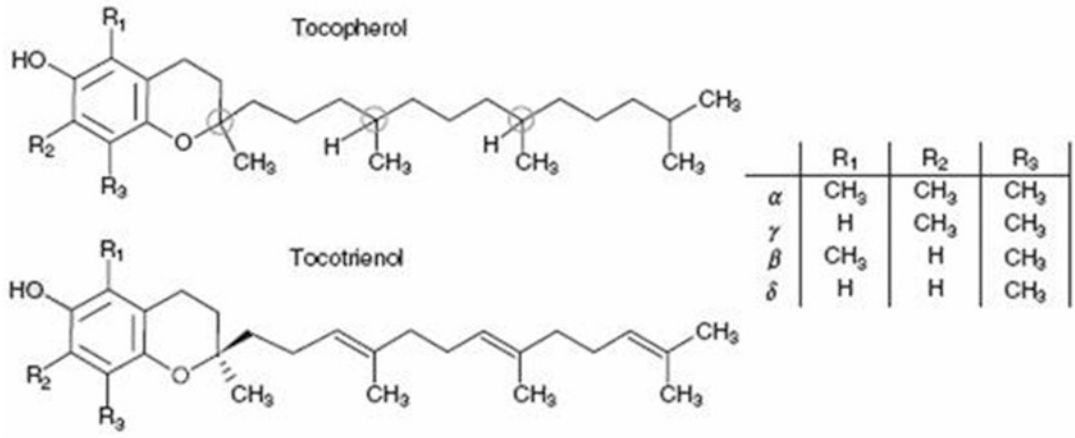


Fig. 9.6 Structural formula of tocol and α-tocopherol

Fig. 9.7 Chemical structure of the tocopherols and tocotrienols

the configuration of the asymmetric carbons in the side chain. The R configuration at each chiral center has the highest biological activity. Because the different isomers have different activities, it is necessary to measure each homolog and convert these to RRR-α-tocopherol equivalents (α-TE). One α-TE is the activity of 1 mg of RRR-α-tocopherol (Eitenmiller 1997). The vitamin E activity of α-tocopherol isomers and synthetic tocopherols is listed in Table 9.7.

Tocopherols are important as antioxidants in foods, especially in vegetable oils. With few exceptions, animal and vegetable products contain from about 0.5 to 1.5 mg/100 g; vegetable oils from 10 to 60 mg/100 g; and cereal germ oils, which are a very good source, from 150 to 500 mg/100 g. Vegetable oils have the highest proportion of α-tocopherol, which amounts to about 60% of the total tocopherols. Refining of vegetable oils, carried out under normal precautions (such as excluding air), appears to result in little destruction of tocopherol. The tocopherol and tocotrienol content of selected fats and oils and their primary homologs are listed in Table 9.8. The seed oils contain only tocopherol. Tree oils, palm, palm kernel, coconut oil, and rice bran oil also contain major amounts of tocotrienols. The processing of vegetable oils by deodorization or physical refining removes a considerable portion of the tocopherols, and these steam-volatile compounds accumulate in the fatty acid distillate (Ong 1993). This product is an important source of natural vitamin E preparations. Baltes (1967) carried out tests in which two

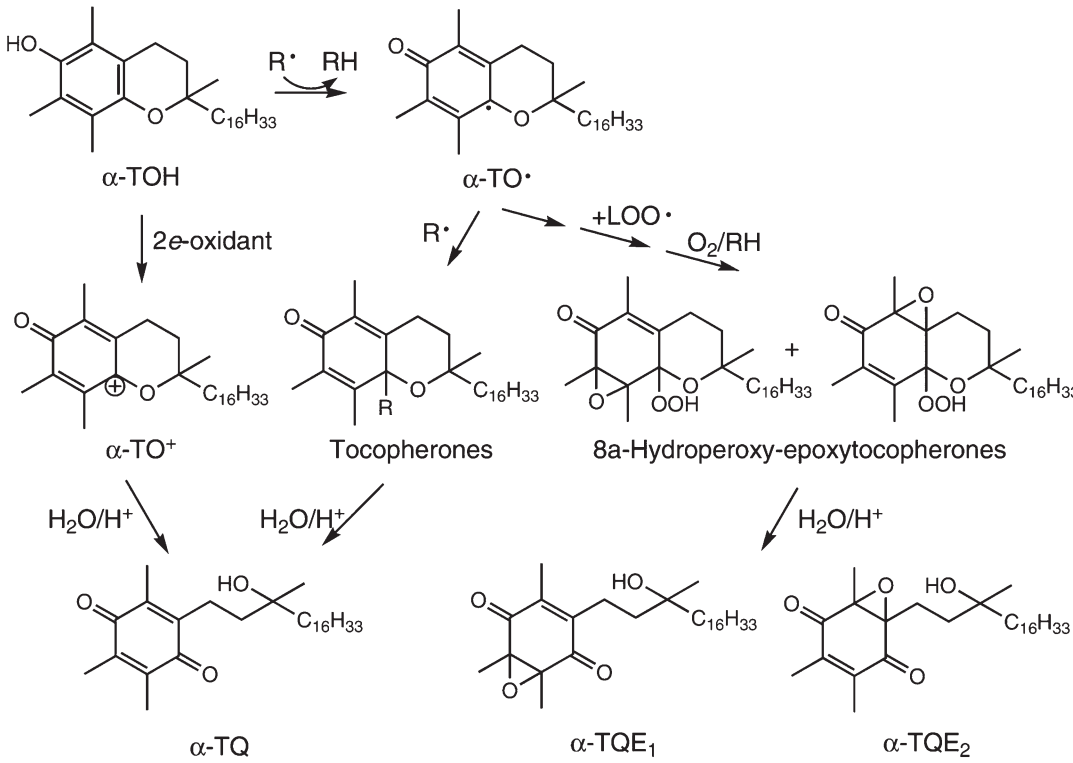


Fig. 9.8 The major pathways of α-TOH oxidation. Two-electron oxidants such as HOCl and $ONOO^-$ oxidize α-TOH to the intermediate tocopheroxylium cation ($\alpha\text{-TO}^+$), which hydrolyzes to α-tocopherylquinone (α-TQ). Radical oxidants (R) generate the α-TO that can further scavenge radicals, to produce 8a-substituted tocopherone adducts, or scavenge LOO, to produce 8a-hydroperoxy-epoxytocopherones. These hydrolyze to α-TQ and α-tocopheryl quinone epoxides (TQEs), respectively (Stocker and Kearney 2004)

Table 9.7 Vitamin E activity of α-tocopherol isomers and synthetic tocopherols

Name	IU/mg
d-α-tocopherol (2R4′R8′R) RRR-α-tocopherol	1.49
1-α-tocopherol (2S4′R8′R)	0.46
dl-α-tocopherol all-rac-α-tocopherol	1.10
2R4′R8′S-α-tocopherol	1.34
2S4′R8′S-α-tocopherol	0.55
2S4′S8′S-α-tocopherol	1.09
2S4′S8′R-α-tocopherol	0.31
2R4′S8′R-α-tocopherol	0.85
2S4′S8′S-α-tocopherol	1.10
d-α-tocopheryl acetate RRR-α-tocopheryl acetate	1.36
dl-α-tocopherol all-rac-α-tocopherol acetate	1.00

Source: Reprinted with permission from R.R. Eiten-miller, Vitamin E Content of Fats and Oils: Nutritional Implications, *Food Technol.*, Vol. 51, no. 5, p. 79, © 1997, Institute of Food Technologists

easily oxidizable fats, lard and partially hydrogenated whale oil, were stabilized with α-tocopherol and ascorbyl palmitate and citric acid as synergists. Without antioxidants, these fats cannot be used in the commercial food chain. Amounts of α-tocopherol ranging from 0.5 to 10 mg/100 g were effective in prolonging the storage life of some samples up to 2 years.

The tocopherol content of some animal and vegetable products as reported by Thaler (1967) is listed in Table 9.9. Cereals and cereal products are good sources of tocopherol (Table 9.10). The distribution of tocopherol throughout the kernels is not uniform, and flour of different degrees of extraction can have different tocopherol levels. This was shown by Menger (1957) in a study of wheat flour (Table 9.11).

Processing and storage of foods can result in substantial tocopherol losses. An example is

Table 9.8 Tocopherol (T) and tocotrienol (T3) content of vegetable oils and their primary homologs

Fats and oils	Total T + T3 (mg/100 g)	α-TE/100 g	%T	%T3	Primary homologs
Sunflower	46–67	35–63	100	0	α-T, γ-T
Cottonseed	78	43	100	0	α-T, γ-T
Safflower	49–80	41–46	100	0	α-T, δ-T, γ-T, β-T
Safflower—high linolenic	41	41	100	0	α-T, β-T
Safflower—high oleic	32	31	100	0	α-T, β-T, γ-T
Palm	89–117	21–34	17–55	45–83	α-T, α-T3, δ-T3, α-T, δ-T3
Canola	65	25	100	0	γ-T, α-T, δ-T, α-T3(Tr), β-T(Tr)
Corn	78–109	20–34	95	5	γ-T, α-T, δ-T, γ-T3, δ-T3
Soybean	96–115	17–20	100	0	γ-T, δ-T, α-T
Rice bran	9–160	0.9–41	19–49	51–81	γ-T3, αT, α-T3, β-T, β-T3
Peanut	37	16	100	0	γ-T, α-T, δ-T
Olive	5.1	5.1	100	0	α-T
Cocoa butter	20	3.0	99	1	γ-T, δ-T, α-T, α-T3
Palm kernel	3.4	1.9	38	62	α-T3, α-T
Butter	1.1–2.3	1.1–2.3	100	0	α-T
Lard	0.6	0.6	100	0	α-T
Coconut	1.0–3.6	0.3–0.7	31	69	γ-T3, α-T3, δ-T, α-T, β-T3

Source: Reprinted with permission from R.R. Eitenmiller, Vitamin E Content of Fats and Oils: Nutritional Implications, *Food Technol.*, Vol. 51, no. 5, p. 80, © 1997, Institute of Food Technologists

Table 9.9 Tocopherol content of some animal and vegetable food products

Product	Total tocopherol as α-tocopherol (mg/100 g)
Beef liver	0.9–1.6
Veal, lean	0.9
Herring	1.8
Mackerel	1.6
Crab, frozen	5.9
Milk	0.02–0.15
Cheese	0.4
Egg	0.5–1.5
Egg yolk	3.0
Cabbage	2–3
Spinach	0.2–6.0
Beans	1–4
Lettuce	0.2–0.8 (0.06)
Peas	4–6
Tomato	0.9 (0.4)
Carrots	0.2 (0.11)
Onion	0.3 (0.22)
Potato	(0.12)
Mushrooms	0.08

Source: From H. Thaler, Concentration and Stability of Tocopherols in Foods, in *Tocopherols*, K. Lang, ed., 1967, Steinkopff Verlag, Darmstadt, Germany

Table 9.10 Tocopherol content of cereals and cereal products

Product	Total tocopherol as α-tocopherol (mg/100 g)
Wheat	7–10
Rye	2.2–5.7
Oats	1.8–4.9
Rice (with hulls)	2.9
Rice (polished)	0.4
Corn	9.5
Whole wheat meal	3.7
Wheat flour	2.3–5.4
Whole rye meal	2.0–4.5
Oat flakes	3.85
Corn grits	1.17
Corn flakes	0.43
White bread	2.15
Whole rye bread	1.3
Crisp bread	4.0

Source: From H. Thaler, Concentration and Stability of Tocopherols in Foods, in *Tocopherols*, K. Lang, ed., 1967, Steinkopff Verlag, Darmstadt, Germany

given in Table 9.12, where the loss of tocopherol during frying of potato chips is reported. After only 2 weeks' storage of the chips at room

Table 9.11 Tocopherol content of wheat and its milling products

Product	Ash (%)	Tocopherol mg/100 g (dry basis)
Whole wheat	2.05	5.04
Flour 1 (fine)	1.68	5.90
Flour 2	1.14	4.27
Flour 3	0.84	3.48
Flour 4	0.59	2.55
Flour 5	0.47	2.35
Flour 6 (coarse)	0.48	2.13
Germ	4.10	25.0

Source: From A. Menger, Investigation of the Stability of Vitamin E in Cereal Milling Products and Baked Goods, *Brot. Gebäck*, Vol. 11, pp. 167–173, 1957 (German)

Table 9.12 Tocopherol losses during processing and storage of potato chips

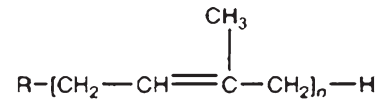
	Tocopherol (mg/100 g)	Loss (%)
Oil before use	82	–
Oil after use	73	11
Oil from fresh chips	75	–
After 2 weeks at room temperature	39	48
After 1 month at room temperature	22	71
After 2 months at room temperature	17	77
After 1 month at −12 °C	28	63
After 2 months at −12 °C	24	68

temperature, nearly half of the tocopherol was lost. The losses were only slightly smaller during storage at freezer temperature. Boiling of vegetables in water for up to 30 min results in only minor losses of tocopherol. Baking of white bread results in a loss of about 5% of the tocopherol in the crumb.

The human daily requirement of vitamin E is estimated at 30 IU. Increased intake of polyunsaturated fatty acids increases the need for this vitamin.

Vitamin K

This vitamin occurs in a series of different forms, and these can be divided into two groups. The first is vitamin K_1 (Fig. 9.9), characterized by one double bond in the side chain. The vitamins K_2 have a side chain consisting of a number of regular units of the type.



where *n* can equal 4, 5, 6, 7, and so forth.

Vitamin K_1 is slowly decomposed by atmospheric oxygen but is readily destroyed by light. It is stable against heat, but unstable against alkali.

The human adult requirement is estimated at about 4 mg per day. Menadione (2-methyl 1,4-naphtoquinone) is a synthetic product and has about twice the activity of naturally occurring vitamin K.

Vitamin K occurs widely in foods and is also synthesized by the intestinal flora. Good sources of vitamin K are dark green vegetables such as spinach and cabbage leaves, and also cauliflower, peas, and cereals. Animal products contain little vitamin K_1, except for pork liver, which is a good source.

The Vitamin K levels in some foods, expressed in menadione units, are given in Table 9.13.

Water-Soluble Vitamins

Vitamin C (L-Ascorbic Acid)

This vitamin occurs in all living tissues, where it influences oxidation-reduction reactions. The major source of L-ascorbic acid in foods is vegetables and fruits (Table 9.14).

L-ascorbic acid (Fig. 9.10) is a lactone (internal ester of a hydroxycarboxylic acid) and is characterized by the enediol group, which makes it a strongly reducing compound. The D form has no biological activity. One of the isomers, D-isoascorbic acid, or erythorbic acid, is produced commercially for use as a food additive. L-ascorbic acid is readily and reversibly oxidized to dehydro-L-ascorbic acid (Fig. 9.11), which retains vitamin C activity. This compound can be further oxidized to diketo-L-gulonic acid, in a nonreversible reaction. Diketo-L-gulonic

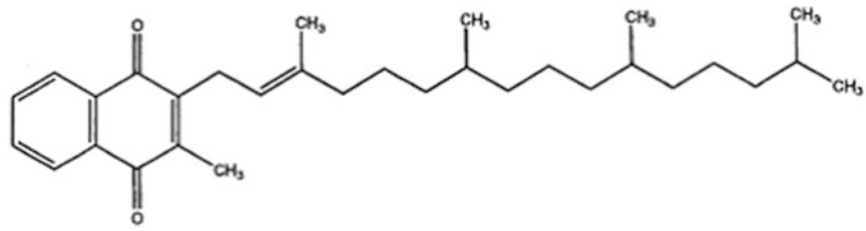


Fig. 9.9 Structural formula of vitamin K_1

Table 9.13 Vitamin K in some foods (expressed as menadione units per 100 g of edible portion)

Product	Units/100 g
Cabbage, white	70
Cabbage, red	18
Cauliflower	23
Carrots	5
Honey	25
Liver (chicken)	13
Liver (pork)	111
Milk	8
Peas	50
Potatoes	10
Spinach	161
Tomatoes (green)	24
Tomatoes (ripe)	12
Wheat	17
Wheat bran	36
Wheat germ	18

Table 9.14 Vitamin C content of some foods

Product	Ascorbic acid (mg/100 g)
Black currants	200
Brussels sprouts	100
Cauliflower	70
Cabbage	60
Spinach	60
Orange	50
Orange juice	40–50
Lemon	50
Peas	25
Tomato	20
Apple	5
Lettuce	15
Carrots	6
Milk	2.1–2.7
Potatoes	30

acid has no biological activity, is unstable, and is further oxidized to several possible compounds, including 1-threonic acid. Dehydration and decarboxylation can lead to the formation of furfural, which can polymerize to form brown pigments or combine with amino acids in the Strecker degradation.

Humans and guinea pigs are the only primates unable to synthesize vitamin C. The human requirement of vitamin C is not well defined. Figures ranging from 45 to 75 mg/day have been listed as daily needs. Continued stress and drug therapy may increase the need for this vitamin.

Vitamin C is widely distributed in nature, mostly in plant products such as fruits (especially citrus fruits), green vegetables, tomatoes, potatoes, and berries. The only animal sources of this vitamin are milk and liver. Although widely distributed, very high levels of the vitamin occur only in a few products, such as rose hips and West Indian cherries. The concentration varies widely in different tissues of fruits; for example, in apples, the concentration of vitamin C is two to three times as great in the peel as in the pulp.

Vitamin C is the least stable of all vitamins and is easily destroyed during processing and storage. The rate of destruction is increased by the action of metals, especially copper and iron, and by the action of enzymes. Exposure to oxygen, prolonged heating in the presence of oxygen, and exposure to light are all harmful to the vitamin C content of foods. Enzymes containing copper or iron in their prosthetic groups are efficient catalysts of ascorbic acid decomposition. The most important enzymes of this group are ascorbic acid oxidase, phenolase, cytochrome oxidase, and peroxidase. Only ascorbic acid oxidase involves a direct reaction among enzyme,

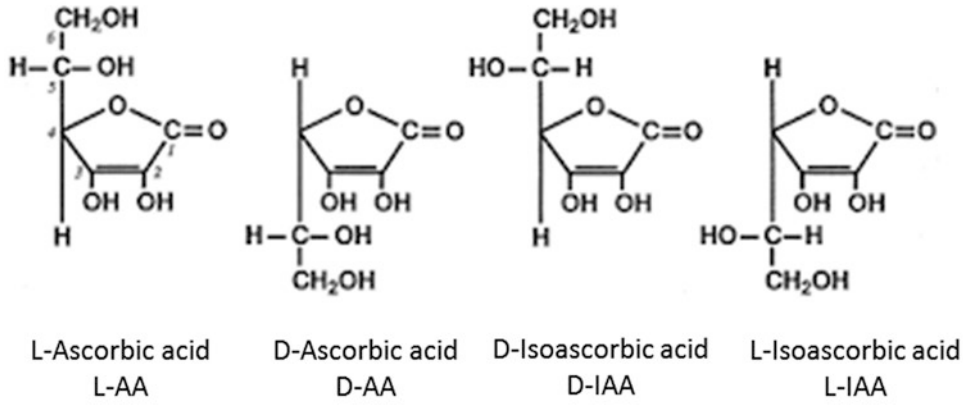


Fig. 9.10 Structural formulas of L-ascorbic acid and its stereoisomers

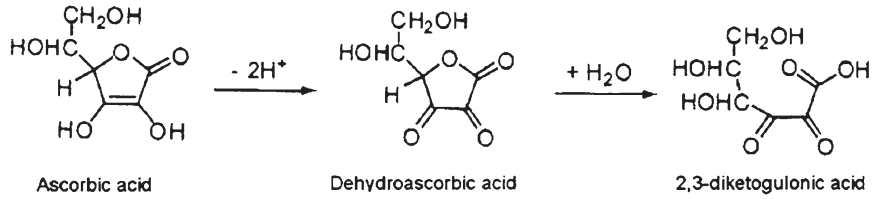


Fig. 9.11 Oxidation of L-ascorbic acid

substrate, and molecular oxygen. The other enzymes oxidize the vitamin indirectly. Phenolase catalyzes the oxidation of mono and dihydroxy phenols to quinones. The quinones react directly with the ascorbic acid. Cytochrome oxidase oxidizes cytochrome to the oxidized form and this reacts with L-ascorbic acid.

Peroxidase, in combination with phenolic compounds, utilizes hydrogen peroxide to bring about oxidation. The enzymes do not act in intact fruits because of the physical separation of enzyme and substrate. Mechanical damage, rot, or senescence lead to cellular disorganization and initiate decomposition. Inhibition of the enzymes in vegetables is achieved by blanching with steam or by electronic heating. Blanching is necessary before vegetables are dried or frozen. In fruit juices, the enzymes can be inhibited by pasteurization, deaeration, or holding at low temperature for a short period. The effect of blanching methods on the ascorbic acid content of broccoli was reported by Odland and Eheart (1975). Steam blanching was found to result in significantly smaller losses of ascorbic acid (Table 9.15). The retention of ascorbic acid in frozen spinach depends on storage temperature. At a very low temperature (−29 °C), only 10% of the initially present ascorbic acid was lost after 1 year. At −12°, the loss after 1 year was much higher, 55%. The presence of metal chelating compounds stabilizes vitamin C. These compounds include anthocyanins and flavonols, polybasic or polyhydroxy acids such as malic and citric acids, and polyphosphates.

Table 9.15 Effect of blanching method on ascorbic acid levels of broccoli

	Ascorbic acid (mg/100 g)		
Factor effect	Reduced	Dehydro	Total
Raw	94.0	4.0	98.2
Water blanch	45.3	5.7	51.0
Steam blanch	48.8	7.4	56.2

Source: From D. Ödland and M.S. Eheart, Ascorbic Acid, Mineral and Quality Retention in Frozen Broccoli Blanched in Water, Steam, and Ammonia-Steam, *J. Food Sci*., Vol. 40, pp. 1004–1007, 1975

Ascorbic acid is oxidized in the presence of air under neutral and alkaline conditions. At acid pH (for example, in citrus juice), the vitamin is more stable. Because oxygen is required for the breakdown, removal of oxygen should have a stabilizing effect. For the production of fruit drinks, the water should be deaerated to minimize vitamin C loss. The type of container may also affect the extent of ascorbic acid destruction. Use of tin cans for fruit juices results in rapid depletion of oxygen by the electrochemical process of corrosion. In bottles, all of the residual oxygen is available for ascorbic acid oxidation. To account for processing and storage losses, it is common to allow for a loss of 7–14 mg of ascorbic acid per 100 mL of fruit juice. Light results in rapid destruction of ascorbic acid in milk. It has been shown (Sattar and deMan 1973) that transparent packaging materials permit rapid destruction of vitamin C (Fig. 9.12). The extent of ascorbic acid destruction is closely parallel to the development of off-flavors. The destruction of ascorbic acid in milk by light occurs under the influence of riboflavin as a sensitizer. The reaction occurs in the presence of light and oxygen, and the riboflavin is converted to lumichrome.

Factors that affect vitamin C destruction during processing include heat treatment and leaching. The severity of processing conditions can often be judged by the percentage of ascorbic acid that has been lost. The extent of loss depends on the amount of water used. During blanching, vegetables that are covered with water may lose 80%; half covered, 40%; and quarter covered, 40% of the ascorbic acid. Particle size affects the size of the loss; for example, in blanching small pieces of carrots, losses may range from 32 to 50%, and in blanching large pieces, only 22 to 33%. Blanching of cabbage may result in a 20% loss of ascorbic acid, and subsequent dehydration may increase this to a total of 50%. In the processing of milk, losses may occur at various stages. From an initial level of about 22 mg/L in raw milk, the content in the product reaching the consumer may be well below 10 mg/L. Further losses may occur in the household during storage of the opened container.

The processing of milk into various dairy products may result in vitamin C losses. Ice cream contains no vitamin C, nor does cheese. The production of powdered milk involves a 20–30% loss, evaporated milk a 50–90% loss. Bullock (1968) studied the stability of added vitamin C in evaporated milk and found that adding 266 mg of sodium ascorbate per kg was sufficient to ensure the presence of at least 140 mg/L of ascorbic acid during 12 months of storage at 21 °C. Data on the stability of vitamin C in fortified foods have been assembled by deRitter (1976) (Table 9.16).

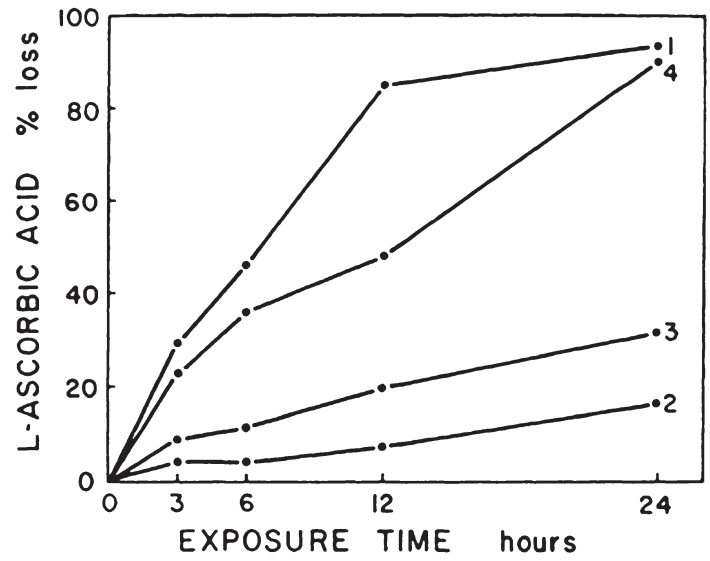


Fig. 9.12 Effect of exposure time at light intensity of 200 Ft-C on the loss of ascorbic acid in milk. Packaging materials: (1) clear plastic pouch, (2) laminated nontransparent pouch, (3) carton, (4) plastic 3-quart jug. *Source:* From A. Sattar and J.M. deMan, Effect of Packaging Material on Light Induced Quality Deterioration of Milk, *Can. Inst. Food Sci. Technol. J.,* Vol. 6, pp. 170–174, 1973

Table 9.16 Vitamin C stability in fortified foods and beverages after storage at 23 °C for 12 months, except as noted

Product	No. of samples	Retention	
		Mean (%)	Range (%)
Ready-to-eat cereal	4	71	60–87
Dry fruit drink mix	3	94	91–97
Cocoa powder	3	97	80–100
Dry whole milk, air pack	2	75	65–84
Dry whole milk, gas pack	1	93	–
Dry soy powder	1	81	–
Potato flakes[a]	3	85	73–92
Frozen peaches	1	80	–
Frozen apricots[b]	1	80	–
Apple juice	5	68	58–76
Cranberry juice	2	81	78–83
Grapefruit juice	5	81	73–86
Pineapple juice	2	78	74–82
Tomato juice	4	80	64–93
Vegetable juice	2	68	66–69
Grape drink	3	76	65–94
Orange drink	5	80	75–83
Carbonated beverage	3	60	54–64
Evaporated milk	4	75	70–82

[a]Stored for 6 months at 23 °C
[b]Thawed after storage in freezer for 5 months
Source: From E. deRitter, Stability Characteristics of Vitamins in Processed Foods, *Food Technol*., Vol. 30, pp. 48–51, 54, 1976

There are many technical uses of ascorbic acid in food processing. It is used to prevent browning and discoloration in vegetables and fruit products; as an antioxidant in fats, fish products, and dairy products; as a stabilizer of color in meat; as an improver of flour; as an oxygen acceptor in beer processing; as a reducing agent in wine, partially replacing sulfur dioxide; and as an added nutrient. The vitamin is protected by sulfur dioxide, presumably by inhibiting polyphenolase.

Vitamin B_1 (Thiamin)

This vitamin acts as a coenzyme in the metabolism of carbohydrates and is present in all living tissues. It acts in the form of thiamin diphosphate in the decarboxylation of α-keto acids and is referred to as cocarboxylase. Thiamin is available in the form of its chloride or nitrate, and its structural formula is shown in Fig. 9.13. The molecule contains two basic nitrogen atoms; one is in the primary amino group, the other in the quaternary ammonium group. It forms salts with inorganic and organic acids. The vitamin contains a primary alcohol group, which is usually present in the naturally occurring vitamin in esterified form with ortho-, di-, or triphosphoric acid. In aqueous solution, the compound may occur in different forms, depending on pH. In acid solution, the equilibrium favors the formation of positive ions (Fig. 9.14). The thiol- form is favored in alkaline medium. This form can react with compounds containing sulfhydryl groups to form disulfide bridges. It has been suggested that thiamin occurs in some foods linked to protein by disulfide bridges.

Small quantities of thiamin are present in almost all foods of plant and animal origin. Good sources are whole cereal grains; organ meats such as liver, heart, and kidney; lean pork; eggs; nuts; and potatoes (Table 9.17). Although thiamin content is usually measured in mg per 100 g of a food, another unit has been used occasionally, the IU corresponding to 3 μg of thiamin-hydrochloride. The human daily requirement is related to the carbohydrate level of the diet. A minimum intake of 1 mg per 2000 kcal is considered essential. Increased metabolic activity, such as that which results from heavy work, pregnancy, or disease, requires higher intake.

Thiamin is one of the more unstable vitamins. Various food processing operations may considerably reduce thiamin levels. Heat, oxygen, sulfur dioxide, leaching, and neutral or alkaline pH may all result in destruction of thiamin. Light has no effect. The enzyme is stable under acid conditions; at pH values of 3.5 or below, foods can be autoclaved at 120 °C with little or no loss of thiamin. At neutral or alkaline pH, the vitamin is destroyed by boiling or even by storage at room temperature. Even the slight alkalinity of water used for processing may have an important effect. Bender (1971) reports that cooking rice in distilled water reduced thiamin content negligibly, whereas cooking in tap water caused an 8–10% loss, and cooking in well water caused a loss of up to 36%.

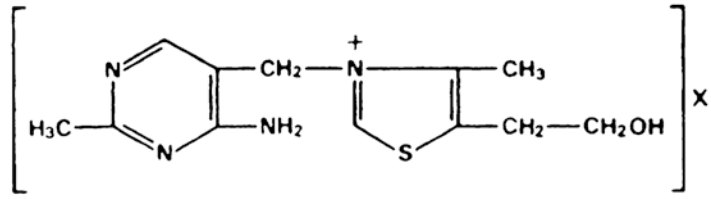


Fig. 9.13 Structural formula of thiamin. Hydrochloride: X=Cl⁻, HCl;mononitrate:X=NO_3^-

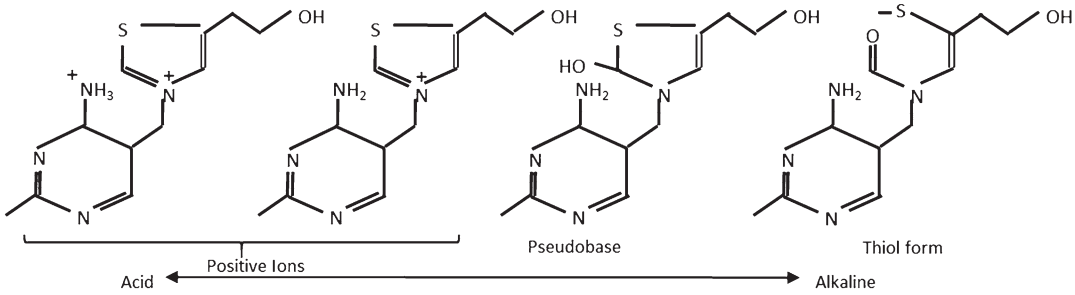


Fig. 9.14 Behavior of thiamin in aqueous solutions

Table 9.17 Thiamin content of some foods

Product	Thiamin (mg/100 g) edible portion
Almonds	0.24
Corn	0.37
Egg	0.11
Filberts	0.46
Beef heart	0.53
Beef liver	0.25
Macaroni (enriched)	0.88
Macaroni (not enriched)	0.09
Milk	0.03
Peas	0.28
Pork, lean	0.87
Potatoes	0.10
Wheat (hard red spring)	0.57
Wheat flour (enriched)	0.44
Wheat flour (not enriched)	0.08

Some fish species contain an enzyme that can destroy thiamin. Sulfur dioxide rapidly destroys thiamin. For this reason, sulfur dioxide is not permitted as an additive in foods that contain appreciable amounts of thiamin.

Baking of white bread may result in thiamin loss of 20%. Thiamin loss in milk processing is as follows: pasteurization, 3–20%; sterilization, 30–50%; spray drying, 10%; and roller drying, 20–30%. Cooking of meat causes losses that are related to size of cut, fat content, and so on. Boiling loss is 15–40%; frying, 40–50%; roasting, 30–60%; and canning, 50–75%. Similar losses apply to fish. Because thiamin and other vitamins are located near the bran of cereal grains, there is a great loss during milling. White flour, therefore, has a greatly reduced content of B vitamins and vitamin E (Fig. 9.15). Not only is thiamin content lowered by milling, but also storage of whole grain may result in losses. This depends on moisture content. At normal moisture level of 12%, 5 months' storage results in a 12% loss; at 17% moisture, a 30% loss; and at 6% moisture, no loss at all. Because of the losses that are likely to occur in cereal grain processing and in the processing of other foods, a program of fortification of flour is an important factor in preventing vitamin deficiencies. Table 9.18 lists the nutrients and recommended levels for grain products fortification (National Academy of Sciences 1974b). A summary of data relating processing treatment to thiamin stability has been given by deRitter (1976) (Table 9.19).

Vitamin B_2 (Riboflavin)

The molecule consists of a d-ribitol unit attached to an isoalloxazine ring (Fig. 9.16). Anything more than a minor change in the molecule results

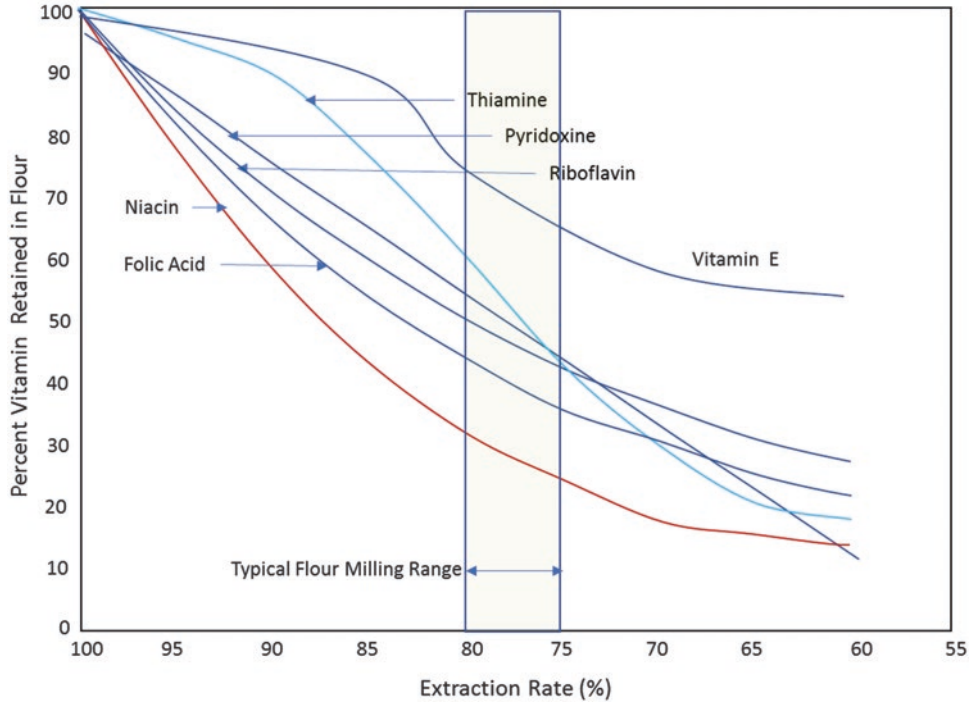


Fig. 9.15 Typical losses of vitamins during flour milling

Table 9.18 Nutrients and levels recommended for inclusion in fortification of cereal-grain products[a]

	Level	
Nutrient	(mg/Ib)	(mg/100 g)
Vitamin A[b]	2.2	0.48
Thiamin	2.9	0.64
Riboflavin	1.8	0.40
Niacin	24.0	5.29
Vitamin B_6	2.0	0.44
Folic acid	0.3	0.07
Iron	40	8.81
Calcium	900	198.2
Magnesium	200	44.1
Zinc	10	2.2

[a]Wheat flour, corn grits, cornmeal, rice. Other cereal-grain products in proportion to their cereal-grain content
[b]Retinol equivalent
Source: Reprinted with permission from National Academy of Sciences, Recommended Dietary Allowances, 8th rev. ed., © 1974, National Academy of Sciences

Table 9.19 Thiamin stability in foods

Product	Treatment	Retention (%)
Nine canned vegetables	Processing	31–89
Four canned vegetables	Storage, 2–3 months @ room temperature	73–94
Cereals	Extrusion cooking	48–90
Fortified ready-to-eat cereal	Storage, 12 months @ 23 °C	100
Bread (white, whole wheat)	Commercial baking	74–79
Devil's food cake (pH 9)	Baking	0–7

Source: From E. deRitter, Stability Characteristics of Vitamins in Processed Foods, *Food Technol.,* Vol. 30, pp. 48–51, 54, 1976

in a loss of vitamin activity. Aqueous solutions of riboflavin are yellow with a yellowish-green fluorescence. The vitamin is a constituent of two coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FMN is riboflavin-5′-phosphate and forms part of several enzymes, including cytochrome c reductase. The flavoproteins serve as electron carriers and are involved in the oxidation of glucose, fatty acids, amino acids, and purines.

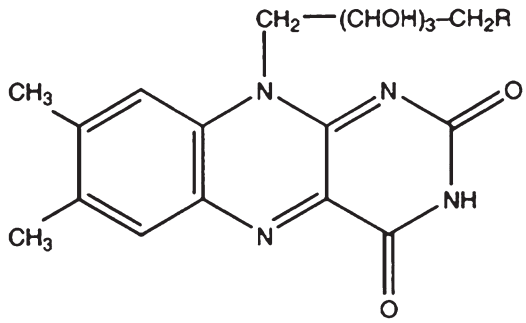


Fig. 9.16 Structural formula of riboflavin. Riboflavin: *R=OH; Riboflavin phosphate: R=PO₃NaOH*

Table 9.20 Riboflavin content of some foods

Product	Riboflavin (mg/100 g) edible portion
Beef	0.16
Cabbage	0.05
Eggs	0.30
Chicken	0.19
Beef liver	3.26
Chicken liver	2.49
Beef kidney	2.55
Peas	0.29
Spinach	0.20
Tomato	0.04
Yeast (dry)	5.41
Milk	0.17
Nonfat dry milk	1.78

Very good sources of riboflavin are milk and milk products; other sources are beef muscle, liver, kidney, poultry, tomatoes, eggs, green vegetables, and yeast (Table 9.20).

Riboflavin is stable to oxygen and acid pH but is unstable in alkaline medium and is very sensitive to light. When exposed to light, the rate of destruction increases as pH and temperature increase. Heating under neutral or acidic conditions does not destroy the vitamin.

The human requirement for riboflavin varies with metabolic activity and body weight and ranges from 1 to 3 mg per day. Normal adult requirement is 1.1–1.6 mg per day. In most cases, the riboflavin of foods is present in the form of the dinucleotide, the phosphoric acid ester, or is bound to protein. Only in milk does riboflavin occur mostly in the free form.

Under the influence of light and alkaline pH, riboflavin is transformed into lumiflavin, an inactive compound with a yellowish green fluorescence. Under acid conditions, riboflavin is transformed into another inactive derivative, lumichrome, and ribitol. This compound has a blue fluorescence. The transformation into lumiflavin in milk results in the destruction of ascorbic acid.

The light sensitivity of riboflavin results in losses of up to 50% when milk is exposed to sunlight for 2 h. The nature of the packaging material significantly affects the extent of riboflavin destruction. It appears that the wavelengths of light responsible for the riboflavin destruction are in the visible spectrum below 500–520 nm. Ultraviolet light has been reported to have no destructive effect on riboflavin (Hartman and Dryden 1978). Riboflavin is stable in dry milk for storage periods of up to 16 months. Pasteurization of milk causes only minor losses of riboflavin.

Vitamin B_6 (Pyridoxine)

There are three compounds with vitamin B_6 activity. The structural formula of pyridoxine is presented in Fig. 9.17. The other two forms of this vitamin are different from pyridoxine—they have another substituent on carbon 4 of the benzene ring. Pyridoxal has a –CHO group in this position and pyridoxamine has a $–CH_2NH_2$ group. All three compounds can occur as salts. Vitamin B_6 plays an important role in the metabolism of amino acids, where it is active in the

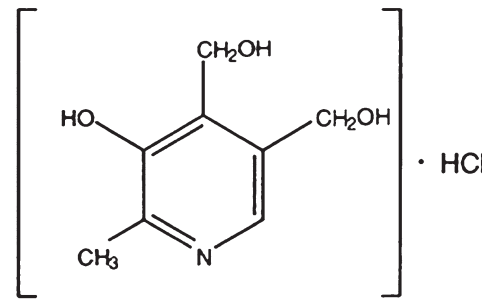


Fig. 9.17 Structural formula of pyridoxine

coenzyme form pyridoxal-5-phosphate. The three forms of vitamin B_6 are equally active in rats; although it can be expected that the same applies for humans, this has not been definitely established.

Vitamin B_6 is widely distributed in many foods (Table 9.21), and deficiencies of this vitamin are uncommon. The recommended allowance for adults has been established at 2 mg per day. The requirement appears to increase with the consumption of high-protein diets.

Vitamin B_6 occurs in animal tissues in the form of pyridoxal and pyridoxamine or as their phosphates. Pyridoxine occurs in plant products.

Pyridoxine is stable to heat and strong alkali or acid; it is sensitive to light, especially ultraviolet light and when present in alkaline solutions. Pyridoxal and pyridoxamine are rapidly destroyed when exposed to air, heat, or light. Pyridoxamine is readily destroyed in food processing operations.

Because it is difficult to determine this vitamin in foods, there is a scarcity of information on its occurrence. Recent data establish the level in milk as 0.54 mg per L. Other sources are meats, liver, vegetables, whole grain cereals, and egg yolk.

Table 9.21 Vitamin B_6 content of some foods

Product	Vitamin B_6 (μg/g)
Wheat	3.2–6.1
Whole wheat bread	4.2
White bread	1.0
Orange juice	0.52–0.60
Apple juice	0.35
Tomatoes	1.51
Beans, canned	0.42–0.81
Peas, canned	0.44–0.53
Beef muscle	0.8–4.0
Pork muscle	1.23–6.8
Milk, pasteurized	0.5–0.6
Yeast	50

The effects of processing on pyridoxine levels in milk and milk products have been reviewed by Hartman and Dryden (1978). No significant losses have been reported to result from pasteurization, homogenization, or production of dried milk. Heat sterilization of milk, however, has been reported to result in losses ranging from 36 to 49%. Losses occur not only during the heat treatment but also during subsequent storage of milk. These storage losses have been attributed to a conversion of pyridoxal to pyridoxamine and then to a different form of the vitamin. Wendt and Bernhart (1960) have identified this compound as *bis*-4-pyridoxal disulfide (Fig. 9.18). This compound is formed by reaction of pyridoxal and active sulfhydryl groups. The latter are formed during heat treatment of milk proteins. Exposure of milk to daylight in clear glass bottles for 8 h resulted in a vitamin B_6 loss of 21%.

Food canning results in losses of vitamin B_6 of 20–30%. Milling of wheat may result in losses of up to 80–90%. Baking of bread may result in losses of up to 17%.

A review of some stability data of vitamin B_6 as prepared by deRitter (1976) is given in Table 9.22.

Niacin

The term *niacin* is used in a generic sense for both nicotinic acid and nicotinamide (Fig. 9.19). Nicotinamide acts as a component of two important enzymes, NAD and NADP, which are involved in glycolysis, fat synthesis, and tissue respiration. Niacin is also known as the pellagra

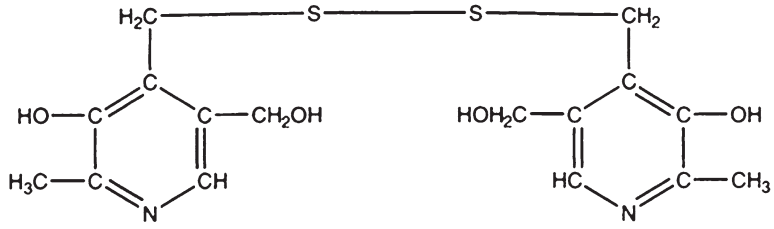


Fig. 9.18 Structural formula of *bis*-4-pyridoxal disulfide

Table 9.22 Vitamin B_6 stability in foods

Product	Treatment	Retention (%)		
Bread (added B_6)	Baking	100		
Enriched corn meal	12 months @ 38 °C + 50% relative humidity	90–95		
Enriched macaroni	12 months @ 38 °C + 50% relative humidity	100		
		Saccharomyces Carlsbergensis	Chick	Rat
Whole milk	Evaporation and sterilization	30	55	65
	Evaporation and sterilization +6 months @ room temperature	18	44	41
Infant formula, liquid	Processing and sterilization	33–50 (natural) 84 (added)		
Infant formula, dry	Spray drying	69–83		
Boned chicken	Canning	57		
	Irradiation (2.79 megarads)	68		

Source: From E. deRitter, Stability Characteristics of Vitamins in Processed Foods, *Food Technol.,* Vol. 30, pp. 48–51, 54, 1976

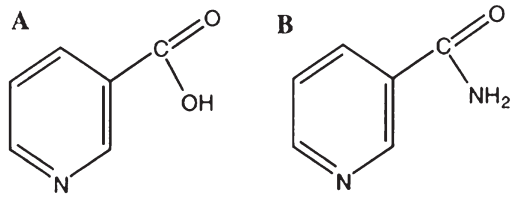


Fig. 9.19 Structural formulas of (**a**) nicotinic acid and (**b**) nicotinamide

preventive factor. The incidence of pellagra has declined but is still a serious problem in parts of the Near East, Africa, southeastern Europe, and in North American populations that subsist on com diets. When com is treated with alkali or lime, as for the tortilla preparation in Central America, the amount of available niacin can be greatly increased. Tryptophan can be converted by the body into niacin. Many diets causing pellagra are low in good quality protein as well as in vitamins. Corn protein is low in tryptophan. The niacin of corn and other cereals may occur in a bound form, called niacytin, that can be converted into niacin by alkali treatment.

The human requirement of niacin is related to the intake of tryptophan. Animal proteins contain approximately 1.4% of tryptophan, vegetable proteins about 1%. A dietary intake of 60 mg of tryptophan is considered equivalent to 1 mg of niacin. When this is taken into account, average diets in the United States supply 500–1000 mg tryptophan per day and 8–17 mg niacin for a total niacin equivalent of 16–33 mg. The RDA for adults, expressed as niacin, is 6.6 mg per 1000 kcal, and not less than 13 mg when caloric intake is less than 2000 kcal.

Table 9.23 Niacin content of some foods

Product	Niacin (mg/100 g) edible portion
Barley (pearled)	3.1
Beans (green, snap)	0.5
Beans (white)	2.4
Beef (total edible)	4.4
Beef kidney	6.4
Beef liver	13.6
Chicken (dark meat)	5.2
Chicken (light meat)	10.7
Corn (field)	2.2
Haddock	3.0
Milk	0.1
Mushrooms	4.2
Peanuts	17.2
Peas	2.9
Potatoes	1.5
Spinach	0.6
Wheat	4.3
Yeast (dry)	36.7

Good dietary sources of this vitamin are liver, kidney, lean meat, chicken, fish, wheat, barley, rye, green peas, yeast, peanuts, and leafy vegetables. In animal tissues, the predominant form of niacin is the amide. Niacin content of some foods are listed in Table 9.23.

Niacin is probably the most stable of the B vitamins. It is unaffected by heat, light, oxygen, acid, or alkali. The main loss resulting from processing involves leaching into the process water. Blanching of vegetables may cause a loss of about 15%. Processes in which brines are used may cause losses of up to 30%. Processing of milk, such as pasteurization, sterilization, evaporation, and drying have little or no effect on nicotinic acid level. Virtually all the niacin in milk occurs in the form of nicotinamide. In many foods, application of heat, such as roasting or baking, increases the amount of available niacin. This results from the change of bound niacin to the free form.

Vitamin B_{12} (Cyanocobalamine)

This vitamin possesses the most complex structure of any of the vitamins and is unique in that it has a metallic element, cobalt, in the molecule (Fig. 9.20). The molecule is a coordination complex built around a central tervalent cobalt atom and consists of two major parts—a complex cyclic structure that closely resembles the porphyrins and a nucleotide-like portion, 5,6-dimethyl-l-(α-D-ribofuranosyl) benzimidazole-3′-phosphate. The phosphate of the nucleotide is esterified with l-amino-2-propanol; this, in turn, is joined by means of an amide bond with the propionic acid side chain of the large cyclic structure. A second

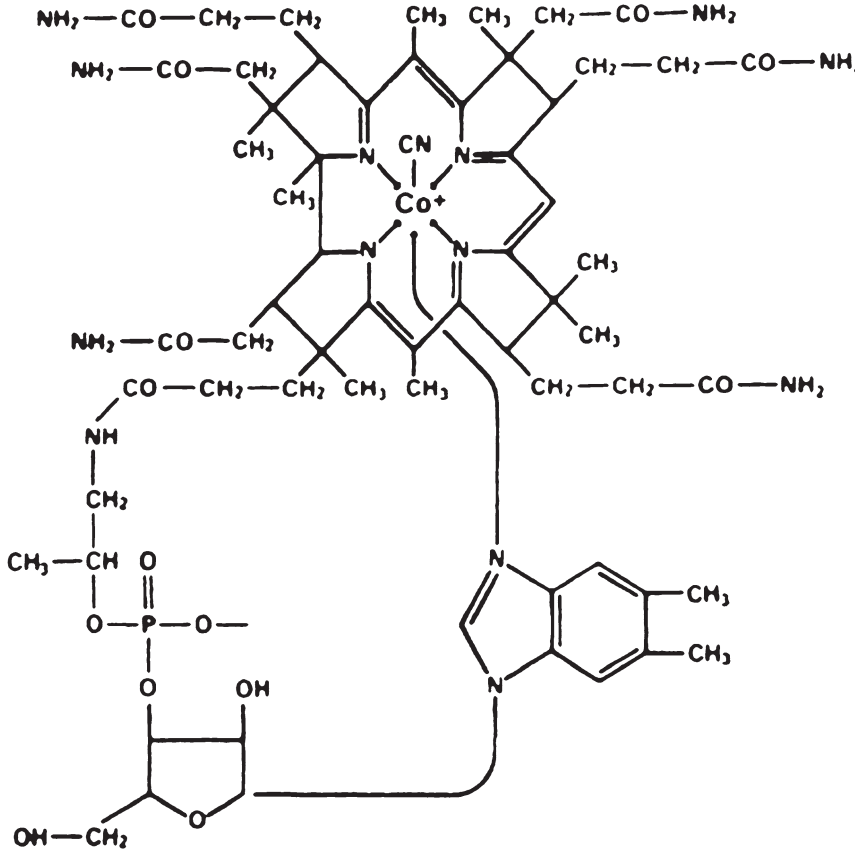


Fig. 9.20 Structural formula of cyanocobalamine

linkage with the large structure is through the coordinate bond between the cobalt atom and one of the nitrogen atoms of the benzimidazole. The cyanide group can be split off relatively easily, for example, by daylight. This reaction can be reversed by removing the light source. The cyano group can also be replaced by other groups such as hydroxo, aquo, and nitroto. Treatment with cyanide will convert these groups back to the cyano form. The different forms all have biological activity.

Cyanocobalamine is a component of several coenzymes and has an effect on nucleic acid formation through its action in cycling 5-methyltetrahydrofolate back into the folate pool. The most important dietary sources of the vitamin are animal products. Vitamin B_{12} is also produced by many microorganisms. It is not surprising that vitamin B_{12} deficiency of dietary origin only occurs in vegetarians.

The average diet in the United States is considered to supply between 5 and 15 μg/day. In foods, the vitamin is bound to proteins via peptide linkages but can be readily absorbed in the intestinal tract. The RDA is 3 μg for adults and adolescents.

Few natural sources are rich in vitamin B_{12}. However, only very small amounts are required in the diet. Good sources are lean meat, liver, kidney, fish, shellfish, and milk (Table 9.24).

Table 9.24 Vitamin B_{12} content of some foods

Product	Vitamin B_{12}
Beef muscle	0.25–3.4 μg/100 g
Beef liver	14–152 μg/100 g
Milk	3.2–12.4 μg/L
Shellfish	600–970 μg/100 g (dry wt)
Egg yolk	0.28–1.556 μg/100 g

In milk, the vitamin occurs as cobalamine bound to protein.

Vitamin B_{12} is not destroyed to a great extent by cooking, unless the food is boiled in alkaline solution. When liver is boiled in water for 5 min, only 8% of the vitamin B_{12} is lost. Broiling of meat may result in higher losses. Pasteurization causes only a slight destruction of vitamin B_{12} in milk; losses range from 7 to 10% depending on pasteurization method. More drastic heat treatment results in higher losses. Boiling milk for two to 5 min causes a 30% loss, evaporation about 50%, and sterilization up to 87%. The loss in drying of milk is smaller; in the production of dried skim milk, the vitamin B_{12} loss is about 30%. Ultra-high-temperature sterilization of milk does not cause more vitamin B_{12} destruction than does pasteurization.

Folic Acid (Folacin)

Folic acid is the main representative of a series of related compounds that contain three moieties: pterin, *p*-aminobenzoic acid, and glutamic acid (Fig. 9.21). The commercially available form contains one glutamic acid residue and is named pteroylglutamic acid (PGA). The naturally occurring forms are either PGA or conjugates with varying numbers of glutamic acid residues, such as tri- and heptaglutamates. It has been suggested that folic acid deficiency is the most common vitamin deficiency in North America and Europe. Deficiency is especially likely to occur in pregnant women.

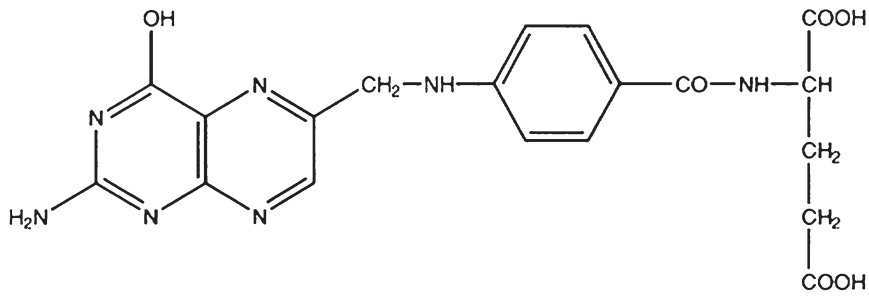


Fig. 9.21 Structural formula of folic acid

The vitamin occurs in a variety of foods, especially in liver, fruit, leafy vegetables, and yeast (Table 9.25) (Hurdle et al. 1968; Streiff 1971). The usual form of the vitamin in these products is a polyglutamate. The action of an enzyme (conjugase) is required to liberate the folic acid for metabolic activity; this takes place in the intestinal mucosa. The folacin of foods can be divided into two main groups on the basis of its availability to *L. casei*: (1) the so-called free folate, which is available to *L. casei* without conjugase treatment; and (2) the total folate, which also includes the conjugates that are not normally available to *L. casei.* About 25% of the dietary folacin occurs in free form. The folate in vegetables occurs mainly in the conjugated form; the folate in liver occurs in the free form.

Table 9.25 Folate content of some foods

Product	Folate (μg/g)
Beef, boiled	0.03
Chicken, roasted	0.07
Cod, fried	0.16
Eggs, boiled	0.30
Brussels sprouts, boiled	0.20
Cabbage, boiled	0.11
Lettuce	2.00
Potato, boiled	0.12
Spinach, boiled	0.29
Tomato	0.18
Orange	0.45
Milk	0.0028
Bread, white	0.17
Bread, brown	0.38
Orange juice, frozen reconstituted	0.50
Tomato juice, canned	0.10

The RDA for folacin is 400 μg for adults. There is an additional requirement of 400 μg/day during pregnancy and 200 μg/day during breastfeeding.

Many of the naturally occurring folates are extremely labile and easily destroyed by cooking. Folic acid itself is stable to heat in an acid medium but is rapidly destroyed under neutral and alkaline conditions. In solution, the vitamin is easily destroyed by light. Folate may occur in a form more active than PGA; this is called folinic acid or citrovorum factor, which is N5-formyl-5, 6, 7, 8-tetrahydro PGA (Fig. 9.22). The folate of milk consists of up to 20% of folinic acid. It has been reported that pasteurization and sterilization of milk involve only small losses or no loss. Hurdle et al. (1968) reported that boiling of milk causes no loss in folate; however, boiling of potato results in a 90% loss and boiling of cabbage a 98% loss. Reconstitution of dried milk followed by sterilization as can occur with baby formulas may lead to significant folacin losses. Fermentation of milk and milk products may result in greatly increased folate levels. Blanching of vegetables and cooking of meat do not appear to cause folic acid losses. Table 9.26 contains a summary of folate stability data prepared by deRitter (1976). Citrus fruit and juices are relatively good sources of folic acid, which is present mostly as the reduced 5-methyl tetrahydro folate (monoglutamate form). There are also polyglutamate derivatives present White (1991).

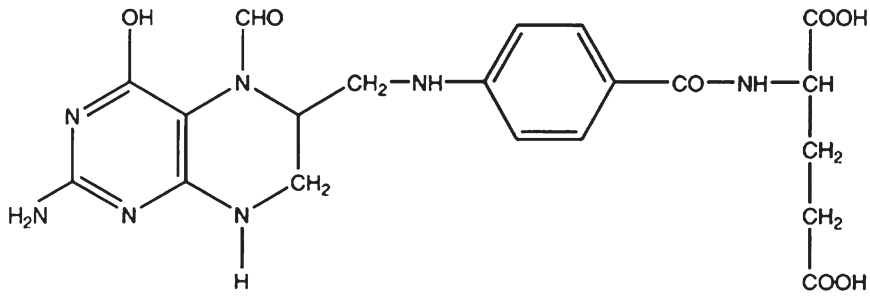


Fig. 9.22 Structural formula of folinic acid

Table 9.26 Folic acid stability in foods

Product	Treatment	Retention of folic acid	
		Free (%)	Total (%)
Cabbage	Boiled 5 min	32	54
Potatoes	Boiled 5 min	50	92
Rice	Boiled 15 min	–	10
Beef, pork, and chicken	Boiled 15 min	<50	<50
Various foods	Cooked	27	55

Source: From E. deRitter, Stability Characteristics of Vitamins in Processed Foods, *Food Technol.*, Vol. 30, pp. 48–51, 54, 1976

Pantothenic Acid

The free acid (Fig. 9.23) is very unstable and has the appearance of a hygroscopic oil. The calcium and sodium salts are more stable. The alcohol (panthenol) has the same biological activity as the acid. Only the dextrorotatory or D form of these compounds has biological activity. Pantothenic acid plays an important role as a component of coenzyme A, and this is the form in which it occurs in most foods.

Pantothenic acid occurs in all living cells and tissues and is, therefore, found in most food products. Good dietary sources include meats, liver, kidney, fruits, vegetables, milk, egg yolk, yeast, whole cereal grains, and nuts (Table 9.27). In animal products, most of the pantothenic acid is present in the bound form, but in milk only about one-fourth of the vitamin is bound.

There is no recommended dietary allowance for this vitamin because of insufficient evidence to base one on. It is estimated that adult dietary intake in the United States ranges from 5 to 20 mg/day, and 5 to 10 mg/day probably represents an adequate intake.

The vitamin is stable to air, and labile to dry heat. It is stable in solution in the pH range of 5–7 and less stable outside this range. Pasteurization and sterilization of milk result in very little or no loss. The production and storage of dried milk involves little or no loss of pantothenic acid. Manufacture of cheese involves large losses during processing, but during ripening the pantothenic acid content increases, due to synthesis by microorganisms. Blanching of vegetables may involve losses of up to 30%. Boiling in water involves losses that depend on the amount of water used.

Biotin

The structural formula (Fig. 9.24) contains three asymmetric carbon atoms, and eight different stereoisomers are possible. Only the dextrorotatory D-biotin occurs in nature and has biological activity. Biotin occurs in some products in free form (vegetables, milk, and fruits) and in other products is bound to protein (organ meats, seeds, and yeast). Good sources of the vitamin are meat, liver, kidney, milk, egg yolk, yeast, vegetables, and mushrooms (Table 9.28).

Biotin is important in a number of metabolic reactions, especially in fatty acid synthesis. The biotin supply of the human organism is only partly derived from the diet.

An important factor in biotin's availability is that some of the vitamin is derived from synthesis by intestinal microorganisms; this is demonstrated by the fact that three to six times more biotin is excreted in the urine than is ingested with the food. The daily intake of biotin is between 100 and 300 μg. No recommended dietary allowance has been established. Biotin is deactivated by raw egg white. This is caused by the glycoprotein avidin. Heating of avidin will destroy the inactivator capacity for biotin.

Data on the stability of biotin are limited. The vitamin appears to be quite stable. Heat treatment results in relatively small losses. The vitamin is stable to air and is stable at neutral and acid pH. Pasteurization and sterilization of milk result in losses of less than 10%. In the production of evaporated and dried milk, losses do not exceed 15%.

Fig. 9.23 Structural formula of pantothenic acid. Pantothenic acid: R=COOH; Panthenol: R=CH_2OH

Table 9.27 Pantothenic acid content of some foods

Product	Pantothenic acid (μg/g)
Beef, lean	10
Wheat	11
Potatoes	6.5
Split peas	20–22
Tomatoes	1
Orange	0.7
Walnuts	8
Milk	1.3–4.2
Beef liver	25–60
Eggs	8–48
Broccoli	46

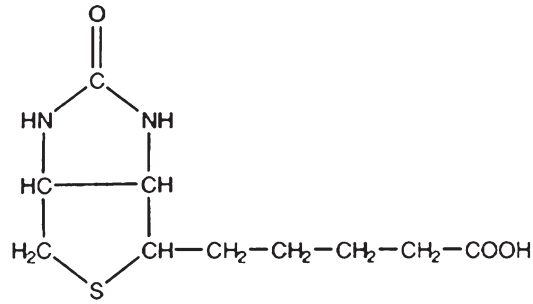


Fig. 9.24 Structural formula of biotin

Table 9.28 Biotin content of some foods

Product	Biotin (μg/100 g)
Milk	1.1–3.7
Tomatoes	1
Broad beans	3
Cheese	1.1–7.6
Wheat	5.2
Beef	2.6
Beef liver	96
Lettuce	3.1
Mushrooms	16
Potatoes	0.6
Spinach	6.9
Apples	0.9
Oranges	1.9
Peanuts	34

Vitamins as Food Ingredients

In addition to their role as essential micronutrients, vitamins may serve as food ingredients for their varied functional properties (Institute of Food Technologists 1987). Vitamin C and vitamin E have found widespread use as antioxidants. In lipid systems, vitamin E may be used as an antioxidant in fats that have little or no natural tocopherol content. Ascorbic acid in the form of its palmitic acid ester, ascorbyl palmitate, is an effective antioxidant in lipid systems. Ascorbyl palmitate prevents the formation of lipid free radicals (Fig. 9.25) and thereby delays the initiation of the chain reaction that leads to the deterioration of the fat (Liao and Seib 1987). Ascorbyl palmitate is used in vegetable oils because it acts synergistically with naturally occurring tocopherols. The tocopherols are fat-soluble antioxidants that are used in animal fats. Ascorbic acid reduces nitrous acid to nitric oxide and prevents the formation of N-nitrosamine. The reaction of nitrous acid and ascorbic acid is given in Fig. 9.26 (Liao and Seib 1987). Ascorbic acid is also widely used to prevent enzymic browning in fruit products. Phenolic compounds are oxidized by polyphenoloxidase to quinones. The quinones rapidly polymerize to form brown pigments. This reaction is easily reversed by ascorbic acid (Fig. 9.27).

The carotenoids β-carotene and β-apo-8-carotenal are used as colorants in fat-based as well as water-based foods.

Other functions of ascorbic acid are inhibition of can corrosion in canned soft drinks, protection of flavor and color of wine, prevention of black spot formation in shrimp, stabilization of cured meat color, and dough improvement in baked goods (Institute of Food Technologists 1987).

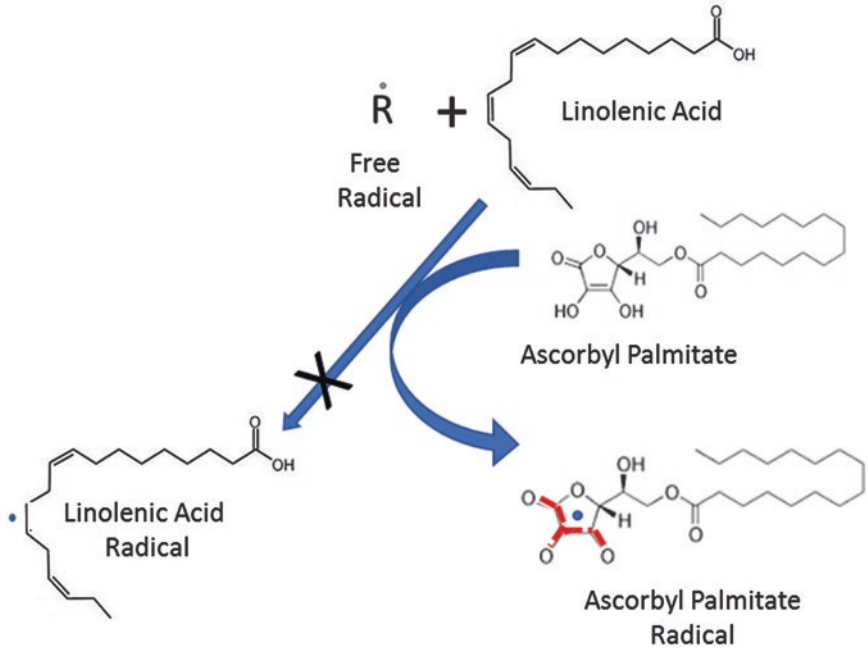


Fig. 9.25 Prevention of lipid free radical formation in linoleic acid by ascorbyl palmitate

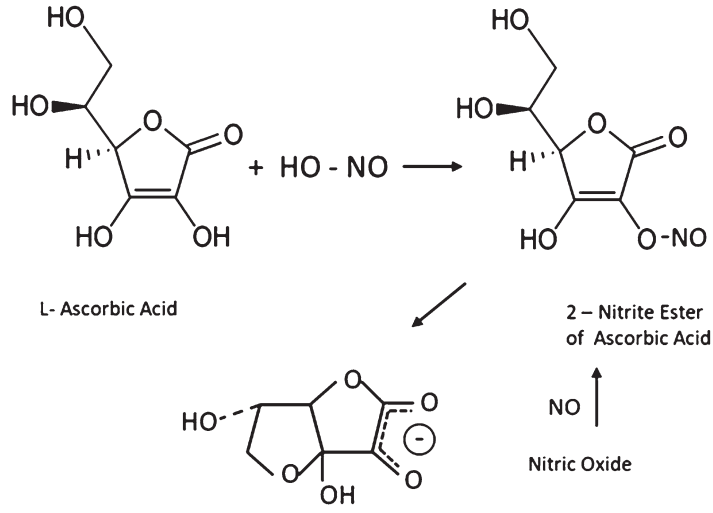


Fig. 9.26 Reaction between nitrous acid and ascorbic acid. *Source:* From M.L. Liao and P.A. Seib, Selected Reactions of L-Ascorbic Acid Related to Foods, *Food Technol*., Vol. 41, no. 11, pp. 104–107, 1987

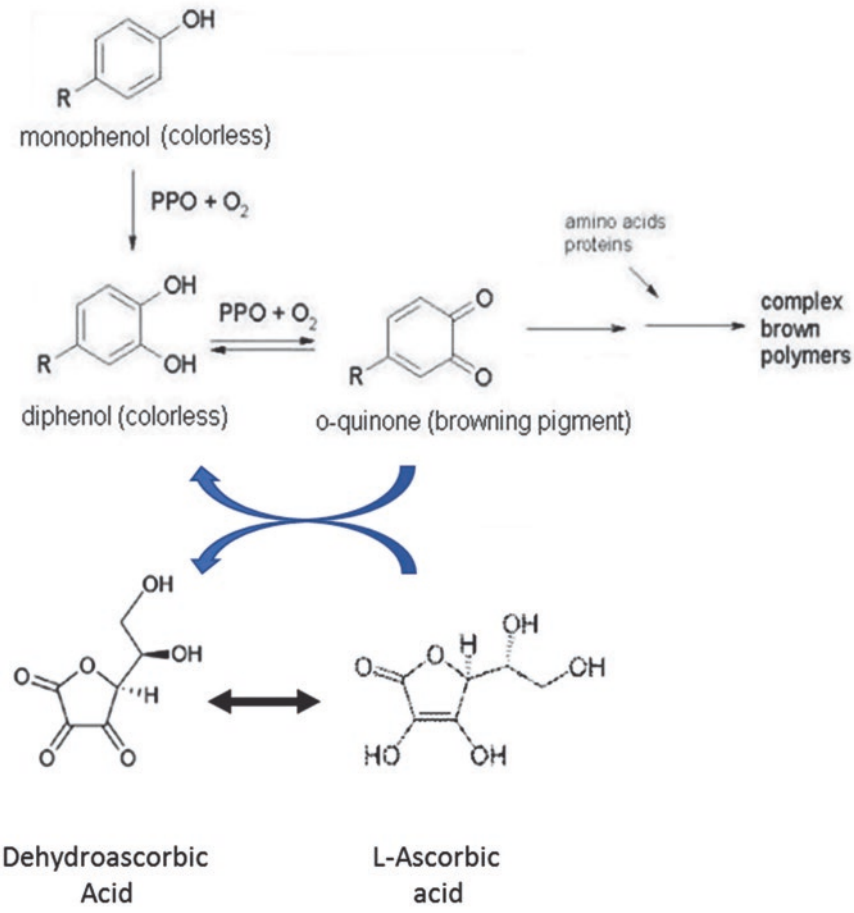


Fig. 9.27 Reduction of ortho-quinone by ascorbic acid during enzymatic browning

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Enzymes

10

Chang Yong Lee and John M. deMan

Introduction

Enzymes, although minor constituents of many foods, play a major and manifold role in foods. They are highly specialized proteins with the special ability to catalyze specific chemical reactions in biological systems. Although they may undergo change during the catalysis, they are unchanged at the end of the reaction. They are highly selective catalysts, greatly accelerating both the rate and specificity of metabolic reactions from digestion of food to the synthesis of DNA. An enzyme can catalyze only a single reaction of a single compound, called the enzyme's substrate. For example, amylase found in the human digestive tract catalyzes only the hydrolysis of starch to yield glucose; cellulose and other polysaccharides are untouched by amylase. Other enzymes have different specificities. Papain, for example, a globular protein isolated from papaya fruit, catalyzes the hydrolysis of many kinds of peptide bonds, which makes papain useful as a meat tenderizer and a cleaner for contact lenses. Enzymes act only to lower the activation energy for a reaction, thereby making the reaction takes place more rapidly. Starch and water, for example, react very slowly in the absence of a catalyst because the activation energy is too high. When amylase is present, however, the energy barrier is lowered, and the hydrolysis reaction occurs rapidly.

The research on enzymes has immense practical importance in agriculture and food processing. There is not a single food system that does not involve enzyme reactions. Enzymes that are naturally present in foods may change the composition of those foods; in some cases, such changes are desirable but in most instances are undesirable, so the enzymes must be deactivated. The blanching of vegetables is an example of enzyme deactivation to prevent undesirable change. Some enzymes are used as indicators in analytical methods; phosphatase, for instance, is used in the phosphatase test of pasteurization of milk. Enzymes are also used as processing aids in food manufacturing. For example, rennin, contained in extract of calves' stomachs, is used as a coagulant for milk in the production of cheese.

Recent advances in biotechnology offer a new promise for tailoring enzymes for specific functions of particular applications and their production in quantities for industrial uses. By manipulating the genetic code of an enzyme, it is now possible to target a specific amino acid residue at any location for modification. During the last three decades, the use of cellulases, hemicellulases and pectinase which account for approximately 20% of the world enzyme market for food, brewery, wine, and textile industries has increased considerably and many commercial enzyme producers are marketing tailor-made enzyme preparations suitable for specific purposes (Wong 1995; Bhat 2000).

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_10

Food science's emphasis in the study of enzymes differs from that in biochemistry. The former deals mostly with decomposition reactions, hydrolysis, and oxidation; the latter is more concerned with synthetic mechanisms. Whitaker (1972) has prepared an extensive listing of the uses of enzymes in food processing (Table 10.1) and this gives a good summary of the many and varied possible applications of enzymes.

Table 10.1 Some uses and suggested uses of enzymes in foods and food processing

Enzyme	Food	Purpose or action
Amylases	Baked goods	Increase sugar content for yeast fermentation
	Brewing	Conversion of starch to maltose for fermentation; removal of starch turbidities
	Cereals	Conversion of starch to dextrins, sugar; increase water absorption
	Chocolate-cocoa	Liquidification of starches for free flow
	Confectionery	Recovery of sugar from candy scraps
	Fruit juices	Remove starches to increase sparkling properties
	Jellies	Remove starches to increase sparkling properties
	Pectin	An aid in preparation of pectin from apple pomace
	Syrups and sugars	Conversion of starches to low molecular weight dextrins (corn syrup)
	Vegetables	Hydrolysis of starch as in tenderization of peas
Cellulase	Brewing	Hydrolysis of complex carbohydrate cell walls
	Coffee	Hydrolysis of cellulose during drying of beans
	Fruits	Removal of graininess of pears; peeling of apricots, tomatoes
Dextran-sucrase	Sugar syrups	Thickening of syrup
	Ice cream	Thickening agent, body
Invertase	Artificial honey	Conversion of sucrose to glucose and fructose
	Candy	Manufacture of chocolate-coated, soft, cream candies
Lactase	Ice cream	Prevent crystallization of lactose, which results in grainy, sandy texture
	Feeds	Conversion of lactose to galactose and glucose
	Milk	Stabilization of milk proteins in frozen milk by removal of lactose
Tannase	Brewing	Removal of polyphenolic compounds
Pentosanase	Milling	Recovery of starch from wheat flour
Naringinase	Citrus	Debittering citrus pectin juice by hydrolysis of the glucoside, naringin
Pectic enzymes (useful)	Chocolate-cocoa	Hydrolytic activity during fermentation of cocoa
	Coffee	Hydrolysis of gelatinous coating during fermentation of beans
	Fruits	Softening
	Fruit juices	Improve yield of press juices, prevent cloudiness, improve concentration processes
	Olives	Extraction of oil
	Wines	Clarification
Pectic enzymes (deteriorative)	Citrus juice	Destruction and separation of pectic substances of juices
	Fruits	Excessive softening action

(continued)

Table 10.1 (continued)

Enzyme	Food	Purpose or action
Proteases (useful)	Baked goods	Softening action in doughs; cut mixing time, increase extensibility of doughs; improvement in grain, texture, loaf volume; liberate β-amylase
	Brewing	Body, flavor and nutrients development during fermentation; aid in filtration and clarification, chill-proofing
	Cereals	Modify proteins to increase drying rate, improve product handling characteristics; manufacture of miso and tofu
	Cheese	Casein coagulation; characteristic flavors during aging
	Chocolate-cocoa	Action on beans during fermentation
	Eggs, egg products	Improve drying properties
	Feeds	Use in treatment of waste products for conversion to feeds
	Meats and fish	Tenderization; recovery of protein from bones, trash fish; liberation of oils
	Milk	In preparation of soybean milk
	Protein hydrolysates	Condiments such as soy sauce and tamar sauce; specific diets; bouillon, dehydrated soups, gravy powders, processed meats
	Wines	Clarification
Proteases (deteriorative)	Eggs	Shelf life of fresh and dried whole eggs
	Crab, lobster	Overtenderization if not inactivated rapidly
	Flour	Influence on loaf volume, texture if too active
Lipase (useful)	Cheese	Aging, ripening, and general flavor characteristics
	Oils	Conversion of lipids to glycerol and fatty acids
	Milk	Production of milk with slightly cured flavor for use in milk chocolate
Lipase (deteriorative)	Cereals	Overbrowning of oat cakes; brown discoloration of wheat bran
	Milk and dairy products	Hydrolytic rancidity
	Oils	Hydrolytic rancidity
Phosphatases	Baby foods	Increase available phosphate
	Brewing	Hydrolysis of phosphate compounds
	Milk	Detection of effectiveness of pasteurization
Nucleases	Flavor enhancers	Production of nucleotides and nucleosides
Peroxidases (useful)	Vegetables	Detection of effectiveness of blanching
	Glucose determinations	In combination with glucose oxidase
Peroxidases (deteriorative)	Vegetables	Off-flavors
	Fruits	Contribution to browning action
Catalase	Milk	Destruction of H_2O_2 in cold pasteurization
	Variety of products	To remove glucose and/or oxygen to prevent browning and/or oxidation; used in conjunction with glucose oxidase
Glucose oxidase	Variety of products	Removal of oxygen and/or glucose from products such as beer, cheese, carbonated beverages, dried eggs, fruit juices, meat and fish, milk powder, wine to prevent oxidation and/or browning; used in conjunction with catalase
	Glucose determination	Specific determination of glucose; used in conjunction with peroxidase
Polyphenol oxidase (useful)	Tea, coffee, tobacco	Development of browning during ripening, fermentation, and/or aging process
Polyphenol oxidase (deteriorative)	Fruits, vegetables	Browning, off-flavor development, loss of vitamins
Lipoxygenase	Vegetables	Destruction of essential fatty acids and vitamin A; development of off-flavors
Ascorbic acid oxidase	Vegetables, fruits	Destruction of vitamin C (ascorbic acid)
Thiaminase	Meats, fish	Destruction of thiamine

Source: Reprinted with permission from Whitaker, J. R. (1972). *Principles of enzymology for the food sciences*, by courtesy of Marcel Dekker, Inc

Nature and Kinetics of Enzymes

Nature of Enzymes

The catalytic properties of enzymes are quite specific, which makes enzymes useful in analytical studies. Some enzymes consist only of protein, but most enzymes contain additional nonprotein components such as carbohydrates, lipids, metals, phosphates, or some other organic moiety. The complete enzyme is called *holoenzyme*; the protein part, *apoenzyme*; and the nonprotein part, *cofactor.* The compound that is being converted in an enzymic reaction is called *substrate.* In an enzyme reaction, the substrate combines with the holoenzyme and is released in a modified form, as indicated in Fig. 10.1.

Kinetics of Enzymes

Enzyme activity can be controlled in a number of ways that are very important to food chemists. The velocity of an enzyme-catalyzed reaction is usually proportional to the active enzyme concentration, and dependent on substrate, inhibitor, and cofactor concentration, and on temperature and pH.

Enzyme Concentration: The relationship between reaction velocity and enzyme concentration can be illustrated as the following Fig. 10.2.

In the first part of the curve, a straight line plot results when the amount of enzyme is increased in the presence of an excess of substrate that shows the concentration of an enzyme is directly proportional to the rate of the reaction. In these conditions, doubling the amount of enzyme will double the reaction velocity. In the second part of the line, at higher levels of enzyme concentration, the amount of substrate becomes the limiting factor and the linear relationship cannot be maintained, causing the line to flatten out. Under these conditions, adding more enzyme has no effect on reaction rate.

Substrate Concentration: For a given amount of enzyme under standard conditions, the initial reaction velocity (V_0) varies with an increase of initial substrate concentration (S). Figure 10.3

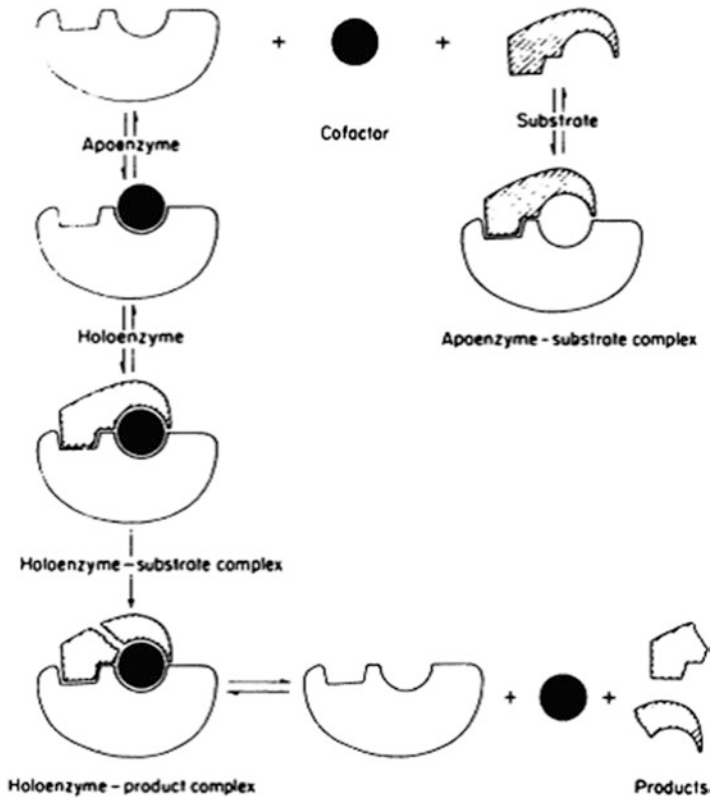


Fig. 10.1 The nature of enzymes—substrate reactions

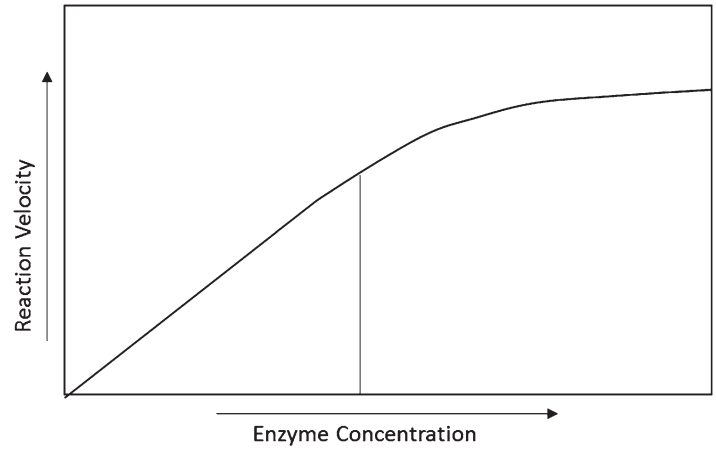


Fig. 10.2 Effect of enzyme concentration on reaction velocity

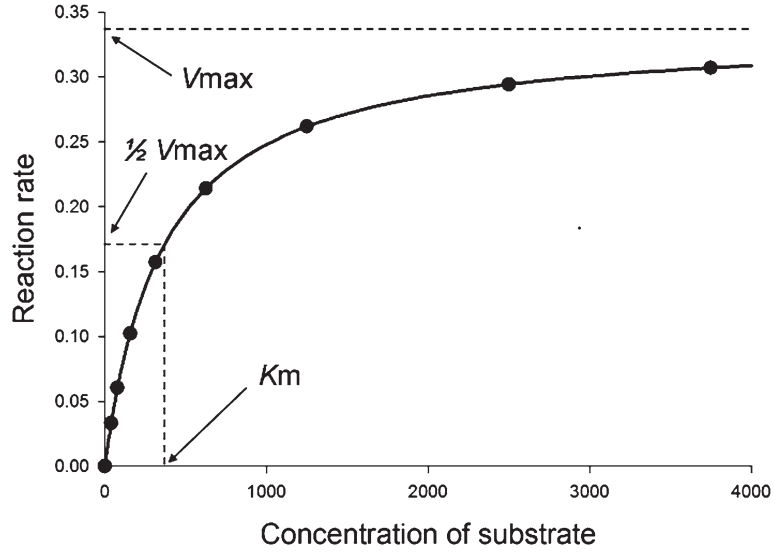


Fig. 10.3 Effect of substrate concentration on the initial velocity of an enzyme catalyzed reaction

shows the effect on V_0 of varying (S) when the enzyme concentration is held constant. When substrate concentrations are low compare to available enzyme, V_0 is nearly linearly related to (S) because not all of the enzyme molecules are combined with substrate. Therefore, substrate concentration is the limiting factor accounting for the lower reaction rate. At high substrate concentration, V_0 increases by smaller amounts in response to increases in (S) and almost unaffected by change in substrate concentration. This plateau is called the maximum velocity (V_{max}), as shown in Fig. 10.3.

This hyperbolic shape of the curve may be explained in terms of the following equation with the formation of an enzyme-substrate complex (E-S) explained by German scientists, Leonor Michaelis and Maud Menten in 1913. They postulated that enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a fast reversible step, then breaks down in a slower second step to yield the free enzyme (E) and the reaction product (P). The second step is slower and limits the rate of the overall reaction.

$$E + S \underset{k_2}{\overset{k_1}{\Leftrightarrow}} E - S \tag{10.1}$$

$$E - S \xrightarrow{k_3} E + P \tag{10.2}$$

At any given instant in an enzyme-catalyzed reaction, the enzyme exists in two forms, the free form, E and the combined form, ES. At low (S), most of the enzyme will be in the E form and the rate will be proportional to (S) because of the equilibrium will be pushed toward formation of more E–S as (S) is increased. The maximum initial rate of the catalyzed reaction (V_{max}) is observed when all of enzyme is present as E–S. At this point the enzyme is saturated with its substrate, so that further increases in (S) have no effect on rate. The saturation effect is a distinguishing characteristic of enzyme catalysis and responsible for the plateau observed in Fig. 10.3. After the E–S breaks down to yield the product P, the enzyme is free to catalyze another reaction (Eq. 10.2).

The above curve is described by the Michaelis-Menten equation:

$$V_0 = \frac{V_{max}(S)}{K_m + (S)} \qquad (10.3)$$

where

V_0 = initial reaction velocity,

(S) = initial substrate concentration,

V_{max} = maximum reaction velocity, attained when E–S is at its maximum value,

K_m = Michaelis-Menten constant, the substrate concentration where one-half of the maximum reaction velocity is attained.

This equation shows the quantitative relationship between the initial velocity V_0, the maximum initial velocity V_{max}, and the initial substrate concentration (S), all related through the Michaelis-Menten constant K_m. The effects of enzyme concentration and of substrate concentration on the reaction velocity are often described in terms of the order of the reaction that expresses the reaction rate as a function of the concentration of one or more of the substances present. An enzymatic reaction follows first-order kinetics whenever the substrate concentration is much less than the K_m value for that particular reaction. When the substrate concentration is much greater than the K_m value, the reaction is zero-order with respect to substrate concentration, since all the enzyme molecules are fully saturated with substrate molecules.

This Michaelis-Menten eq. (10.3) can be algebraically transformed into forms that are useful in the practical determination of K_m and V_{max}.

By taking the reciprocal of both sides of the Michaelis-Menten equation:

$$\frac{1}{V_0} = \frac{K_m + (S)}{V_{max}(S)} \text{ and } \frac{1}{V_0} = \frac{K_m}{V_{max}(S)} + \frac{(S)}{V_{max}(S)}$$

and simplifies to

$$\frac{1}{V_0} = \frac{K_m}{V_{max}}\frac{1}{(S)} + \frac{1}{V_{max}}$$

This equation is a transform of the Michaelis-Menten equation called the Lineweaver-Burk equation. For enzymes obeying the Michaelis-Menten relationship, a plot of $1/V_0$ versus 1/(S) yields a straight line as shown in Fig. 10.4.

This double-reciprocal presentation is also called a Lineweaver-Burk plot. The straight line will have a slop of K_m/V_{max}, an intercept of $1/V_{max}$ on the $1/V_0$ axis, and an intercept of $-1/K_m$ on the 1/(S) axis. The K_m value which is sometimes used as an indication of the affinity of an enzyme for its substrate can vary greatly from enzyme to enzyme, therefore, useful for the study and comparison of different enzymes. V_{max} also varies greatly from one enzyme to the next.

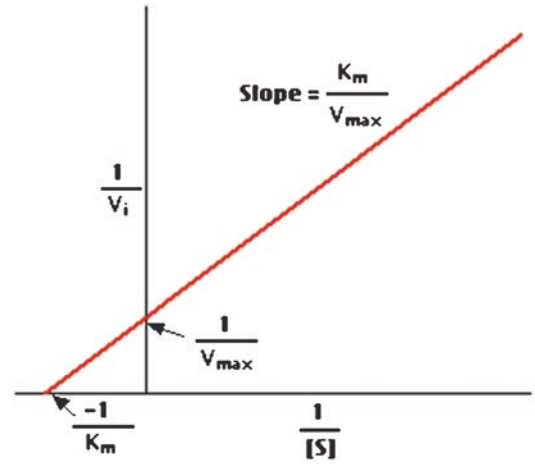
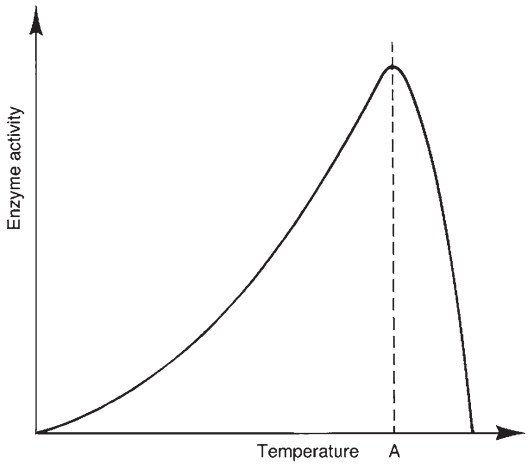


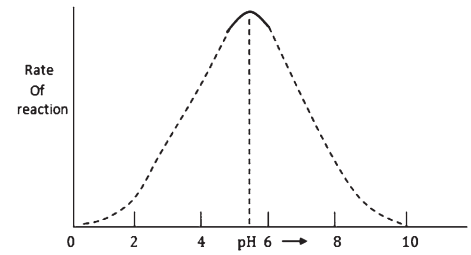
Fig. 10.4 Lineweaver-Burk (or double-reciprocal) plot

Effect of Temperature: Changes in temperature affect the rate of enzyme reaction. It is well known that enzyme-catalyzed reaction occur more slowly when a food is placed in a refrigerator, but the reactions do not stop at 0–4 °C. Most enzyme-catalyzed reactions decrease 1.4–2 time per 10 °C decrease in temperature. The usual effect of temperature on enzymatic reactions is in two stages: (1) the rate of the reaction increases with increasing temperature up to maximum (see the following graph); (2) above this temperature followed by decreasing activity at higher temperatures, due to denaturation of the enzyme.



The increasing temperature at the start produces greater molecular activity, which increases the rate of reaction. The enzyme activity increases with temperature increase such that the reaction velocity is approximately doubled for every 10 °C rise up to 45–50 °C for most enzymes. The temperature coefficient, Q_{10}, is used as an expression of the change in rate of reaction for a 10 °C change in temperature. The value of Q_{10} is determined by dividing the reaction rate at a given temperature plus 10 °C by the reaction rate at that given temperature. At higher temperature, the enzyme begins to denature (loss of activity), followed by rapid thermal inactivation at above 60–75 °C. Some enzymes, such as peroxidase, are much more thermostable than others. The criterion of heat stability is very valuable in characterizing an enzyme and is more pertinent in food processing. Heat-stability studies determine the temperature-time combination required for the inactivation of undesirable enzymes in foods. Some enzymes are known to regenerate when they are cooled following denaturation by heat. Peroxidase in vegetables which has been inactivated by scalding (blanching) can recover at least part of its enzyme activity during frozen storage.

Effect of pH: The hydrogen ion concentration of the medium in which the enzyme works affects greatly the activity of the enzyme because each enzyme works within a small pH range. Therefore, changing the pH of the system by 1 or 2 pH units from the optimum pH, can decrease enzyme velocity to 0.5 or 0.1, respectively, of that at the pH optimum. The greatest reaction velocity is attained at the optimal pH of the enzyme. Most enzymes pH optima lie often within the pH range of 4.5–8.0: β-amylase, 4.8; invertase, 5.0, and pectin methylesterase, 6.5–8.0. Denaturation of the enzyme by high or low pH can result in inactivation. Thus, it is important that pH must be controlled for two main reasons: (1) the enzyme reaction proceeds at a maximum rate at a specific pH and (2) the range of maximum stability of an enzyme also occurs at a definite pH. In food industry, the pH is controlled either to inhibit the enzyme activity or to produce the maximum activity. In fruit and vegetable products, the pH is lowered by the addition of compounds such as citric or phosphoric acids. As shown in the following figure, when the activity of an enzyme is plotted against varying pH values the result is ordinarily a bell-shaped.



Effect of Inhibitors: Some substances reduce or even stop the catalytic activity of enzymes in biochemical reactions. They block or distort the active site. Inhibitors that occupy the active site

and prevent a substrate molecule from binding to the enzyme are said to be active site-directed. It is called competitive inhibitor, as they 'compete' with the substrate for the active site. Competitive inhibitors are often compounds that resemble the substrate and combine with the enzyme. Inhibitors that attach to other parts of the enzyme molecule, perhaps distorting its shape, are said to be non-active site-directed are called noncompetitive inhibitors.

Practical methods of enzyme inhibition that may be used in the food industry are very limited due to problems associated with sensory quality and economic feasibility. In practice, the food industry is limited in enzyme inhibition or control to inactivation by heat, pH, dehydration, high pressure processing or the use of chemicals such as sulfur dioxide and phenolic antioxidants.

Specificity

The nature of the enzyme-substrate reaction as explained in Fig. 10.1 requires that each enzyme reaction is highly specific. The shape and size of the active site of the enzyme, as well as the substrate, are important. But this complementarity may be even further expanded to cover amino acid residues in the vicinity of the active site, hydrophobic areas near the active site, or the presence of a positive electrical charge near the active site (Parkin 1993). Types of specificity may include group, bond, stereo, and absolute specificity, or some combination of these. An example of the specificity of enzymes is given in Fig. 10.5, which illustrates the specificity of proline-specific peptidases (Habibi-Najafi and Lee 1996). The amino acid composition of casein is high in proline, and the location of this amino acid in the protein chain is inaccessible to common aminopeptidases and the di- and tripeptidases with broad specificity. Hydrolysis of the proline bonds requires proline-specific peptidases, including several exopeptidases and an endopeptidase. Figure 10.5 illustrates that this type of specificity is related to the type of amino acid in a protein as well as its location in the chain. Neighboring amino acids also determine the type of peptidase required to hydrolyze a particular peptide bond.

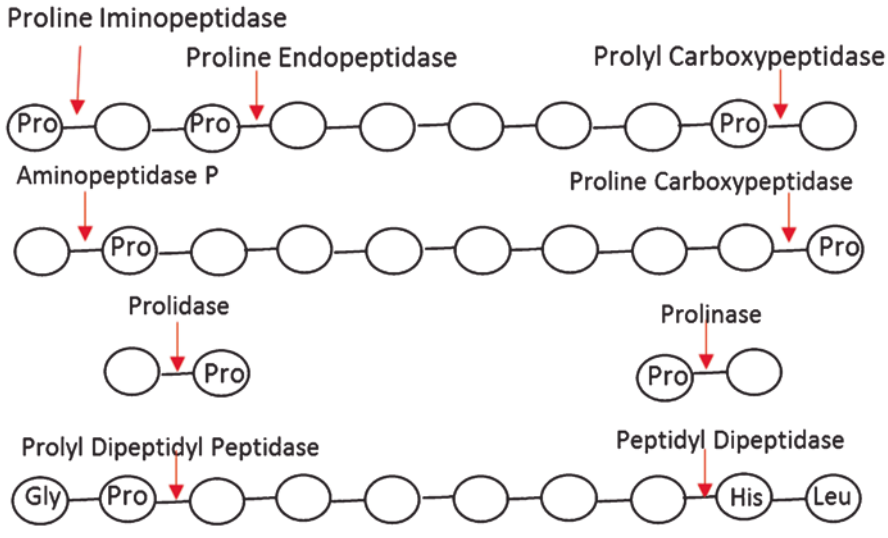


Fig. 10.5 Mode of action of proline-specific peptidases. Adopted from from Habibi-Najafi, M. B., & Lee, B. H. Bitterness in cheese: A review. *Critical Reviews in Food Science and Nutrition*, *36*(5), 408

Classification

Enzymes are classified by the Commission on Enzymes of the International Union of Biochemistry. The basis for the classification is the division of enzymes into groups according to the type of reaction catalyzed. This, together with the name or names of substrate(s), is used to name individual enzymes. Each well-defined enzyme can be described in three ways—by a systematic name, by a trivial name, and by a number of the Enzyme Commission (EC). Thus, the enzyme α-amylase (trivial name) has the systematic name α-l,4-glucan-4-glucanohydrolase, and the number EC 3.2.1.1. The system of nomenclature has been described by Whitaker (1972, 1974) and Parkin (1993).

Enzyme Production

Some of the traditionally used industrial enzymes (e.g., rennet and papain) are prepared from animal and plant sources. Recent developments in industrial enzyme production have emphasized the microbial enzymes (Frost 1986). Microbial enzymes are very heat stable and have a broader pH optimum. Most of these enzymes are made by submerged cultivation of highly developed strains of microorganisms. Developments in biotechnology will make it possible to transfer genes for the elaboration of specific enzymes to different organisms. The major industrial enzyme processes are listed in Table 10.2.

Hydrolases

The hydrolases as a group include all enzymes that involve water in the formation of their products. For a substrate *AB*, the reaction can be represented as follows:

$$AB + HOH \rightarrow HA + BOH$$

The hydrolases are classified on the basis of the type of bond hydrolyzed. The most important are those that act on ester bonds, glycosyl bonds, peptide bonds, and C–N bonds other than peptides.

Esterases

The esterases are involved in the hydrolysis of ester linkages of various types. The products formed are acid and alcohol. These enzymes may hydrolyze triglycerides and include several lipases; for instance, phospholipids are hydrolyzed by phospholipases, and cholesterol esters are hydrolyzed by cholesterol esterase. The carboxylesterases are enzymes that hydrolyze triglycerides such as tributyrin. They can be distinguished from lipases because they hydrolyze soluble substrates, whereas lipases only act at the water-lipid interfaces of emulsions. Therefore, any condition that results in increased surface area of the water-lipid interface will increase the activity of the enzyme. This is the reason that lipase activity is much greater in homogenized (not pasteurized) milk than in the non-homogenized product. Most of the lipolytic enzymes are specific for either the acid or the alcohol moiety of the substrate, and, in the case of esters of polyhydric alcohols, there may also be a positional specificity.

Lipases are produced by microorganisms such as bacteria and molds; are produced by plants; are present in animals, especially in the pancreas; and are present in milk. Lipases may cause spoilage of food because the free fatty acids formed cause rancidity. In other cases, the action of lipases is desirable and is produced intentionally. The boundary between flavor and off-flavor is often a very narrow range. For instance, hydrolysis of milk fat in milk leads to very unpleasant off-flavors at very low free fatty acid concentration. The hydrolysis of milk fat in cheese contributes to the desirable flavor. These differences are probably related to the

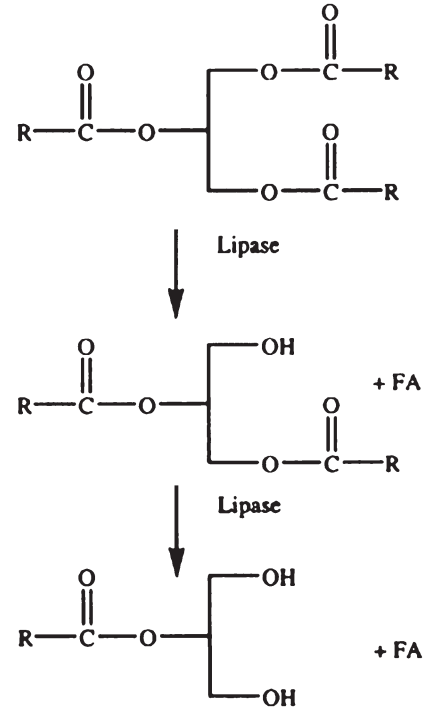
Table 10.2 Major industrial enzymes and the process used for their production

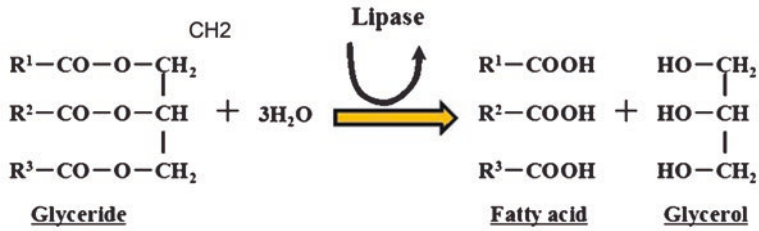
Enzyme	Source	Submerged fermentation	Surface fermentation	Intracellular	Extracellular	Concentration	Precipitation	Drying	Pelleting	Further purification	Solid product	Solution product	Immobilized product
Proteases													
Rennet	Calf stomach	–	–	–	✓	–	–	✓	–	–	✓	✓	–
Trypsin	Animal pancreas	–	–	–	✓	✓	✓	✓	–	✓	✓	–	–
Papain	Carica papaya fruit	–	–	–	✓✓	–	–	✓	–	–	✓	✓	–
Fungal	*Aspergillus oryzae*	✓	✓	–	✓	✓	✓	✓	–	–	✓	–	–
Fungal (rennins)	*Mucor* spp.	✓	✓	–	✓	✓	–	✓	–	–	✓	✓	–
Bacterial	*Bacillus* spp.	✓	–	–	✓	✓	✓	✓	✓	–	✓	✓	–
Glycosidases													
Bacterial α-amylase	*Bacillus* spp.	✓	–	–	✓	✓	–	✓	–	–	✓	✓	–
Fungal α-amylase	*Aspergillus oryzae*	✓	–	–	✓	✓	–	✓	–	–	✓	✓	–
β-amylase	Barley	–	–	–	✓	✓	✓	✓	–	–	✓	–	–
Amyloglucosidase	*Aspergillus niger*	✓	–	–	✓	✓	–	–	–	–	–	✓	–
Pectinase	*Aspergillus niger*	–	✓	–	✓	✓	–	✓	–	–	✓	✓	–
Cellulase	Molds	✓	–	–	✓	✓	✓	–	–	✓	✓	–	–
Yeast lactase	*Kluyveromyces* spp.	✓	–	✓	–	–	✓	✓	–	–	✓	✓	–
Mold lactase	*Aspergillus* spp.	✓	✓	–	✓	–	✓	✓	–	–	✓	–	✓
Others													
Glucose isomerase	Various microbial sources	✓	–	✓	–	✓	✓	✓	–	–	–	–	✓
Glucose oxidase	*Aspergillus niger*	✓	–	✓	–	–	✓	–	–	✓	✓	✓	–
Mold catalase	*Aspergillus niger*	✓	–	✓	–	–	✓	–	–	–	–	✓	–
Animal catalase	Liver	–	–	✓	–	✓	✓	✓	–	✓	✓	✓	–
Lipase	Molds	✓	–	–	✓	✓	✓	✓	–	–	✓	–	–

Source: From Frost, G. M. (1986). Commercial production of enzymes. In B. J. F. Hudson (Ed.), *Developments in food proteins.* Elsevier Applied Science Publishers Ltd

background upon which these fatty acids are superimposed and to the specificity for particular groups of fatty acids of each enzyme. In seeds, lipases may cause fat hydrolysis unless the enzymes are destroyed by heat. Palm oil produced by primitive methods in Africa used to consist of more than 10% of free fatty acids. Such spoilage problems are also encountered in grains and flour. The activity of lipase in wheat and other grains is highly dependent on water content. In wheat, for example, the activity of lipase is five times higher at 15.1% than at 8.8% moisture. The lipolytic activity of oats is higher than that of most other grains.

Lipases can be divided into those that have a positional specificity and those that do not. The former preferentially hydrolyze the ester bonds of the primary ester positions. This results in the formation of mono- and diglycerides, as represented by the following reaction:





During the progress of the reaction, the concentration of diglycerides and monoglycerides increases, as is shown in Fig. 10.6. The β-monoglycerides formed are resistant to further hydrolysis. This pattem is characteristic of pancreatic lipase and has been used to study the triglyceride structure of many fats and oils.

The hydrolysis of triglycerides in cheese is an example of a desirable flavor-producing process. The extent of free fatty acid formation is much higher in blue cheese than in Cheddar cheese, as is shown in Table 10.3. This is most likely the result of lipases elaborated by organisms growing in the blue cheese, such as *P. roqueforti, P. camemberti,* and others. The extent of lipolysis increases with age, as is demonstrated by the increasing content of partial glycerides during the aging of cheese (Table 10.4). In many cases, lipolysis is induced by the addition of lipolytic enzymes. In the North American chocolate industry, it is customary to induce some lipolysis in chocolate by means of lipase. In the production of Italian cheeses, lipolysis is induced by the use of pregastric esterases. These are lipolytic enzymes obtained from the oral glands located at the base of the tongue in calves, lambs, or kids.

Specificity for certain fatty acids by some lipolytic enzymes has been demonstrated. Pancreatic lipase and milk lipase are broad-spectrum enzymes and show no specificity for

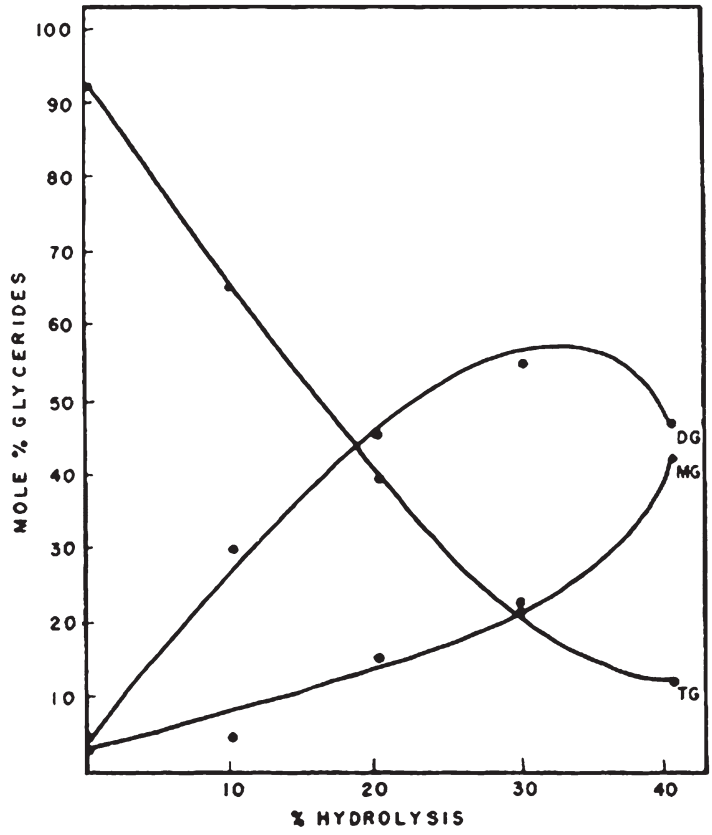


Fig. 10.6 The course of pancreatic lipase hydrolysis of tricaprylin. *MG* monoglycerides, *DG* diglycerides, *TG* triglycerides. *Source*: From Boudreau, A., & deMan, J. M. (1965). The mode of action of pancreatic lipase on milkfat glycerides. *Canadian Journal of Biochemistry*, *43*, 1799–1805

Table 10.3 Free fatty acids in some dairy products

Product	Free fatty acids (mg/kg)
Fresh milk	415
Moderately rancid cream	1027
Butter	2733
Cheddar cheese	1793 (avg. of 12 samples)
Blue cheese	23,500–66,700 (range 3 samples)

Source: From Day, E. A. (1966). Role of milk lipids in flavors of dairy products. In R. F. Gould (Ed.), *Flavor chemistry*. American Chemical Society

Table 10.4 Formation of partial glycerides in cheddar cheese

Product type	Diglycerides (wt %)	Mono-glycerides (wt %)
Mild	7.4–7.6	1.0–2.0
Medium	7.6–9.7	0.5–1.4
Old	11.9–15.6	1.1–3.2

any of the fatty acids found in fats. Instead, the fatty acids that are released from the glycerides occur in about the same ratio as they are present in the original fat. Specificity was shown by Nelson (1972) in calf esterase and in a mixed pancreatin-esterase preparation (Table 10.5). Pregastric esterases and lipase from *Aspergillus* species primarily hydrolyze shorter chain-length fatty acids (Arnold et al. 1975).

Specificity of lipases may be expressed in a number of different ways—substrate specific, regiospecific, nonspecific, fatty acyl specific, and stereospecific. Examples of these specificities have been presented by Villeneuve and Foglia (1997) (Table 10.6).

Substrate specificity is the ability to hydrolyze a particular glycerol ester, such as when a lipase can rapidly hydrolyze a triacylglycerol, but acts on a monoacylglycerol only slowly. Regiospecificity involves a specific action on

Table 10.5 Free fatty acids released from milkfat by several lipolytic enzymes

Fatty acid	Milk lipase	Steapsin	Pancreatic lipase	Calf esterase	Esterase pancreatin
4:0	13.9	10.7	14.4	35.00	15.85
6:0	2.1	2.9	2.1	2.5	3.6
8:0	1.8	1.5	1.4	1.3	3.0
10:0	3.0	3.7	3.3	3.1	5.5
12:0	2.7	4.0	3.8	5.1	4.4
14:0	7.7	10.7	10.1	13.2	8.5
16:0	21.6	21.6	24.0	15.9	19.3
18:1 and 18:2	29.2	24.3	25.5	14.2	21.1
18:0	10.5	13.4	9.7	3.2	10.1

Source: From Nelson, J. H. (1972). Enzymatically produced flavors for fatty systems. Journal of the American Oil Chemists' Society, *49*, 559–562

Table 10.6 Examples of lipase specificities

Specificity	Lipase
Substrate specific	
Monoacylglyercols	Rat adipose tissue
Mono- and diacylglycerols	*Penicillium camembertii*
Triacylglycerols	*Penicillium* sp.
Regiospecific	
1,3-regioselective	*Aspergilllus niger*
	Rhizopus arrhizus
	Mucor miehei
sn-2-regioselective	*Candida antarctica* A
Nonspecific	*Penicillium expansum*
	Aspergillus sp.
	Pseudomonas cepacia
Fatty acylspecific	
Short-chain fatty acid (FA)	*Penicillium roqueforti*
	Premature infant gastric
cis-9 unsaturated FA	*Geotrichum candidum*
Long-chain unsaturated FA	*Botrytis cinerea*
Stereospecific	
sn-1 stereospecific	*Humicola lanuginosa*
	Pseudomonas aeruginosa
sn-3 stereospecific	*Fusarium solani* cutinase
	Rabbit gastric

Source: Reprinted with permission from Villeneuve, R., & Foglia, T. A. Lipase specificities: Potential application in lipid bioconversions. Journal of the American Oil Chemists' Society, *8*, 641, © 1997, AOCS Press

either the sn-1 and sn-3 positions or reaction with only the sn-2 position. The 1,3-specific enzymes have been researched extensively, because it is now recognized that lipases in addition to hydrolysis can catalyze the reverse reaction, esterification or transesterification. This has opened up the possibility of tailor-making triacylglycerols with a specific structure, and this is especially important for producing high-value fats such as cocoa butter equivalents. The catalytic activity of lipases is reversible and depends on the water content of the reaction mixture. At high water levels, the hydrolytic reaction prevails, whereas at low water levels the synthetic reaction is favored. A number of lipase catalyzed reactions are possible, and these have been summarized in Fig. 10.7 (Villeneuve and Foglia 1997). Most of the lipases used for industrial processes have been developed from microbes because these usually exhibit high temperature tolerance. Lipases from *Mucor miehei* and *Candida antarctica* have been cloned and expressed in industry-friendly organisms. Lipases from genetically engineered strains will likely be of major industrial importance in the future (Godtfredsen 1993). Fatty acid–specific lipases react with either short-chain fatty acids (*Penicillium roqueforti*) or some long-chain fatty acids such as *cis*-9-unsaturated fatty acids (*Geotrichum candidum*). Stereospecific lipases react with only fatty acids at the sn-1 or sn-3 position.

The applications of microbial lipases in the food industry involve the hydrolytic as well as the synthetic capabilities of these enzymes and have been summarized by Godtfredsen (1993) in Table 10.7.

The lipase-catalyzed interesterification process can be used for the production of triacylglycerols with specific physical properties, and it also opens up possibilities for making so-called

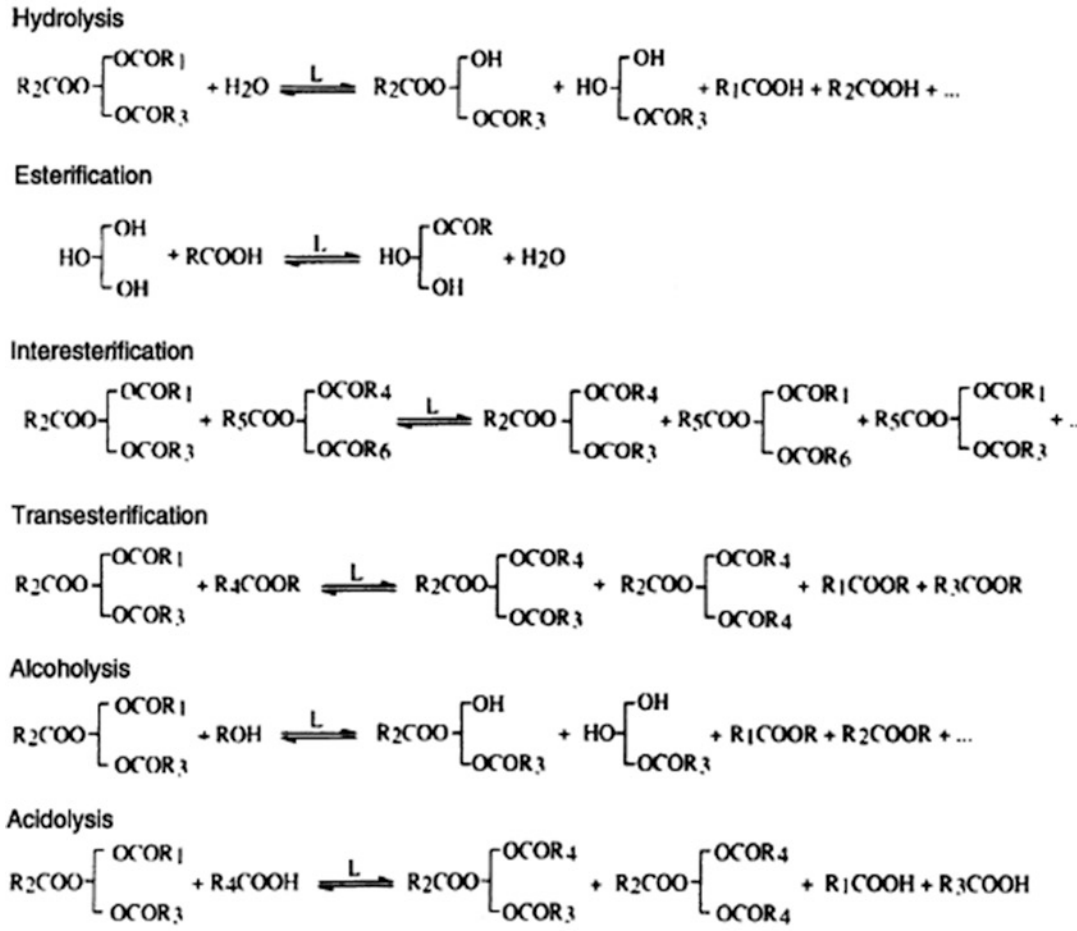


Fig. 10.7 Lipase catalyzed reactions used in oil and fat modification. *Source:* Reprinted with permission from Villeneuve, R., & T. A. Foglia. Lipase specificities: Potential application in lipid bioconversions. *Journal of the American Oil Chemists' Society*, *8*, 642, © 1997, AOCS Press

Table 10.7 Application of microbial lipases in the food industry

Industry	Effect	Product
Dairy	Hydrolysis of milk fat	Flavor agents
	Cheese ripening	Cheese
	Modification of butter fat	Butter
Bakery	Flavor improvement and shelf-life prolongation	Bakery products
Beverage	Improved aroma	Beverages
Food dressing	Quality improvement	Mayonnaise, dressing, and whipped toppings
Health food	Transesterification	Health foods
Meat and fish	Flavor development and fat removal	Meat and fish products
Fat and oil	Transesterification	Cocoa butter, margarine
	Hydrolysis	Fatty acids, glycerol, mono- and diglycerides

Source: Reprinted with permission from Godtfredsen, S. E. Lipases, enzymes in food processing. T. Nagodawithana and G. Reed (Eds.), p. 210, © 1993, Academic Press

structured lipids (Akoh 1997). An example is a triacylglycerol that carries an essential fatty acid (e.g., DHA-docosahexaenoic acid) in the sn-2 position and short-chain fatty acids in the sn-1 and sn-3 positions. Such a structural triacylglycerol would rapidly be hydrolyzed in the digestive tract and provide an easily absorbed monoacylglycerol that carries the essential fatty acid (Godtfredsen 1993).

The lipases that have received attention for their ability to synthesize ester bonds have been obtained from yeasts, bacteria, and fungi. Lipases can be classified into three groups according to their specificity (Macrae 1983). The first group contains nonspecific lipases. These show no specificity regarding the position of the ester bond in the glycerol molecule, or the nature of the fatty acid. Examples of enzymes in this group are lipases of *Candida cylindracae, Corynebacterium acnes,* and *Staphylococcus aureus.* The second group contains lipases with position specificity for the 1- and 3-positions of the glycerides. This is common among microbial lipases and is the result of the sterically hindered ester bond of the 2-position's inability to enter the active site of the enzyme. Lipases in this group are obtained from *Aspergillus niger, Mucor javanicus,* and *Rhizopus arrhizus.* The third group of lipases show specificity for particular fatty acids. An example is the lipase from *Geotrichum candidum,* which has a marked specificity for long-chain fatty acids that contain a *cis* double bond in the 2-position. The knowledge of the synthetic ability of lipases has opened a whole new area of study in the modification of fats. The possibility of modifying fats and oils by immobilized lipase technology may result in the production of food fats that have a higher essential fatty acid content and lower *trans* levels than is possible with current methods of hydrogenation.

Amylases

The amylases are the most important enzymes of the group of glycoside hydrolases. These starch-degrading enzymes can be divided into two groups, the so-called debranching enzymes that specifically hydrolyze the 1,6-linkages between chains, and the enzymes that split the 1,4-linkages between glucose units of the straight chains. The latter group consists of endoenzymes that cleave the bonds at random points along the chains and exoenzymes that cleave at specific points near the chain ends. This behavior has been represented by Marshall (1975) as a diagram of the structure of amylopectin (Fig. 10.8). In this molecule, the 1,4-α-glucan chains are interlinked by 1,6-α-glucosidic linkages resulting in a highly branched molecule. The molecule is com posed of three types of chains; the *A* chains carry no substituent, the *B* chains carry other chains linked to a primary hydroxyl group, and the molecule contains only one *C* chain with a free reducing glucose unit. The chains are 25–30 units in length in starch and only 10 units in glycogen.

Alpha-Amylase (α-1,4-Glucan 4-Glucanohydrolase)

This enzyme is distributed widely in the animal and plant kingdoms. The enzyme contains 1 gram-atom of calcium per mole. Alpha-amylase (α-1,4-glucan-4-glucanohydrolase) is an endoenzyme that hydrolyzes the α-l,4-glucosidic bonds in a random fashion along the chain. It hydrolyzes amylopectin to oligosaccharides that contain two to six glucose units. This action, therefore, leads to a rapid decrease in viscosity, but little monosaccharide formation. A mixture of amylose and amylopectin will be hydrolyzed into a mixture of dextrins, maltose, glucose, and oligosaccharides. Amylose is completely hydrolyzed to maltose, although there usually is some maltotriose formed, which hydrolyzes only slowly.

Beta-Amylase (α-1,4-Glucan Maltohydrolase)

This is an exoenzyme and removes successive maltose units from the nonreducing end of the glucosidic chains. The action is stopped at the branch point where the α-1,6 glucosidic linkage cannot be broken by α-amylase. The resulting compound is named *limit dextrin.* Beta-amylase is found only in higher plants. Barley malt, wheat, sweet potatoes, and soybeans are good sources.

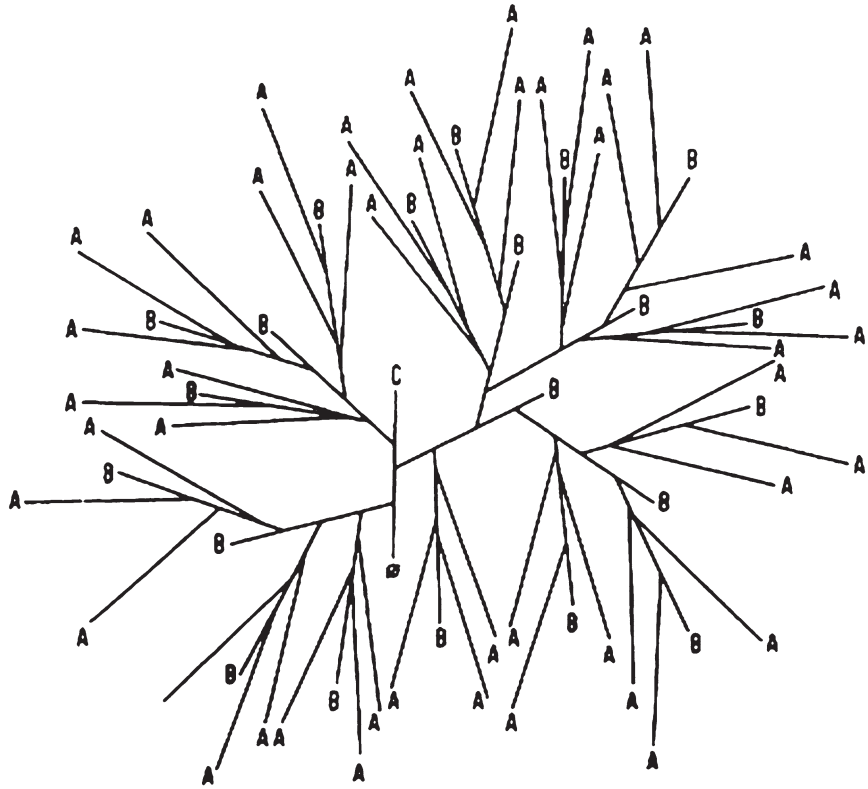


Fig. 10.8 Diagrammatic representation of amylopectin structure. Lines represent α-D-glucan chains linked by 1,4-bonds. The branch points are 1,6-α glucosidic bonds. *Source:* From Marshall, J. J. (1975). Starch degrading enzymes, old and new. *Starke*, *27*, 377–383

Beta-amylase is technologically important in the baking, brewing, and distilling industries, where starch is converted into the fermentable sugar maltose. Yeast ferments maltose, sucrose, invert sugar, and glucose but does not ferment dextrins or oligosaccharides containing more than two hexose units.

Glucoamylase (α-1,4-Glucan Glucohydrolase)

This is an exoenzyme that removes glucose units in a consecutive manner from the nonreducing end of the substrate chain. The product formed is glucose only, and this differentiates this enzyme from α- and β-amylase. In addition to hydrolyzing the α-1,4 linkages, this enzyme can also attack the α-1,6 linkages at the branch point, albeit at a slower rate. This means that starch can be completely degraded to glucose. The enzyme is present in bacteria and molds and is used industrially in the production of corn syrup and glucose.

A problem in the enzymic conversion of corn starch to glucose is the presence of transglucosidase enzyme in preparations of α-amylase and glucoamylase. The transglucosidase catalyzes the formation of oligosaccharides from glucose, thus reducing the yield of glucose.

Nondamaged grains such as wheat and barley contain very little α-amylase but relatively high levels of β-amylase. When these grains germinate, the β-amylase level hardly changes, but the α-amylase content may increase by a factor of 1000. The combined action of α- and β-amylase in the germinated grain greatly increases the production of fermentable sugars. The development

Table 10.8 Development of α-amylase during malting of barley at 20 °C

Days of steeping and germination	α-Amylase (20° dextrose units)
0	0
3	55
5	110
7	130
8	135

Source: From Green, S. R. (1969). New use of enzymes in the brewing industry. *MBAA Technical Quarterly, 6*, 33–39

of α-amylase activity during malting of barley is shown in Table 10.8. In wheat flour, high α-amylase activity is undesirable, because too much carbon dioxide is formed during baking.

Raw, nondamaged, and ungelatinized starch is not susceptible to β-amylase activity. In contrast, α-amylase can slowly attack intact starch granules. This differs with the type of starch; for example, waxy corn starch is more easily attacked than potato starch. In general, extensive hydrolysis of starch requires gelatinization. Damaged starch granules are more easily attacked by amylases, which is important in bread making Alphaamylase can be obtained from malt, from fungi (*Aspergillus oryzae*), or from bacteria (*B. subtilis*). The bacterial amylases have a higher temperature tolerance than the malt amylases.

Beta-Galactosidase (β-D-Galactoside Galactohydrolase)

This enzyme catalyzes the hydrolysis of β-D-galactosides and α-L-arabinosides. It is best known for its action in hydrolyzing lactose and is, therefore, also known as lactase. The enzyme is widely distributed and occurs in higher animals, bacteria, yeasts, and plants. Beta-galactosidase or lactase is found in humans in the cells of the intestinal mucous membrane. A condition that is widespread in non-Caucasian adults is characterized by an absence of lactase. Such individuals are said to have lactose intolerance, which is an inability to digest milk properly.

The presence of galactose inhibits lactose hydrolysis by lactase. Glucose does not have this effect.

Pectic Enzymes

The pectic enzymes are capable of degrading pectic substances and occur in higher plants and in microorganisms. They are not found in higher animals, with the exception of the snail. These enzymes are commercially important for the treatment of fruit juices and beverages to aid in filtration and clarification and increasing yields. The enzymes can also be used for the production of low methoxyl pectins and galacturonic acids. The presence of pectic enzymes in fruits and vegetables can result in excessive softening. In tomato and fruit juices, pectic enzymes may cause "cloud" separation.

There are several groups of pectic enzymes, including pectinesterase, the enzyme that hydrolyzes methoxyl groups, and the depolymerizing enzymes polygalacturonase and pectate lyase.

Pectinesterase (Pectin Pectyl-Hydrolase)

This enzyme removes methoxyl groups from pectin. The enzyme is referred to by several other names, including pectase, pectin methoxylase, pectin methyl esterase, and pectin demethylase. Pectinesterases are found in bacteria, fungi, and higher plants, with very large amounts occurring in citrus fruits and tomatoes. The enzyme is specific for galacturonide esters and will not attack non-galacturonide methyl esters to any large extent. The reaction catalyzed by pectin esterase is presented in Fig. 10.9. It has been suggested that the distribution of methoxyl groups along the chain affects the reaction velocity of the enzyme (MacMillan and Sheiman 1974). Apparently, pectinesterase requires a free carboxyl group next to an esterified group on the galacturonide chain to act, with the pectinesterase moving down the chain linearly until an obstruction is reached.

To maintain cloud stability in fruit juices, high-temperature–short-time (HTST) pasteurization is used to deactivate pectolytic enzymes. Pectin is a protective colloid that helps to keep insoluble particles in suspension. Cloudiness is required in commercial products to provide a desirable appearance. The destruction of the high levels of pectinesterase during the production of

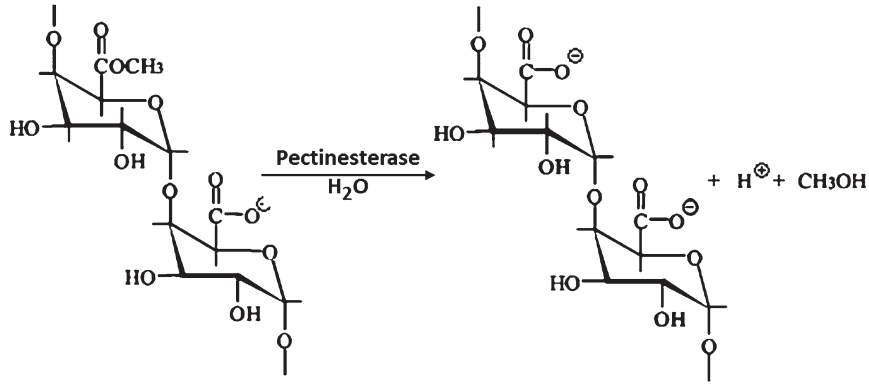


Fig. 10.9 Reaction catalyzed by pectinesterase

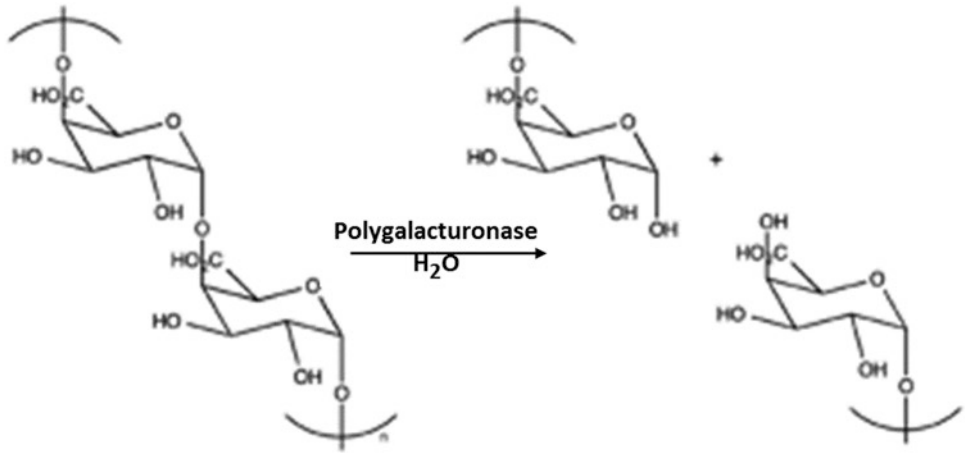


Fig. 10.10 Reaction catalyzed by polygalacturonase

tomato juice and puree is of vital importance. The pectinesterase will act quite rapidly once the tomato is broken. In the so-called hot-break method, the tomatoes are broken up at high temperature so that the pectic enzymes are destroyed instantaneously.

Polygalacturonase (Poly-α-1,4-Galacturonide Glycanohydrolase)

This enzyme is also known as pectinase, and it hydrolyzes the glycosidic linkages in pectic substances according to the reaction pattern shown in Fig. 10.10. The polygalacturonases can be divided into endoenzymes that act within the molecule on α-1,4 linkages and exoenzymes that catalyze the stepwise hydrolysis of galacturonic acid molecules from the nonreducing end of the chain. A further division can be made by the fact that some polygalacturonases act principally on methylated substrates (pectins), whereas others act on substrates with free carboxylic acid groups (pectic acids). These enzymes are named polymethyl galacturonases and polygalacturonases, respectively. The preferential mode of hydrolysis and the preferred substrates are listed in Table 10.9. Endopolygalacturonases occur in fruits and in filamentous fungi, but not in yeast or bacteria. Exopolygalacturonases occur in plants (for example, in carrots and peaches), fungi, and bacteria.

Table 10.9 Action of polygalacturonases

Type of attack	Enzyme	Preferred substrate
Random	Endo-polymethylgalacturonase	Pectin
Random	Endo-polygalacturonase	Pectic acid
Terminal	Exo-polymethylgalacturonase	Pectin
Terminal	Exo-polygalacturonase	Pectic acid

Pectate Lyase (Poly-α-l,4-D-Galacturonide Lyase)

This enzyme is also known as *trans*-eliminase; it splits the glycosidic bonds of a glucuronide chain by *trans* elimination of hydrogen from the 4- and 5-positions of the glucuronide moiety. The reaction pattern is presented in Fig. 10.11. The glycosidic bonds in pectin are highly susceptible to this reaction. The pectin lyases are of the endotype and are obtained exclusively from filamentous fungi, such as *Aspergillus niger*. The purified enzyme has an optimum pH of 5.1 to 5.2 and isoelectric point between 3 and 4 (Albersheim and Kilias 1962).

Commercial Use

Pectic enzymes are used commercially in the clarification of fruit juices and wines and for aiding the disintegration of fruit pulps. By reducing the large pectin molecules into smaller units and eventually into galacturonic acid, the compounds become water soluble and lose their suspending power; also, their viscosity is reduced and the insoluble pulp particles rapidly settle out.

Most microorganisms produce at least one but usually several pectic enzymes. Almost all fungi and many bacteria produce these enzymes, which readily degrade the pectin layers holding plant cells together. This leads to separation and degradation of the cells, and the plant tissue becomes soft. Bacterial degradation of pectin in plant tissues is responsible for the spoilage known as "soft rot" in fruits and vegetables. Commercial food grade pectic enzyme preparations may contain several different pectic enzymes. Usually, one type predominates; this depends on the intended use of the enzyme preparation.

Proteases

Proteolytic enzymes are important in many industrial food processing procedures. The reaction catalyzed by proteolytic enzymes is the hydrolysis of peptide bonds of proteins; this reaction is shown in Fig. 10.12. Whitaker (1972) has listed the specificity requirements for the hydrolysis of peptide bonds by proteolytic enzymes. These include the nature of R_1, and R_2 groups, configuration of the amino acid, size of substrate molecule, and the nature of the *X* and *Y* groups. A major distinguishing factor of proteolytic enzymes is the effect of R_1, and R_2 groups. The enzyme α-chymotrypsin hydrolyzes peptide bonds readily only when R_1 is part of a tyrosyl, phenylalanyl, or tryptophanyl residue. Trypsin requires R_1 to belong to an arginyl or lysyl residue. Specific requirement for the R_2 groups is exhibited by pepsin and the carboxypeptidases; both require R_2 to belong to a phenylalanyl residue. The enzymes

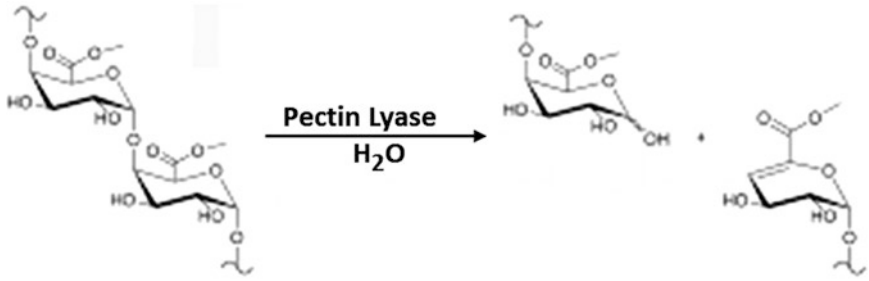


Fig. 10.11 Reaction catalyzed by pectin lyase

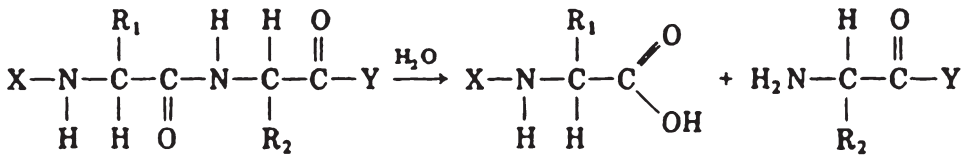


Fig. 10.12 Reaction catalyzed by proteases

require the amino acids of proteins to be in the L-configuration but frequently do not have a strict requirement for molecular size. The nature of *X* and *Y* permits the division of proteases into endopeptidases and exopeptidases. The former split peptide bonds in a random way in the interior of the substrate molecule and show maximum activity when *X* and *Y* are derived. The carboxypeptidases require that *Y* be a hydroxyl group, the aminopeptidases require that *X* be a hydrogen, and the dipeptidases require that *X* and *Y* both be underived.

Proteolytic enzymes can be divided into the following four groups: the acid proteases, the serine proteases, the sulfhydryl proteases, and the metal-containing proteases.

Acid Proteases

This is a group of enzymes with pH optima at low values. Included in this group are pepsin, rennin (chymosin), and a large number of microbial and fungal proteases. Rennin, the pure enzyme contained in rennet, is an extract of calves' stomachs that has been used for thousands of years as a coagulating agent in cheese making. Because of the scarcity of calves' stomachs, rennet substitutes are now widely used, and the coagulants used in cheese making usually contain mixtures of rennin and pepsin and/or microbial proteases. Some of the microbial proteases have been used for centuries in the Far East in the production of fermented foods such as soy sauce.

Rennin is present in the fourth stomach of the suckling calf. It is secreted in an inactive form, a zymogen, named prorennin. The crude extract obtained from the dried stomachs (vells) contains both rennin and prorennin. The conversion of prorennin to rennin can be speeded up by addition of acid. This conversion involves an autocatalytic process, in which a limited proteolysis of the prorennin occurs, thus reducing the molecular weight about 14 percent. The conversion can also be catalyzed by pepsin. The process involves the release of peptides from the N-terminal end of prorennin, which reduces the molecular weight from about 36,000 to about 31,000. The molecule of prorennin consists of a single peptide chain joined internally by three disulfide bridges. After conversion to rennin, the disulfide bridges remain intact. As the calves grow older and start to eat other feeds as well as milk, the stomach starts to produce pepsin instead of rennin. The optimum activity of rennin is at pH 3.5, but it is most stable at pH 5; the clotting of cheese milk is carried out at pH values of 5.5–6.5.

The coagulation or clotting of milk by rennin occurs in two stages. In the first, the enzymic stage, the enzyme acts on κ-casein so that it can no longer stabilize the casein micelle. The second, or nonenzymic stage, involves the clotting of the modified casein micelles by calcium ions. The enzymic stage involves a limited and specific action on the κ-casein, resulting in the formation of insoluble *para*-κ-casein and a soluble macropeptide. The latter has a molecular weight of 6000–8000, is extremely hydrophilic, and contains about 30 percent carbohydrate. The glycomacropeptide contains galactosamine, galactose, and *N*-acetyl neuraminic acid (sialic acid). The splitting of the glycomacropeptide from κ-casein involves the breaking of a phenylalanine-methionine bond in the peptide chain. Other clotting enzymes—including pepsin, chymotrypsin, and microbial proteases—break the same bond and produce the same glycomacropeptide.

Pepsin is elaborated in the mucosa of the stomach lining in the form of pepsinogen. The high acidity of the stomach aids in the autocatalytic conversion into pepsin. This conversion involves splitting several peptide fragments from the N-terminal end of pepsinogen. The fragments consist of one large peptide and several small ones. The large peptide remains associated with pepsinogen by noncovalent bonds and acts as an inhibitor. The inhibitor dissociates from pepsin at a pH of 1–2. In the initial stages of the conversion of pepsinogen to pepsin, six peptide bonds are broken, and continued action on the large peptide (Fig. 10.13) results in three more bonds being hydrolyzed. In this process, the molecular weight changes from 43,000 to 35,000 and the isoelectric point changes from 3.7 to less than 1. The pepsin molecule consists of a single polypeptide chain that contains 321 amino acids. The tertiary structure is stabilized by three disulfide bridges and a phosphate linkage. The phosphate group is

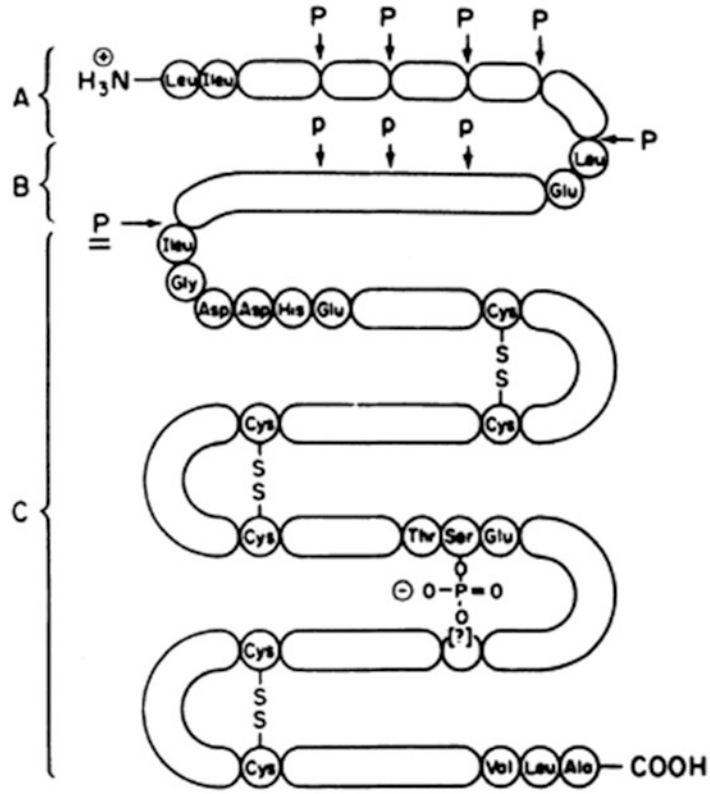


Fig. 10.13 Structure of pepsinogen and its conversion to pepsin. *Source:* From Bovey, F. A., & Yanari, S. S. (1960). Pepsin. In P. D. Boyer et al. (Eds.) *The enzymes* (vol. 4). Academic Press

attached to a seryl residue and is not essential for enzyme activity. The pH optimum of pepsin is pH 2 and the enzyme is stable from pH 2–5. At higher pH values, the enzyme is rapidly denatured and loses its activity. The primary specificity of pepsin is toward the R_2 group (see the equation shown in Fig. 10.12), and it prefers this to be a phenylalanyl, tyrosyl, or tryptophanyl group.

The use of other acid proteases as substitutes for rennin in cheese making is determined by whether bitter peptides are formed during ripening of the cheese and by whether initial rapid hydrolysis causes excessive protein losses in the whey. Some of the acid proteases used in cheese making include preparations obtained from the organisms *Endothia parasitica, Mucor miehei,* and *Mucor pusillus.* Rennin contains the enzyme chymosin, and the scarcity of this natural enzyme preparation for cheese making resulted in the use of pepsin for this purpose. Pepsin and chymosin have primary structures that have about 50% homology and quite similar tertiary structures. The molecular mass of the two enzymes is similar, 35 kDa, but chymosin has a higher pI. Much of the chymosin used in cheese making is now obtained by genetic engineering processes. In the production of soy sauce and other eastern food products, such as miso (an oriental fermented food) and ketjap (Indonesian type soy sauce), the acid proteases of *Aspergillus oryzae* are used. Other products involve the use of the fungus *Rhizopus oligosporus.* Acid proteases also play a role in the ripening process of a variety of soft cheeses. This includes the *Penicillia* used in the blue cheeses, such as Roquefort, Stilton, and Danish blue, and in Camembert and Brie. The molds producing the acid proteases may grow either on the surface of the cheese or throughout the body of the cheese.

Serine Proteases

This group includes the chymotrypsins, trypsin, elastase, thrombin, and subtilisin. The name of this group of enzymes refers to the seryl residue

that is involved in the active site. As a consequence, all of these enzymes are inhibited by diisopropylphosphorofluoridate, which reacts with the hydroxyl group of the seryl residue. They also have an imidazole group as part of the active site and they are all endopeptides. The chymotrypsins, trypsin and elastase, are pancreatic enzymes that carry out their function in the intestinal tract. They are produced as inactive zymogens and are converted into the active form by limited proteolysis.

Sulfhydryl Proteases

These enzymes obtain their name from the fact that a sulfhydryl group in the molecule is essential for their activity. Most of these enzymes are of plant origin and have found widespread use in the food industry. The only sulfhydryl proteases of animal origin are two of the cathepsins, which are present in the tissues as intracellular enzymes. The most important enzymes of this group are papain, ficin, and bromelain. Papain is an enzyme present in the fruit, leaves, and trunk of the papaya tree (*Carica papaya*). The commercial enzyme is obtained by purification of the exudate of full-grown but unripe papaya fruits. The purification involves use of affinity chromatography on a column containing an inhibitor (Liener 1974). This process leads to the full activation of the enzyme, which then contains 1 mole of sulfhydryl per mole of protein. The crude papain is not fully active and contains only 0.5 mole of sulfhydryl per mole of protein. Bromelain is obtained from the fruit or stems of the pineapple plant (*Ananas comosus*). The stems are pressed and the enzyme precipitated from the juice by acetone. Ficin is obtained from the latex of tropical fig trees (*Ficus glabrata*). The enzyme is not homogeneous and contains at least three different proteolytic components.

The active sites of these plant enzymes contain a cysteine and a histidine group that are essential for enzyme activity. The pH optimum is fairly broad and ranges from 6 to 7.5 The enzymes are heat stable up to temperatures in the range of 60–80 °C. The papain molecule consists of a single polypeptide chain of 212 amino acids. The molecular weight is 23,900. Ficin and bromelain contain carbohydrate in the molecule; papain does not. The molecular weights of the enzymes are quite similar; that of ficin is 25,500 and that of bromelain, 20,000–33,200. These enzymes catalyze the hydrolysis of many different compounds, including peptide, ester, and amide bonds. The variety of peptide bonds split by papain appears to indicate a low specificity. This has been attributed (Liener 1974) to the fact that papain has an active site consisting of seven subsites that can accommodate a variety of amino acid sequences in the substrate. The specificity in this case is not determined by the nature of the side chain of the amino acid involved in the susceptible bond but rather by the nature of the adjacent amino acids.

Commercial use of the sulfhydryl proteases includes stabilizing and chill proofing of beer. Relatively large protein fragments remaining after the malting of barley may cause haze in beer when the product is stored at low temperatures. Controlled proteolysis sufficiently decreases the molecular weight of these compounds so that they will remain in solution. Another important use is in the tenderizing of meat. This can be achieved by injecting an enzyme solution into the carcass or by applying the enzyme to smaller cuts of meat. The former method suffers from the difficulty of uneven proteolysis in different parts of the carcass with the risk of overtenderizing some parts of the carcass.

Metal-Containing Proteases

These enzymes require a metal for activity and are inhibited by metal-chelating compounds. They are exopeptidases and include carboxypeptidase A (peptidyl-L-amino-acid hydrolase) and B (peptidyl-L-lysine hydrolase), which remove amino acids from the end of peptide chains that carry a free α-carboxyl group. The aminopeptidases remove amino acids from the free α-amino end of the peptide chain. The metalloexopeptidases require a divalent metal as a cofactor; the carboxypeptidases contain zinc. These enzymes are quite specific in the action; for example, carboxypeptidase B requires the C-terminal amino acid to be either arginine or lysine; the requirement for carboxypeptidase A is phenylalanine, tryptophan, or isoleucine. These specificities are compared with those of some other proteolytic

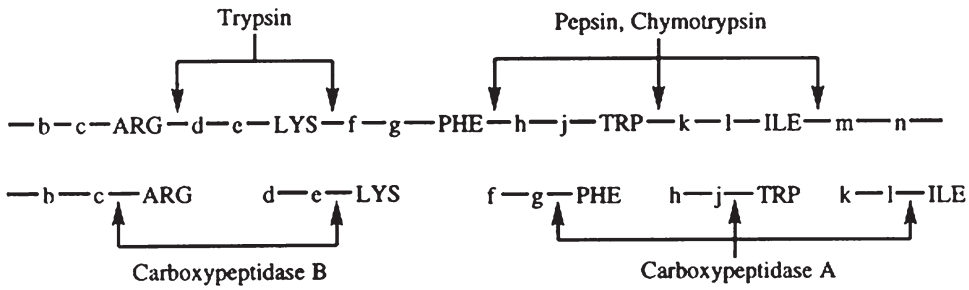


Fig. 10.14 Specificity of some proteolytic enzymes

enzymes in Fig. 10.14. The carboxypeptidases are relatively small molecules; molecular weight of carboxypeptidase A is 34,600. The amino peptidases have molecular weights around 300,000. Although many of the aminopeptidases are found in animal tissues, several are present in microorganisms (Riordan 1974).

Protein Hydrolysates

Protein hydrolysates is the name given to a family of protein breakdown products obtained by the action of enzymes. It is also possible to hydrolyze proteins by chemical means, acids, or alkali, but the enzymatic method is preferred. Many food products such as cheese and soy sauce are obtained by enzymatic hydrolysis. The purpose of the production of protein hydrolysates is to improve nutritional value, cost, taste, antigenicity, solubility, and functionality. The proteins most commonly selected for producing hydrolysates are casein, whey protein, and soy protein (Lahl and Braun 1994). Proteins can be hydrolyzed in steps to yield a series of proteoses, peptones, peptides, and finally amino acids (Table 10.10). These products should not be confused with hydrolyzed vegetable proteins, which are intended as flavoring substances.

The extent of hydrolysis of protein hydrolysates is measured by the ratio of the amount of amino nitrogen to the total amount of nitrogen present in the raw material (AN/TN ratio). Highly hydrolyzed materials have AN/TN ratios of 0.50–0.60. To obtain the desired level of hydrolysis in a protein, a combination of proteases is selected. Serine protease prepared from *Bacillus licheniformis* has broad specificity and some preference for terminal hydrophobic amino acids. Peptides containing terminal hydrophobic amino acids cause bitterness. Usually a mixture of different proteases is employed. The hydrolysis reaction is terminated by adjusting the pH and increasing the temperature to inactivate the enzymes. The process for producing hydrolysates is shown in Fig. 10.15 (Lahl and Braun 1994). Protein hydrolysates can be used as food ingredients with specific functional properties or for physiological or medical reasons. For example, hydrolyzed proteins may lose allergenic properties by suitably arranged patterns of hydrolysis (Cordle 1994).

Table 10.10 Protein hydrolysate products produced from casein and whey protein concentrate (WPC)

Hydrolysate[a]	Protein source	Average molecular weight[b]	AN/TN[c]
Intact protein	Casein	28,500	0.07
	WPC	25,000	0.06
Proteose	Casein	6000	0.13
	WPC	6800	0.11
Peptone	Casein	2000	0.24
	WPC	1400	0.24
Peptides	Casein	400	0.48
	WPC	375	0.43
Peptides and free amino acids	Casein	260	0.55
	WPC	275	0.58

Source: Reprinted with permission from Lahl, W. J., & S. D. Braun. Enzymatic production of protein hydrolysates for food use. *Food Technology, 48*(10), 69, © 1994, Institute of Food Technologists

[a]Commercial hydrolysates produced by Deltown Specialties, Fraser, NY

[b]Determined by reverse-phase HPLC

[c]Ratio of amino nitrogen present in the hydrolysate to the total amount of nitrogen present in the substrate

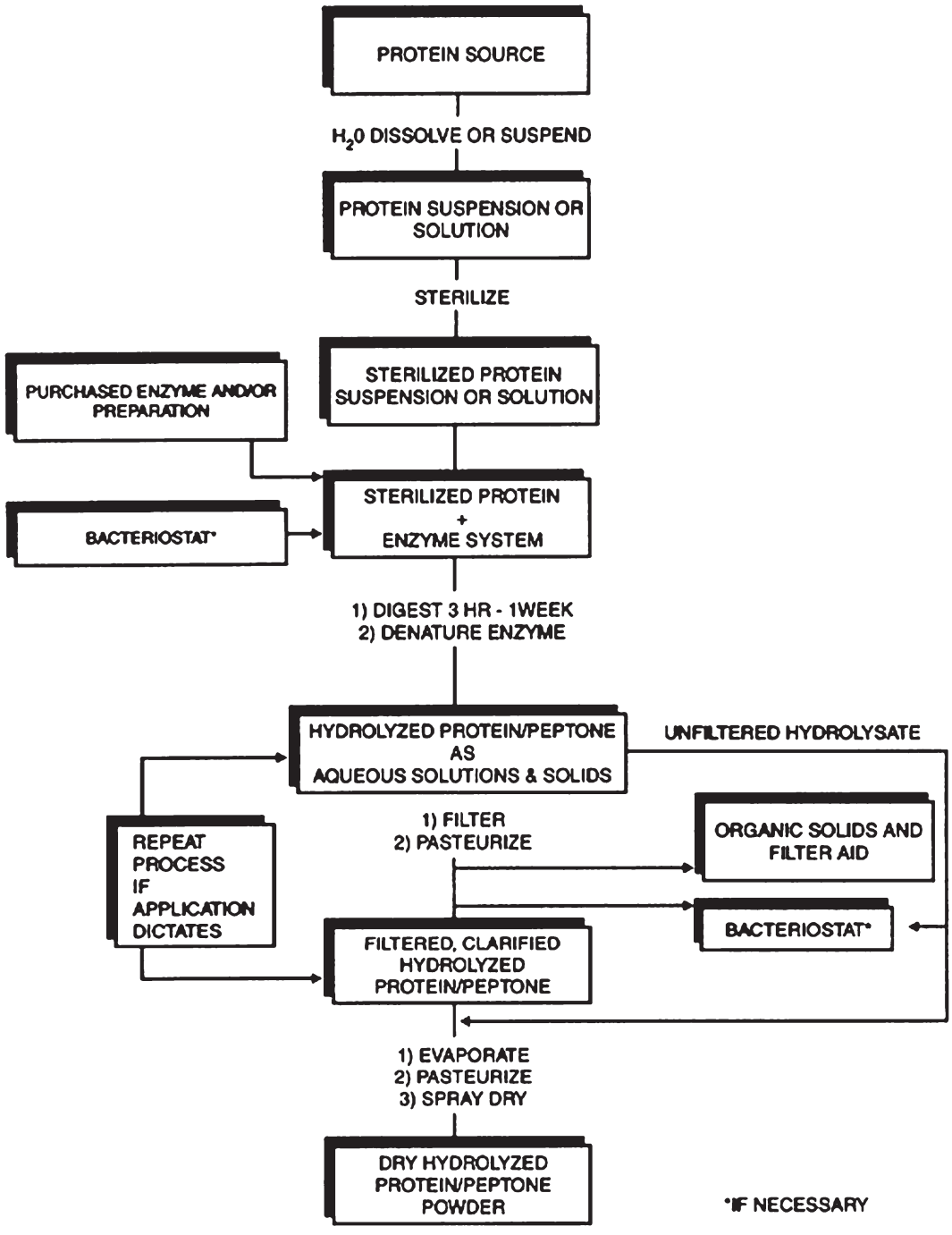


Fig. 10.15 Process for the production of protein hydrolysates. *Source:* Reprinted with permission from Lahl, W. J., & Braun, S. D. Enzymatic production of protein hydrolysates for food use. *Food Technology, 48*(10), 70, © 1994, Institute of Food Technologists

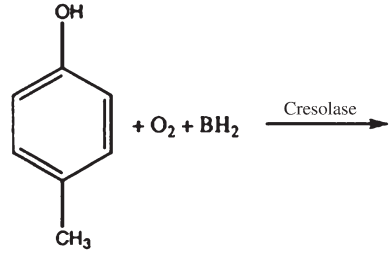
Oxidoreductases

Phenolases

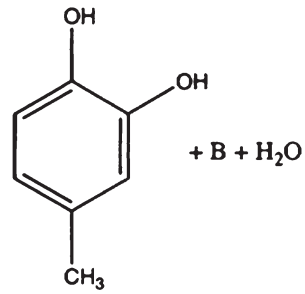
The enzymes involved in enzymic browning are known by the name polyphenoloxidase and are also called polyphenolase, phenolase, tyrosinase, or catechol oxidase. It is generally agreed (Mathew and Parpia 1971) that these terms include all enzymes that have the capacity to oxidize phenolic compounds to *o*-quinones. This can be represented by the conversion of *o*-dihydroxyphenol to *o*-quinone,

The action of polyphenolases is detrimental when it leads to browning in bruised and broken plant tissue but is beneficial in the processing of tea and coffee. The enzyme occurs in almost all plants, but relatively high levels are found in potatoes, mushrooms, apples, peaches, bananas, avocados, tea leaves, and coffee beans.

In addition to changing *o*-diphenols into *o*-quinones, the enzymes also catalyze the conversion of monophenols into *o*-diphenols (called monophenol monooxygenase or cresolase), as follows:



p-cresol



4-methyl-catechol

where BH_2 stands for an *o*-diphenolic compound.

To distinguish this type of activity from the one mentioned earlier, it is described as cresolase activity, whereas the other is referred to as catecholase activity. For both types of activity, the involvement of copper is essential. Copper has been found as a component of all polyphenolases. The activity of cresolase involves three steps, which can be represented by the following overall equation (Mason 1956):

$$\text{Protein} - \text{Cu}_2^{+} - \text{O}_2 + \text{monophenol} \rightarrow \text{Protein} - \text{Cu}_2^{+} + o - \text{quinone} + \text{H}_2\text{O}$$

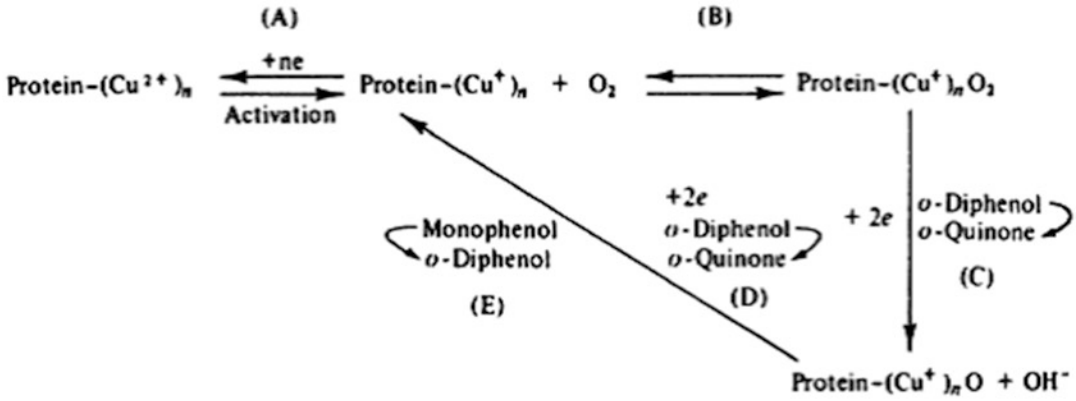


Fig. 10.16 Phenolase catalyzed reactions. (**a**) Activation of phenolase. (**b–d**) Two-step four-electron reduction of oxycuprophenolase, and the associated hydroxylation of monophenols. *Source:* From Mason, H. S. Mechanisms of oxygen metabolism. *Advances in Enzymology, 19*, 79–233, © 1957

The protein copper-oxygen complex is formed by combining one molecule of oxygen with the protein to which two adjacent cuprous atoms are attached.

Catecholase activity involves oxidizing two molecules of *o*-diphenols to two molecules of *o*-quinones, resulting in the reduction of one molecule of oxygen to two molecules of water. The action sequence as presented in Fig. 10.16 has been proposed by Mason (1957). The enzyme-oxygen complex serves as the hydroxylating or dehydroxylating intermediate, and (Cu)*n* represents the actual charge designation of the copper at the active site. In preparations high in cresolase activity, $n = 2$, and in preparations high in catecholase activity, $n = 1$. The overall reaction involves the use of one molecule of oxygen, one atom of which goes into the formation of the diphenol, and the other, which is reduced to water. This can be expressed in the following equation given by Mathew and Parpia (1971):

$$\text{Monophenol} + \text{O}_2 + o-\text{diphenol} \xrightarrow{\text{enzyme}} o-\text{diphenol} + \text{quinone} + \text{H}_2\text{O}$$

The substrates of the polyphenol oxidase enzymes are phenolic compounds present in plant tissues, mainly flavonoids. These include catechins, anthocyanidins, leucoanthocyanidins, flavonols, and cinnamic acid derivatives. Polyphenol oxidases from different sources show distinct differences in their activity for different substrates. Some specific examples of polyphenolase substrates are chlorogenic acid, caffeic acid, dicatechol, protocatechuic acid, tyrosine, catechol, dihydroxyphenylalanine, pyrogallol, and catechins.

To prevent or minimize enzymic browning of damaged plant tissue, several approaches are possible. The first and obvious one, although rarely practical, involves the exclusion of molecular oxygen. Another approach is the addition of reducing agents that can prevent the accumulation of *o*-quinones. Heat treatment is effective in deactivating the enzymes. Metal complexing agents may deactivate the enzyme by making the copper unavailable.

One of the most useful methods involves the use of L-ascorbic acid as a reducing agent. This is practiced extensively in the commercial production of fruit juices and purees. The ascorbic acid reacts with the *o*-quinones and changes them back into *o*-diphenols (Fig. 10.17).

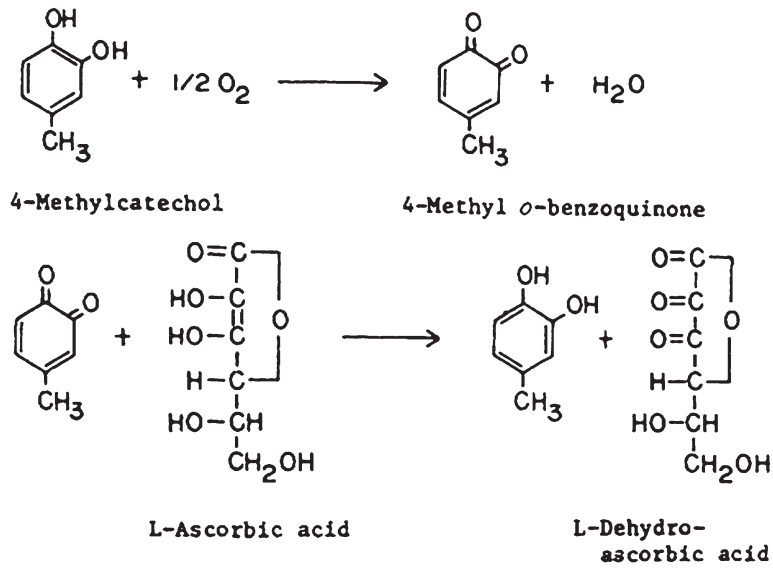


Fig. 10.17 Reaction of L-ascorbic acid with *o*-quinone in the prevention of enzymic browning

Glucose Oxidase (β-D-Glucose: Oxygen Oxidoreductase)

This enzyme catalyzes the oxidation of D-glucose to δ-D-gluconolactone and hydrogen peroxide in the presence of molecular oxygen, as follows:

$$C_6H_{12}O_6 \xrightarrow{\text{enzyme}} C_6H_{10}O_6 + H_2O_2$$

The enzyme is present in many fungi and is highly specific for β-D-glucopyranose. It has been established that the enzyme does not oxidize glucose by direct combination with molecular oxygen. The mechanism as described by Whitaker (1972) involves the oxidized form of the enzyme, flavin adenine dinucleotide (FAD), which serves as a dehydrogenase. Two hydrogen atoms are removed from the glucose to form the reduced state of the enzyme, FADH2, and δ-D-gluconolactone. The enzyme is then reoxidized by molecular oxygen. The gluconolactone is hydrolyzed in the presence of water to form D-gluconic acid.

In food processing, glucose oxidase is used to remove residual oxygen in the head space of bottled or canned products or to remove glucose. Light has a deteriorative effect on citrus beverages. Through the catalytic action of light, peroxides are formed that lead to oxidation of other components, resulting in very unpleasant off-flavors. Removing the oxygen by the use of a mixture of glucose oxidase and catalase will prevent these peroxides from forming. The glucose oxidase promotes the formation of gluconic acid with uptake of one molecule of oxygen. The catalase decomposes the hydrogen peroxide formed into water and one half-molecule of oxygen. The net result is the uptake of one half-molecule of oxygen. The overall reaction can be written as follows:

$$\text{Glucose} + \tfrac{1}{2}O_2 \xrightarrow[\text{catalase}]{\text{glucose oxidase}} \text{gluconic acid}$$

Recent information suggests that this application is not effective because of reversible inhibition of the glucose oxidase by the dyes used in soft drinks below pH 3 (Hammer 1993).

This enzyme mixture can also remove glucose from eggs before drying to prevent Maillard type browning reactions in the dried product.

Catalase (Hydrogen Peroxide: Hydrogen Peroxide Oxidoreductase)

Catalase catalyzes the conversion of two molecules of hydrogen peroxide into water and molecular oxygen as follows:

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

This enzyme occurs in plants, animals, and microorganisms. The molecule has four subunits; each of these contains a protohemin group, which forms part of four independent active sites. The molecular weight is 240,000. Catalase is less stable to heat than is peroxidase. At neutral pH, catalase will rapidly lose activity at 35 °C. In addition to catalyzing the reaction shown above (catalatic activity), catalase can also have peroxidatic activity. This occurs at low concentrations of hydrogen peroxide and in the presence of hydrogen donors (e.g., alcohols).

In plants, catalase appears to have two functions. First is the ability to dispose of the excess H_2O_2 produced in oxidative metabolism, and second is the ability to use H_2O_2 in the oxidation of phenols, alcohols, and other hydrogen donors. The difference in heat stability of catalase and peroxidase was demonstrated by Lopez et al. (1959). They found that blanching of southern peas for 1 min in boiling water destroys 70–90% of the peroxidase activity and 80–100% of the catalase activity.

The combination of glucose oxidase and catalase is used in a number of food processing applications, including the removal of trace glucose or oxygen from foods and in the production of gluconic acid from glucose. Greenfield and Lawrence (1975) have studied the use of these enzymes in their immobilized form on an inorganic support.

Peroxidase (Donor: Hydrogen Peroxide Oxidoreductase)

The reaction type catalyzed by peroxidase involves hydrogen peroxide as an acceptor, and a compound AH_2 as a donor of hydrogen atoms, as shown:

$$H_2O_2 + AH_2 \xrightarrow{\text{peroxidase}} 2\ H_2O + A$$

In contrast to the action of catalase, no molecular oxygen is formed.

The peroxidases can be classified into the two groups, iron-containing peroxidases and flavoprotein peroxidases. The former can be further subdivided into ferriprotoporphyrin peroxidases and verdoperoxidases. The first group contains ferriprotoporphyrin III (protohemin) as the prosthetic group (Fig. 10.18). The common plant peroxidases (horseradish, fig, and turnip) are in this group and the enzymes are brown when highly purified. The second group includes the peroxidases of animal tissue and milk (lactoperoxidase). In these enzymes, the prosthetic group is an iron porphyrin nucleus but not protohemin. When highly purified, these enzymes are green in color. Flavoprotein peroxidases occur in microorganisms and animal tissues. The prosthetic group is FAD.

The linkage between the iron-containing prosthetic group and the protein can be stabilized

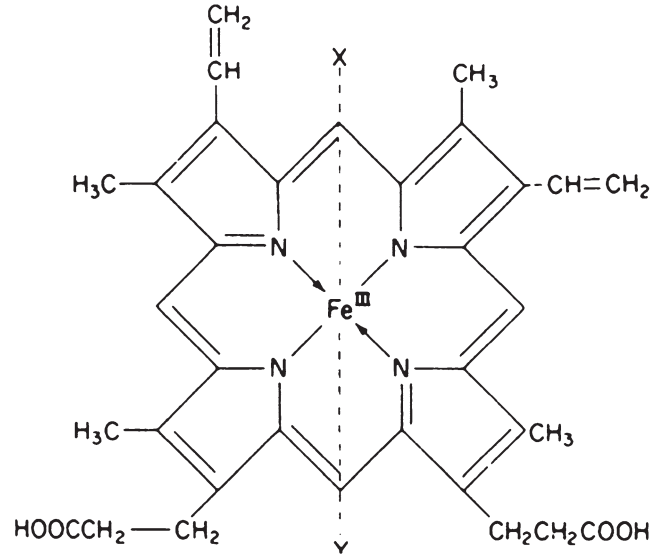


Fig. 10.18 Structural formula of ferriprotoporphyrin III (protohemin). *Source:* From Whitaker, J. R. (1974). *Food related enzymes.* American Chemical Society

by bisulfite (Embs and Markakis 1969). It is suggested that the bisulfite forms a complex with the peroxidase iron, which stabilizes the enzyme.

Because of the widespread occurrence of peroxidase in plant tissues, Nagle and Haard (1975) have suggested that it plays an important role in the development and senescence of plant tissues. It plays a role in biogenesis of ethylene; in regulating ripening and senescence; in the degradation of chlorophyll; and in the oxidation of indole-3-acetic acid.

The enzyme can occur in a variety of multiple molecular forms, named isoenzymes or isozymes. Such isoenzymes have the same enzymatic activity but can be separated by electrophoresis. Nagle and Haard (1975) separated the isoperoxidases of bananas into six anionic and one cationic component by gel electrophoresis. By using other methods of separation, an even greater number of isoenzymes was demonstrated.

Peroxidase has been implicated in the formation of the "grit cells" or "stone cells" of pears (Ranadive and Haard 1972). Bound peroxidase but not total peroxidase activity was higher in the fruit that contained excessive stone cells. The stone cells or sclereids are lignocellulosic in nature. The presence of calcium ions causes the release of wall bound peroxidase and a consequent decrease in the deposition of lignin.

The peroxidase test is used as an indicator of satisfactory blanching of fruits and vegetables. However, it has been found that the enzymes causing off-flavors during frozen storage can, under some conditions, be regenerated. Regeneration of enzymes is a relatively common phenomenon and is more likely to occur the faster the temperature is raised to a given point in the blanching process. The deactivation and 3 reactivation of peroxidase by heat was studied by Lu and Whitaker (1974). The rate of reactivation was at a maximum at pH 9 and the extent of reactivation was increased by addition of hematin.

The deactivation of peroxidase is a function of heating time and temperature. Lactoperoxidase is completely deactivated by heating at 85 °C for 13 s. The effect of heating time at 76 °C on the deactivation of lactoperoxidase is represented in Fig. 10.19, and the effect of heating temperature on the deactivation constant is shown in Fig. 10.20. Lactoperoxidase can be regenerated under conditions of high temperature short time (HTST) pasteurization. Figure 10.21 shows the regeneration of lactoperoxidase activity in milk that is pasteurized for 10 s at 85 °C. The regeneration effect depends greatly on storage temperature; the lower the storage temperature, the smaller the regeneration effect.

Lactoperoxidase is associated with the serum proteins of milk. It has an optimum pH of 6.8 and a molecular weight of 82,000.

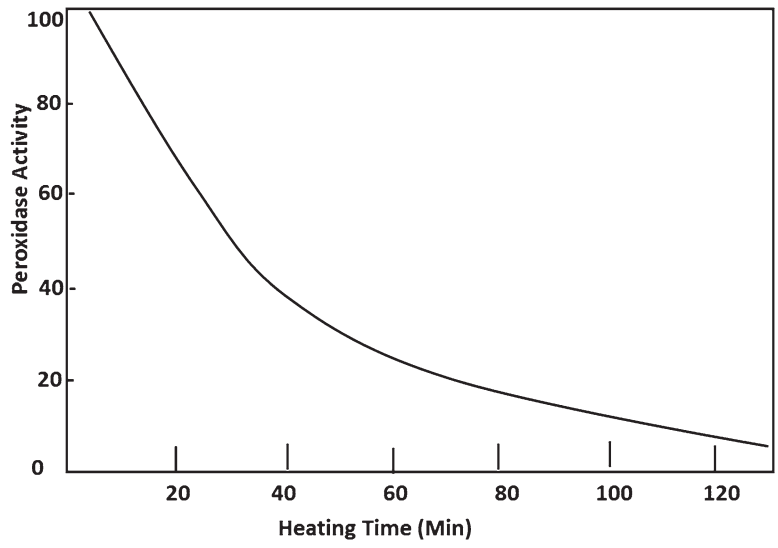


Fig. 10.19 Deactivation of lactoperoxidase as a function of heating time. *Source:* Adapted from Kiermeier, F., & Kayser, C. (1960). Heat inactivation of lactoperoxidase (in German). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *113*

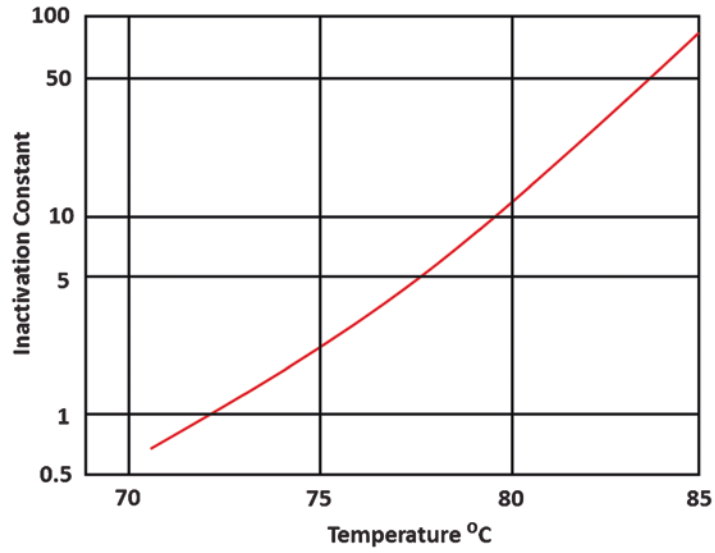


Fig. 10.20 Dependence of the rate constant of heat deactivation of lactoperoxidase on the heating temperature. *Adapted from* Kiermeier, F., & Kayser, C. (1960). Heat inactivation of lactoperoxidase (in German). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 113*

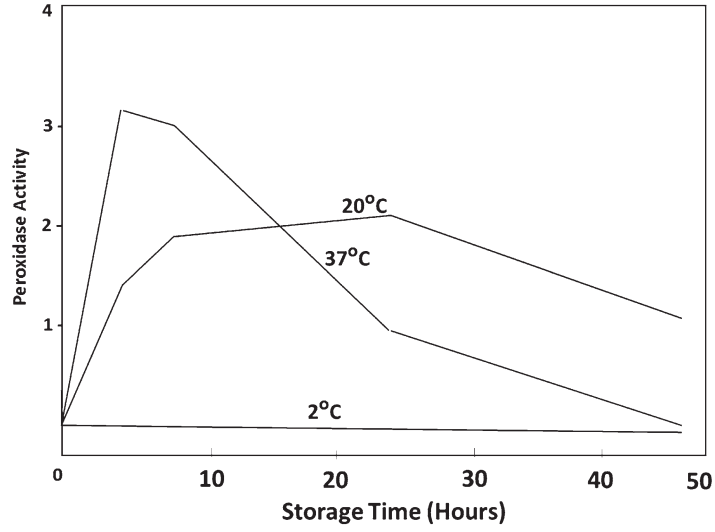


Fig. 10.21 Regeneration in ultra high temperature treated milk as a function of storage temperature. (**a**) 2 °C; (**b**) 20 °C; (**c**) 37 °C. *Adapted from* Kiermeier, F., & Kayser, C. (1960). Heat inactivation of lactoperoxidase (in German). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *113*

Lipoxygenase (Linoleate: Oxygen Oxidoreductase)

This enzyme, formerly named lipoxidase, is present in plants and catalyzes the oxidation of unsaturated fats. The major source of lipoxygenase is legumes, soybeans, and other beans and peas. Smaller amounts are present in peanuts, wheat, potatoes, and radishes. Lipoxygenase is a metallo-protein with an iron atom in its active center. In plants two types of lipoxygenase exist: type I lipoxygenase peroxidizes only free fatty acids with a high stereo- and regioselectivity; type II lipoxygenase is less specific for free linoleic acid and acts as a general catalyst for autoxidation. Type I reacts with fats in a food only after free fatty acids have been formed by lipase action; type II acts directly on triacylglycerols.

Lipoxygenase is highly specific and attacks the *cis-cis*-1,4-pentadiene group contained in the fatty acids linoleic, linolenic, and arachidonic, as follows:

$$-CH=CH-CH_2-CH=CH-$$

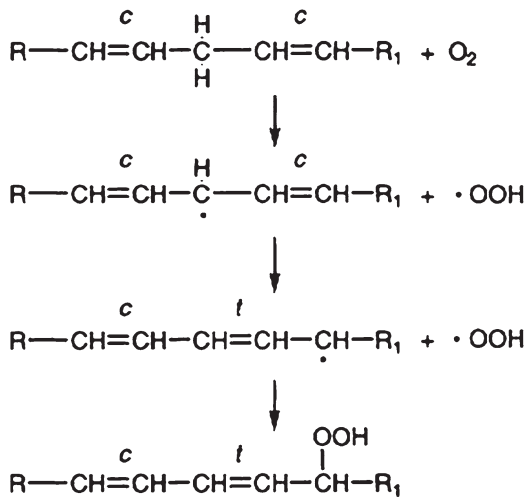


Fig. 10.22 Essential steps in the mechanism of the lipoxygenase-catalyzed oxidation of the 1,4-pentadiene group

The specificity of this enzyme requires that both double bonds are in the *cis* configuration; in addition, there is a requirement that the central methylene group of the 1,4-pentadiene group occupies the ω-8 position on the fatty acid chain and also that the hydrogen to be removed from the central methylene group be in the L-position. Although the exact mechanism of the reaction is still in some doubt, there is agreement that the essential steps are as represented in Fig. 10.22. Initially, a hydrogen atom is abstracted from the ω-8 methylene group to produce a free radical. The free radical isomerizes, causing conjugation of the double bond and isomerization to the *trans* configuration. The free radical then reacts to form the ω-6 hydroperoxide.

Lipoxygenase is reported to have a pH optimum of about 9. However, these values are determined with linoleic acid as substrate, and in natural systems the substrate is usually present in the form of triglycerides. The enzyme has a molecular weight of 102,000 and an isoelectric point of 5.4. The peroxide formation by lipoxygenase is inhibited by the common lipid antioxidants. The antioxidants are thought to react with the free radicals and interrupt the oxidation mechanism.

Most manifestations of lipoxygenase in foods are undesirable. However, it is used in baking to bring about desirable changes. Addition of soybean flour to wheat flour dough results in a bleaching effect, because of oxidation of the xanthophyll pigments. In addition, there is an effect on the rheological and baking properties of the dough. It has been suggested that lipoxygenase acts indirectly in the oxidation of sulfhydryl groups in the gluten proteins to produce disulfide bonds. When raw soybeans are ground with water to produce soy milk, a strong and unpleasant flavor develops that is called painty, green, or beany. Carrying out the grinding in boiling water instantly deactivates the enzyme, and no off-flavor is formed. Blanching of peas and beans is essential in preventing the lipoxygenase-catalyzed development of off-flavor. In addition to the development of off-flavors, the enzyme may be responsible for destruction of carotene and vitamin A, chlorophyll, bixin, and other pigments.

In some cases the action of lipoxygenase leads to development of a characteristic aroma. Galliard et al. (1976) found that the main aroma compounds of cucumber, 2-*trans* hexenol and 2-*trans,* 6-*cis*-nonadienal, are produced by reaction of linolenic acid and lipoxygenase to form hydroperoxide (Fig. 10.23); these are changed into *cis* unsaturated aldehydes by hydroperoxide lyase. The *cis* unsaturated aldehydes are transformed by isomerase into the corresponding *trans* isomers. These same substances in another matrix would be experienced as off-flavors. The use of lipoxygenase as a versatile biocatalyst has been described by Gardner (1996).

Xanthine Oxidase (Xanthine: Oxygen Oxidoreductase)

This enzyme catalyzes the conversion of xanthine and hypoxanthine to uric acid. The reaction equation is given in Fig. 10.24; heavy arrows indicate the reactions catalyzed by the enzyme and the dashed arrows represent the net result of the catalytic process (Whitaker 1972). Although xanthine oxidase is a nonspecific enzyme and many substances can serve as substrate, the rate

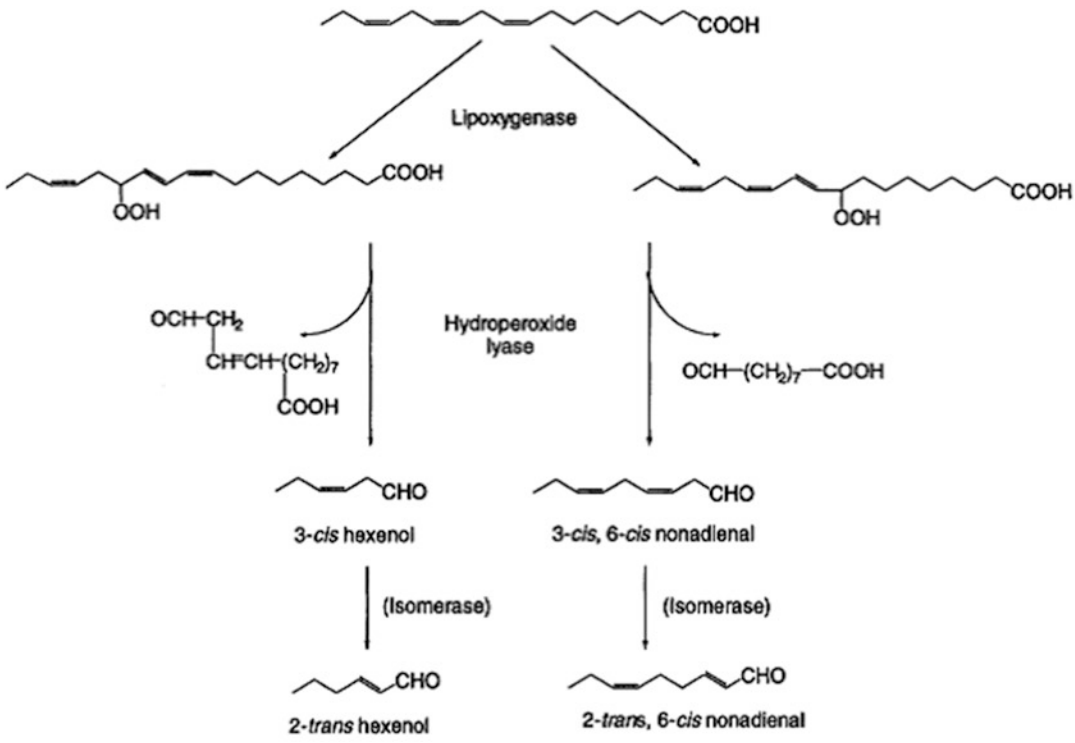


Fig. 10.23 Lipoxygenase catalyzed formation of aroma compounds in cucumber. *Source:* Reprinted from Galliard, T., Phillips, D. R., & Reynolds, J. The formation of *cis*-3-nonenal, *trans*-2-nonenal and hexanol from linoleic acid hydroperoxide isomers by a hydroperoxide cleavage enzyme system in cucumber (Cucumis Sativus) fruits. *Biochimica et Biophysica Acta, 441*, 184, Copyright 1976, with permission from Elsevier Science

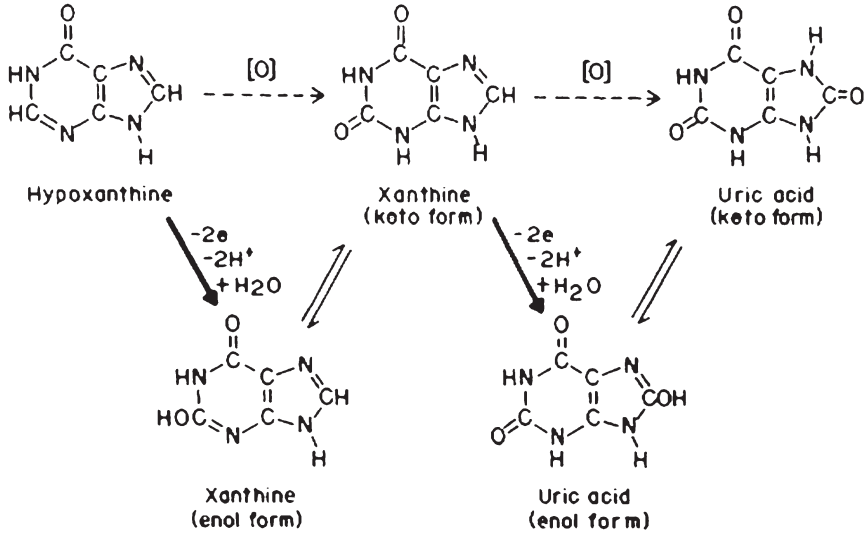


Fig. 10.24 Oxidation of hypoxanthine and xanthine to uric acid by xanthine oxidase. *Source*: From Whitaker, J. R. (1972). *Principles of enzymology for the food sciences.* Marcel Dekker, Inc

of oxidation of xanthine and hypoxanthine is many times greater than that of other substrates.

Xanthine oxidase has been isolated from milk and obtained in the crystalline state. The molecular weight is 275,000. One mole of the protein contains 2 moles of FAD, 2 gram-atoms of molybdenum, 8 gram-atoms of nonheme iron, and eight labile sulfide groups. The eight labile sulfide groups are liberated in the form of H_2S upon acidification or boiling at pH 7. The optimum pH for activity is 8.3. The xanthine oxidase in milk is associated with the fat globules and, therefore, follows the fat into the cream when milk is separated. It seems to be located in small particles (microsomes) that are attached to the fat globules. The microsomes also contain the enzyme alkaline phosphatase. The microsomes can be dislodged from the fat globules by mechanical treatment such as pumping and agitation and by heating and cooling. The enzyme is moderately stable to heat but no less so than peroxidase.

Immobilized Enzymes

One of the most important recent developments in the use of enzymes for industrial food processing is the fixing of enzymes on water-insoluble inert supports. The fixed enzymes retain their activity and can be easily added to or removed from the reaction mixture. The use of immobilized enzymes permits continuous processing and greatly increased use of the enzyme. Various possible methods of immobilizing enzymes have been listed by Weetall (1975) and Hultin (1983). A schematic representation of the available methods is given in Fig. 10.25. The immobilizing methods include adsorption on organic polymers, glass, metal oxides, and siliceous materials such as bentonite and silica; entrapment in natural or synthetic polymers, usually polyacrylamide; microencapsulation in polymer membranes; ion exchange; cross-linking; adsorption and cross-linking combined; copolymerization; and covalent attachment to organic polymers.

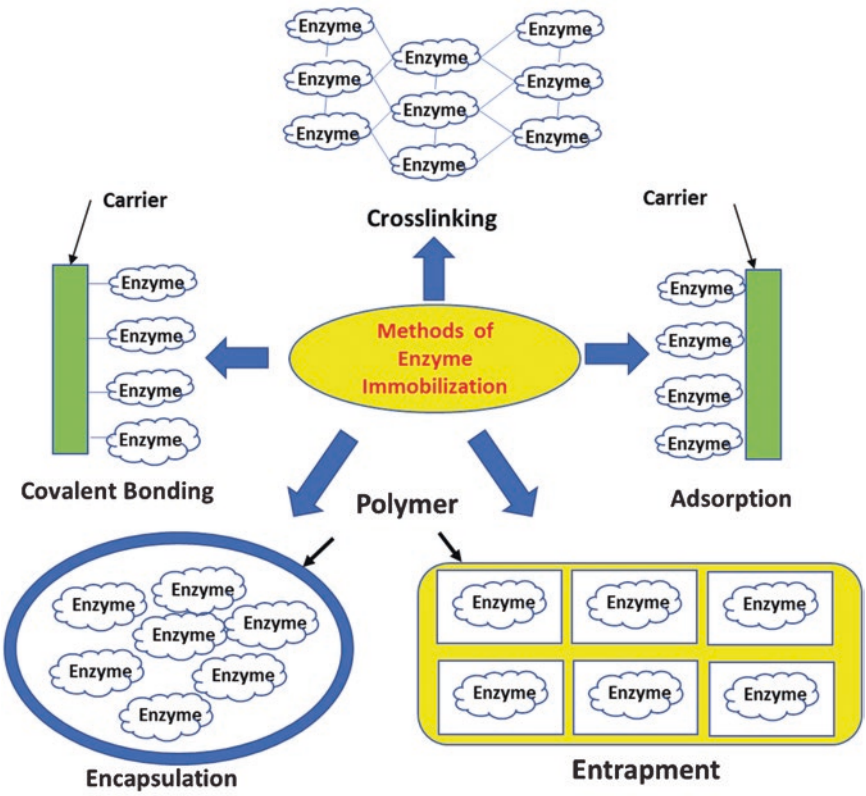


Fig. 10.25 Methods of immobilizing enzymes

The chemistry of immobilizing enzymes has been covered in detail by Stanley and Olson (1974).

A summary of immobilization methods has been provided by Adlercreutz (1993) and is presented in Exhibit 10.1. In membrane reactors, the reaction product is separated from the reaction mixture by a semipermeable membrane. In two-phase systems, a hydrophobic reaction product can be separated from the aqueous reaction mixture by transfer to the organic solvent phase. In aqueous-aqueous systems, two incompatible polymers in aqueous solution form a two-phase system.

Exhibit 10.1Summary of Enzyme Immobilization Methods

1. chemical methods
   - covalent binding
   - cross-linking
2. physical methods
   - adsorption
   - physical deposition
   - entrapment
   - in polymer gels
   - in microcapsules
   - membranes
3. two-phase systems
   - organic-aqueous
   - aqueous-aqueous

Immobilizing enzymes is likely to change their stability, and the method of attachment to the carrier also affects the degree of stability. When a high molecular weight substrate is used, the immobilizing should not be done by entrapment, microencapsulation, or copolymerization, because enzyme and substrate cannot easily get in contact. One of the promising methods appears to be covalent coupling of enzymes to inorganic carriers such as porous silica glass particles. Not all of the immobilized enzyme is active, due to either inactivation or steric hindrance. Usually, only about 30–50% of the bound enzyme is active.

Immobilized enzymes can be used in one of two basic types of reactor systems. The first is the stirred tank reactor where the immobilized enzyme is stirred with the substrate solution. This is a batch system and, after the reaction is complete, the immobilized enzyme is separated from the product. The other system employs continuous flow columns in which the substrate flows through the immobilized enzyme contained in a column or similar device. A simplified flow diagram of such a system is given in Fig. 10.26.

The characteristics of immobilized enzymes are likely to be somewhat different than those of the original enzyme. The pH optimum can be shifted; this depends on the surface charge of the carrier (Fig. 10.27). Another property that can be changed is the Michaelis constant, K_m. This value can become either larger or smaller. Immobilizing may result in increased thermal stability (Fig. 10.28), but in some cases the thermal stability is actually decreased.

Many examples of the use of immobilized enzymes in food processing have been reported. One of the most important of these is the use of immobilized glucose isomerase obtained from

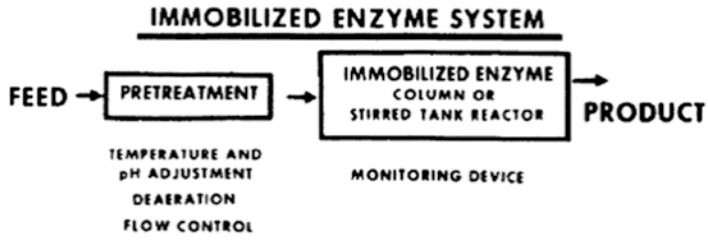


Fig. 10.26 Flow diagram of an immobilized enzyme system (column operation of lactase immobilized on phenol-formaldehyde resin with glutaraldehyde). *Source:* From Stanley, W. L., & Olson, A. C. (1974). The chemistry of immobilizing enzymes. *Journal of Food Science, 39*, 660–666

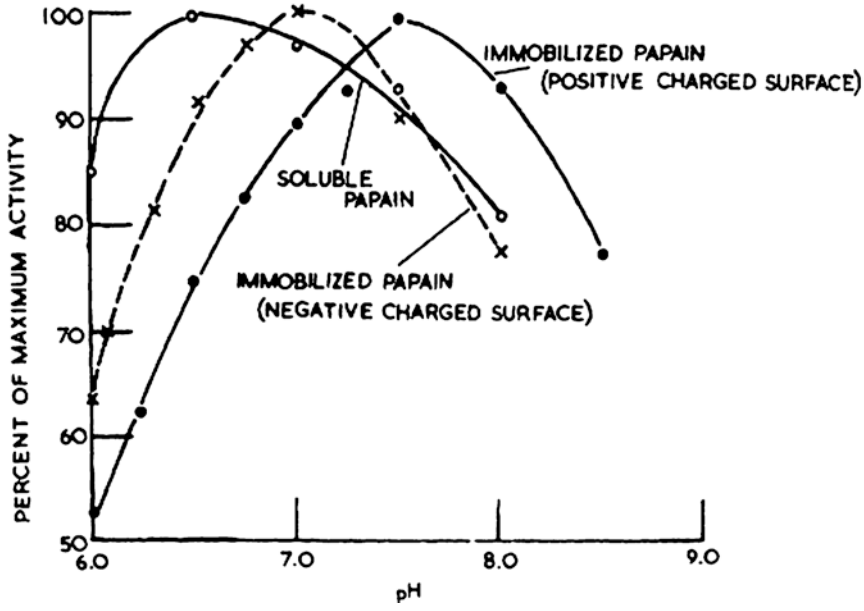


Fig. 10.27 Effect of immobilizing on the pH optimum of papain. *Source:* From Weetall, H. H. (1975). Immobilized enzymes and their application in the food and beverage industry. *Process Biochemistry, 10*, 3–6

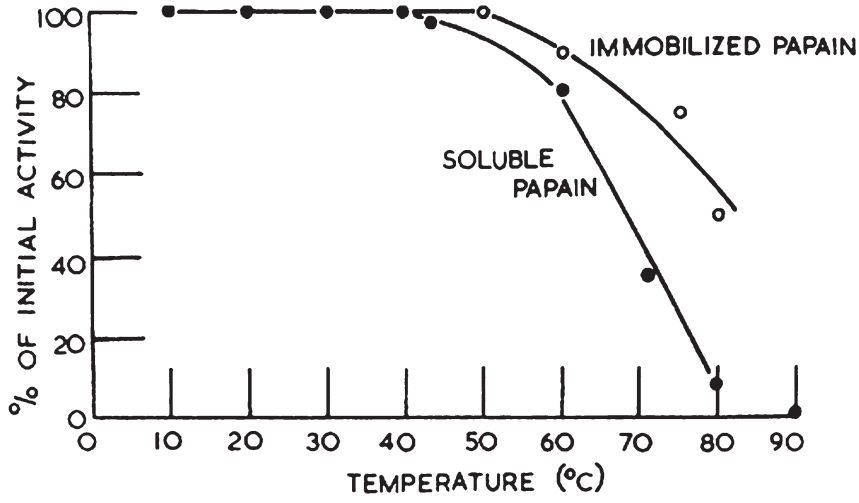


Fig. 10.28 Effect of immobilizing of the thermal stability of papain. *Source*: From Weetall, H. H. (1975). Immobilized enzymes and their application in the food and beverage industry. *Process Biochemistry, 10*, 3–6

Streptomyces for the production of high-fructose com syrup (Mermelstein 1975). In this process, the enzyme is bound to an insoluble carrier such as diethyl amino ethyl cellulose or a slurry of the fixed enzyme coated onto a pressure-leaf filter. The filter then serves as the continuous reactor through which the com syrup flows. The product obtained by this process is a syrup with 71% solids that contains about 42% fructose and 50% glucose; it has high sweetening power, high fermentability, high humectancy, reduced tendency to crystallize, low viscosity, and good flavor.

Examples of the use of immobilized enzymes in food processing and analysis have been listed by Olson and Richardson (1974) and Hultin (1983). L-aspartic acid and L-malic acid are produced by using enzymes contained in whole microorganisms that are immobilized in a polyacrylamide gel. The enzyme aspartase from *Escherichia coli* is used for the production of aspartic acid. Fumarase from *Brevibacterium ammoniagenes* is used for L-malic acid production.

The most widely used immobilized enzyme process involves the use of the enzyme glucose isomerase for the conversion of glucose to fructose in com syrup (Carasik and Carroll 1983). The organism *Bacillus coagulans* has been selected for the production of glucose isomerase. The development of the immobilized cell slurry has not proceeded to the point where half-lives of the enzyme are more than 75 days. A half-life is defined as the time taken for a 50% decrease in activity. Such immobilized enzyme columns can be operated for periods of over three half-lives.

The second important application of immobilized enzymes is the hydrolysis of lactose to glucose and galactose in milk and milk products by lactase (Sprössler and Plainer 1983). Several lactase sources are available; from yeast, *Saccharomyces lactis* and *S. fragilis,* or from fungi, *Aspergillus oryzae* or *A. niger.* The enzymes vary in their optimum pH and optimum temperature, as well as other conditions.

It is to be expected that the use of immobilized enzymes in food processing will continue to grow rapidly in the near future.

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Fruits and Vegetables

11

Chang Yong Lee

Major and Minor Components

Chemical composition of fruits and vegetables is highly dependent on photosynthesis and absorption of water and minerals during the period of growth and maturation on the parent plant. However, following harvest, the sources of nutrients and water that supplied the growing entities are no longer available and they become independent units in which respiratory processes now play a proportionately major role, with the uptake of oxygen and loss of carbon dioxide and water. Deterioration accompanies the use of the reserves of reparable materials (mainly carbohydrates) accumulated during photosynthesis that are no longer replenished. Therefore, chemical constituents of fresh fruits and vegetables are decided by these growth, maturation and respiration processes.

Fruits and vegetables contain a very wide range of different chemical compounds and show considerable variations in composition and in structure. Apart from the obvious inter-specific differences, no two entities are exactly the same, e.g. two fruits from the same plant. Moreover, an individual fruit or vegetable, being largely composed of living tissues which are metabolically active, is constantly changing in composition, the rate and extent of such changes depending on the physiological role and stage of maturity.

The most ubiquitous of all biological compounds is water ranging 80–90%. Most of the solid matter of fruits and vegetables is made up of carbohydrates along with small amounts of protein and fat (Table 11.1). Also represented, usually only in relatively small amounts, are many other classes of organic compounds and a wide range of mineral elements drawn from the soil. Many of these "minor" constituents can have a most important influence on the properties of fruits and vegetables—on their color, flavor, nutritive value and biological activity, and in some cases on their texture.

Water

Water is the most abundant single constituent of fruits and vegetables. Most fresh produce contains more than 80% water, with some tissues, such as cucumber, lettuce, and melons, containing about 95% of the total weight. The starchy tubers and seeds, for example yam, cassava and corn, contain less water, but even they usually comprise more than 50% water. It was suggested that water plays four important functions in the plant cell walls as a part of the matrix gel: it is a structural component; it can act as wetting agent interrupting direct hydrogen bonding between polymers; it can help in stabilizing conformations of polymers; and it acts as a solvent for the presence and transport of salts and low molecular weight organic compounds (Northcote 1972). Especially, its contribution to texture of fresh

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_11

Table 11.1 Ranges of proximate composition in fresh fruits and vegetables

	Water	Carbohydrate	Lipid	Protein
	(% Fresh weight)			
Fruits	80–90	5–20	0.1–0.5	0.5–3
Vegetables	70–90	2–25	0.1–0.3	5–7

fruits and vegetables can be very significant. Given an unlimited supply of available moisture, the water content of a living plant tissue assumes a characteristic maximum value which is associated with a state of complete turgor of the component cells. The internal pressure (up to 9 atm) developed by osmotic forces is exactly balanced by the inward pressure of the fully extended cell wall in which the tissue is physically incapable of absorbing any further water. Significant variations in water content can occur within a species, since the water content of individual cells varies considerably. The actual water content is dependent on the availability of water to the tissue at the time of harvest. It may also be markedly affected by cultural conditions which influence structural differentiation. Interspecific differences in moisture content are generally smaller than differences between different types of tissue.

The susceptibility to wilting of harvested fruits and vegetables varies according to the extent to which their external surfaces are structurally modified to reduce water loss. Leaves are especially liable to postharvest wilting. Since water supports various chemical reactions, therefore, removal of water, such as dehydration, retards many reactions and inhibits growth of microorganisms, thus improving shelf lives of processed fruit and vegetable products.

Carbohydrates

During the growth and maturation of plants, carbohydrates, mainly in the forms of sugars and transient starch, are elaborated as a result of photosynthesis. Starch, having been formed in the storage cells and tissues, may become transformed into sugars, particularly sucrose, glucose, and fructose, during the postharvest period. In addition to simple sugars, the structural framework of plant tissues is largely composed of complex molecules built up from monosaccharides. The total carbohydrate content of fruits and vegetables can range from as little as 2% of the fresh weight in some cucurbitaceous fruits (melons, cucumbers, squashes) to over 30% in vegetables containing storage starch. The total carbohydrate includes polysaccharides, which apart from starch are largely confined to the cell walls, and sugars, mainly sucrose, glucose and fructose, which accumulate mainly in the cell sap of fruits.

In general, fruits and vegetables contain more reducing sugars than sucrose (Table 11.2), although in many cases the reverse is true. Other sugars, such as xylose, mannose, arabinose, galactose, maltose, sorbose, and cellobiose, may also be present. Plant tissues may also contain sugar alcohols, such as sorbitol, and sugar acids, such as ascorbic acid. Sucrose is the dominant oligosaccharide. Maltose occurs in small amounts in grapes, bananas and guavas. D-Sorbitol is abundant in *Rosaceae* fruits (pomme fruits, stone fruits), at the concentration range in 300–800 mg/100 mL in apple juice. Tropical and subtropical fruits tend to have the higher level of glucose and fructose with persimmon, litchi, banana and pomegranate having combined levels of the two sugars of more than 10%, while grape is probably the only temperate fruit with more than 10%. Sucrose occurs at 8–10% in the tropical fruits, banana, mango, guava, and others (Wills et al. 1989). Some vegetables, such as broccoli and onions contain insignificant amounts of raffinose and stachyose that cause flatulence when ingested because humans do not produce the α-galactosidase needed to hydrolyze the galactose-galactose bond.

The principal carbohydrate of plant tissues, which is not associated with cell walls, is starch, a linear (α 1,4) or branched (α 1,4;1,6) polymer of D-glucose. Starch is localized in intracellular plastids that have species-specific shape, size, and optical properties. Industrially, starch is obtained from such crops as potatoes, sweet potatoes, or cereals that may contain up to 40% starch on a fresh weight basis. Since starch can be utilized as energy source, some vegetables with

Table 11.2 Distribution of free sugars in some fresh fruits and vegetables

Name	Variety	Glucose	Fructose	Sucrose	Maltose	Raffinose	Stachyose
Apple	G. Delicious	0.98	7.10	3.80	0.38		
Apricot	Alfred	1.80	0.92	5.82			
Blueberry	Blue Crop	3.92	4.04	0.24			
Grape	Risling	6.39	6.34	1.90	3.08		
Peach	Elberta	1.26	1.40	7.12			
Pear	Bartlett	1.00	7.88	1.28	0.48		
Sweet Cherry	Schmidt	6.32	5.94	0.64			
Strawberry	Sparkle	2.20	2.52	1.56	0.14		
Asparagus	M. Wash.	1.02	1.40	0.30			
Broccoli	Primo	0.60	0.52	0.40		0.10	0.18
Cabbage	Early Head	1.40	1.14	0.26			
Carrot	Nantes	0.92	0.82	3.64			
Celery	Early Fortune	0.36	0.36	0.24			
Lettuce	Grand Rapids	0.17	0.24	0.10			
Onion	Premier	1.76	1.18	0.84		0.66	0.24
Spinach	Virg. Savory	0.05	0.03				
Sweet corn	Seneca Chief	0.52	0.56	3.10			
Tomato	New Yorker	1.20	1.34	0.02			

Lee et al. (1970)

high starch content such as cassava, yam, and sweet potato are the major energy source in many developing countries. Although starch contributes more calories to the normal human diet than any other single substance, it is absent or negligible in most ripe fruits and many vegetables. Other polysaccharides are cellulose, hemicellulose and pectins.

A substantial proportion of carbohydrates in fruits and vegetables is made of the cell wall and middle lamella that have important effects on the texture of the products and human health. Cellulose, hemicellulose, six substances and lignins are found in cell wall; the cellulose gives rigidity as well as resistance to tearing; pectic substance and hemicellulose give plasticity together with ability to stretch. Cellulose is one of the most abundant substances in the biosphere, is largely insoluble and indigestible by human beings due to lack of enzymes. Cellulose is a β-(1→4) linkages with about 8000–12,000 glucose units per chain. In the primary cell walls, it occurs as linear associations of the polymer molecules called fibrils and up to 70% of the fibril is crystalline form (Preston 1974). The hemicelluloses are a heterogeneous group of polysaccharides that contain various kinds of hexose and pentose sugars. The middle lamella, composed mainly with pectic substances, is the outermost portion of the cell wall that plays the primary role in intercellular adhesion. More than one-third of the dry material of the primary cell walls and the middle lamella of fruits and vegetables are made up with pectic substances. Chemically they are bound by covalent bonding, hydrogen bonding, and ionic bonding. Therefore, canning, cooking, storage, or ripening processes that bring about texture changes are accompanied by significant changes in the characteristics of the pectic substances.

Fruits and vegetables are important sources of dietary fiber in our diet. During the last 30 years, results from extensive research have demonstrated that fiber is important for gastrointestinal tract function and that foods rich in fiber help prevent and manage a variety of diseases: cardiovascular disease, diabetes mellitus, obesity and weight control, and others, including lowering the risk of death. Major fruits and vegetables that provide significant amounts of dietary fibers are listed in Table 11.3.

Table 11.3 Total dietary fiber content of selected fruits and vegetables (g/100 g fresh)

Fruit	Total fiber	Vegetables	Total fiber
Apple	2.4	Asparagus	2.1
Apricot	2.0	Beans, Lima	4.9
Banana	2.6	Beet	2.8
Blueberry	2.4	Broccoli	2.6
Cherry, sweet	2.1	Brussels sprout	3.8
Mango	1.6	Cabbage	2.5
Grapes, American	0.9	Carrot	2.8
Cantaloupe	0.9	Cauliflower	2.0
Orange	2.4	Celery	1.6
Peach	1.5	Cucumber	0.5
Pear	3.1	Kale	3.6
Plum	1.4	Lettuce, Romaine	2.1
Pineapple	1.4	Lettuce Green Leaf	1.3
Raspberry	6.5	Onion	1.7
Strawberry	2.0	Peas	2.6
Watermelon	0.4	Pepper, sweet	1.7
		Potato	2.2

Source: USDA (2006)

Proteins and Nitrogenous Compounds

Proteins, though commonly representing less than 1% of the fresh weight of fruit and vegetable tissue, must be considered as structural constituents since they are the major solid components of the cytoplasm of living cells. Leguminous seeds are richest in protein, containing up to about 8%. Some leafy vegetables and sweet corn can contain up to about 4% of protein, but in most other products the level is below 3%. The protein content of fruits is usually particularly low, seldom rising above about 1%, but is mostly functional as enzymes. Enzyme systems, which are of such primary importance in the physiology and postmortem behavior of fruits and vegetables, always contain a protein moiety, and traces of protein, probably enzymatic, are found in parts of the cell other than the cytoplasmic layer, e.g. in the cell wall. Enzymes may have either a beneficial or detrimental effect on processing. Major enzymes associated with quality of fruit and vegetable products will be discussed later.

A large number of simple nitrogenous substances have been found to occur in the tissues of fruits and vegetables. Free amino acids and related amines such as asparagine and glutamine, normally those which are also present in the proteins of the tissue, appear to make up the bulk (up to 80%) of this soluble fraction of the total nitrogen. Asparagine and glutamine or the related acids aspartic and glutamic appear to be especially abundant in many species—citrus fruits, potato, tomato, strawberry, gooseberry and blackberry, and together these compounds often represent more than half of the non-protein nitrogen.

Lipids

The lipid content of fresh fruits and vegetables is small (0.1–1%) but significant in terms of storage and quality, due to undesirable flavor changes resulting from oxidation. In fruits, only seeds and nuts contain significantly higher levels of lipids. The lipids of fruits and vegetables (with the exception of the avocado and the olive), are, like the proteins, largely confined to the cytoplasmic layers in which they are especially associated with the surface membranes. In addition to triacylglycerides, glyco- and phospholipids, carotenoids, triterpenoids and wax are present. Lipid and lipid-like materials are particularly prominent in the protective tissues at the surfaces of plant organs—in the cuticle, epidermis and corky layers. These include wax-like substances which are soluble in fat solvents and contain mixtures of fatty acids, hydroxyacids, alcohols, esters, ketones, ethers and hydrocarbons. In climacteric fruits, such as tomatoes, the increase in respiration rate is known to accompany a large increase in phosphatidyl choline and a breakdown of total lipid during the postclimacteric stage (Guclu et al. 1989).

Minor Composition

Organic Acids

Small quantities of various organic acids of the Krebs' cycle or shikimic acid pathway intermediates are produced and accumulate in vacuoles of

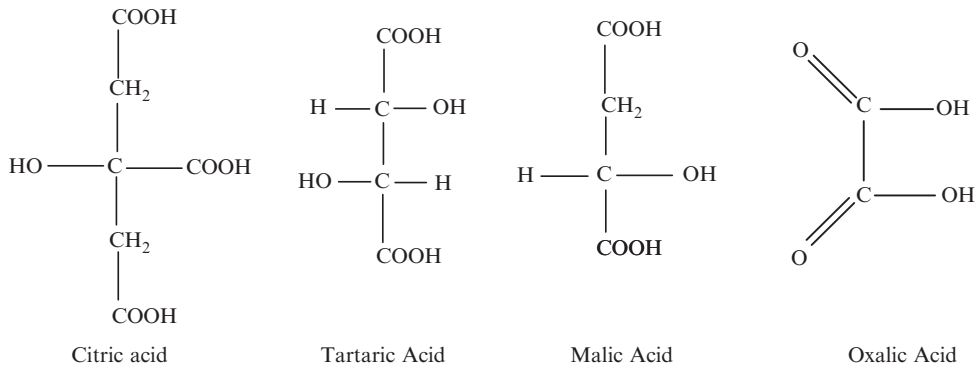


Fig. 11.1 Chemical structures of common aliphatic organic acids in fruits and vegetables

fruits and vegetables. Some of these acids and various others such as oxalic and tartaric acids, which have not thus far been linked with particular metabolic cycles, can accumulate in the tissues in considerable amounts. As a result, fruits and vegetables are normally acidic; the acid content ranging widely from very low levels in some vegetables, such as sweet corn and leguminous seeds, to high level in certain fruits such as lemon, black current, grape, and loganberry. Among vegetables, spinach and rhubarb show an unusually high level of acidity due to high content of oxalic acid.

Aliphatic Acids: The most widely occurring and abundant acids in fruit and vegetable tissues are citric and malic, each of which can, in particular examples, constitute over 2% of the fresh weight. Lemons generally contain over 3% of citric acid. In most species either citric acid or malic acid is the predominant individual acid constituent but there are one or two notable exceptions. The blackberry produces mainly isocitric in place of citric acid, while the avocado is exceptional in being deficient in both of the major plant acids citric and malic. Citric acid is the principal acid of citrus fruits, of black and red currant, raspberries, loganberries, strawberries, cranberries, pineapples, pomegranates and pears. Malic acid predominates in apples, most drupe fruits (plums, cherries, apricots, etc.), and cucurbitaceous fruits, banana and rhubarb (Fig. 11.1 and Table 11.4).

Vegetables also differ in the relative abundance of citric and malic acids. In tomato, along with potato, many leafy vegetables, and beet root, citric acid is the main acid. Malic acid predominates

Table 11.4 Major organic acids in some fresh fruits and vegetables (mg/100 g fresh weight)

Fruits and vegetables	Malic	Citric	Others
Grape	1095	61	Tartaric 1061
Lemon	228	5149	
Melon	14	1005	
Orange	131	2049	
Peach	2183	673	
Broccoli	120	210	
Cabbage, white	100	140	
Carrot	240	90	Oxalic 0–60
Cauliflower	190	10	
Green beans	112	34	Oxalic 20–45
Green pepper	190	77	
Lettuce	575	118	
Rhubarb	910	137	Oxalic 230–500
Tomato	81	1064	

Belitz and Grosch 1999; Flores et al. 2012

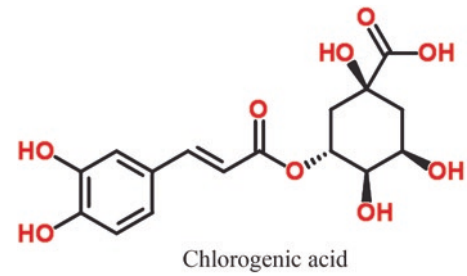
in lettuce, cauliflower, carrot, artichoke, cucurbits, okra, onion, celery, parsnip, turnip, green pepper, and green beans. Others are succinic, glycolic, gluoxylic, oxalo-acetic, benzoic (antifungal activity in cranberry), fumaric, α-ketoglutaric, pyruvic, aconitic, lacto-isocitric, malonic, quinic, and shikimic. In addition, various free uronic, glutamic, and short-chain fatty acids are often present in small amounts.

Aromatic (cyclic) acids: Some phenolic acids contribute to color and taste of products. Hydroxycinnamic acids and their derivatives *p*-coumaric, ferulic, caffeic and sinapic acids are widespread in fruits and vegetables. These acids are rarely free but are present mainly as

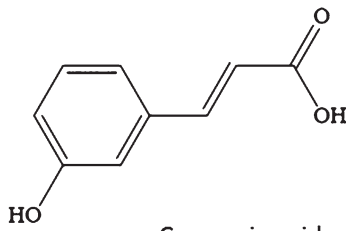
Table 11.5 Chlorogenic acid content in common fruits

Fruit	Chlorogenic acid (mg/100 g fresh wt.)	References
Apples		
Empire	2.56	Coseteng and Lee (1987)
McIntosh	10.40	
Golden Delicious	7.71	
Granny Smith	3.2	Mattila and Kumpulainen (2002)
Pear		
d'Anjou, Bosc	3–7	Spanos and Wrolstad (1992)
Red raspberry	1.5	Mattila and Kumpulainen (2002)
Strawberry	2.9	

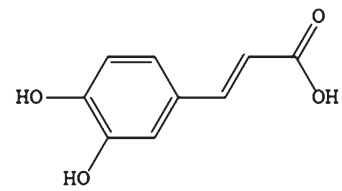
acid derivatives. For example, caffeic acid is esterified with quinic acid, giving rise to chlorogenic acid. Esters of other hydroxycinnamic acids with quinic acids are also widespread. Hydroxycinnamic acid biosynthesis starts with phenylalanine. Chlorogenic acid, the ester of caffeic acid and quinic acid, is the most widely occurring member of phenolic compounds. It is the major phenolic compounds in apples and pears (Table 11.5). It is the main substrate involved in the enzymatic oxidative discoloration of products such as apple, pear, peach, potato and sweet potato. A recent report (Mattila et al. 2006) on phenolic acids in fruits determined by HPLC after hydrolyses showed that the content of total phenolic acids as aglycones ranged from 0 to 103 mg/100 g fresh weight and caffeic acid dominated in most of fruit samples (Table 11.6). Chlorogenic and caffeic acids have also been implicated in the non-enzymatic blackening of potato tissue after cooking, due to the formation of complexes with ferrous iron.



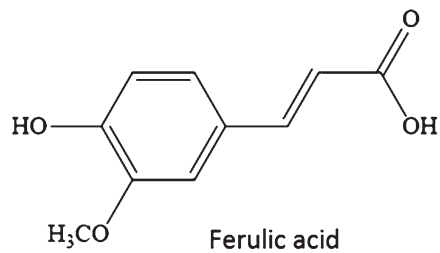
Chlorogenic acid



m-Coumaric acid



Caffeic acid



Ferulic acid

Table 11.6 Phenolic acid content in some fruits (mg/100 g fresh weight)

Fruit	CAF	FER	SIN	VAN	P-COUM	GAL	TOT
Apple, Lobo	4.30	0.27	0.66	0.09	0.66	7.2	13
Apple, Granny Smith	1.3	n.d.	n.d.	n.d.	0.41	5.4	7
Apple, Red Delicious	5.4	0.27	0.10	n.d.	1.0	6.5	14
Banana	0.20	5.4	n.d.	0.44	0.46	n.d.	7
Grape, red	3.4	0.43	n.d.	1.07	3.8	3.1	19
Grape, green	3.4	n.d.	n.d.	n.d.	1.17	2.8	7
Plum, dark	23.4	1.47	0.14	1.27	2.1	n.d.	28
Cherry	17.1	0.46	n.d.	1.17	5.1	n.d.	28
Pear	6.5	0.29	0.10	0.27	0.7	n.d.	8
Orange	3.3	9.4	2.2	0.44	1.78	n.d.	18
Grapefruit	5.5	11.6	0.99	1.66	1.35	n.d.	21
Kiwi fruit	1.5	n.d.	n.d.	0.19	0.25	n.d.	3
Watermelon	0.12	0.35	n.d.	0.23	0.37	n.d.	2

CAF caffeic acid, *FER* ferulic acid, *SIN* sinapic acid, *VAN* vanillic acid, *P-COUM* p-coumaric acid, *GAL* gallic acid, *TOT* total of phenolic acids after hydrolysis (Mattila et al. 2006)

Phenolic Compounds

Plants consumed by humans contain thousands of phenolic compounds because phenolic compounds comprise one of the largest and most ubiquitous groups of plant metabolites. They are formed to protect the plant from photosynthetic stress, reactive oxygen species, wounds, and herbivores. The term "phenolic compounds" embraces a wide range of compounds that possess an aromatic ring bearing a hydroxyl substituent, including their functional derivatives. Phenolic compounds are commonly present in most fruits and vegetables (Table 11.7). Many phenolic compounds participate in both enzymatic and nonenzymatic browning reactions. In addition to color, polyphenol compounds also contribute to food flavor and other qualities. For example, astringency of polyphenols and its ratio with sugar and acid are important and useful criteria for determining the overall quality of fresh fruits, fruit beverages, and wines. Some polyphenols, such as chalcones and related compounds found in citrus fruits, are exceedingly sweet or bitter. Both bitterness and astringency of wine are due to phenolic compounds present in grapes. Traditionally, they were related to antinutritional effects, by decreasing absorption and digestibility of food because of their ability to bind proteins and minerals. In addition, the astringency of many fruits and beverages is due to the precipitation of salivary proteins with plant polyphenols. Therefore, polyphenol compounds are directly related to their nutritional and sensory characteristics of foods. However, current interest stems from the observations that dietary phenolic compounds have antioxidative, anti-inflammatory, and anticarcinogenic activities. Numerous epidemiological data have shown a very strong inverse relationship between consumption of fruits and vegetables and the risk of heart disease and cancer (Joshipura et al. 2001) and many investigators have attributed benefits to various antioxidants in fruits and vegetables, including phenolic compounds (Willcox et al. 2004).

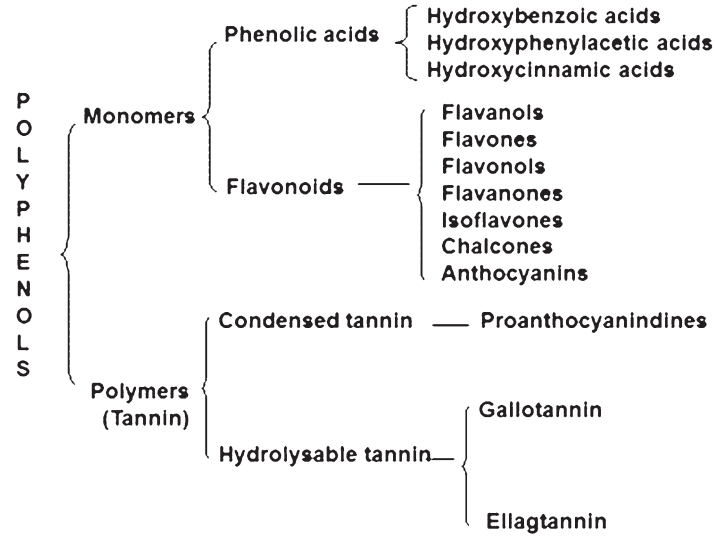
In broad sense, polyphenol compounds can be divided into two main groups, monomer and polymer. Monomer can be divided to flavonoid subgroup and phenolic acid subgroup: flavonoid subgroup includes flavone, flavonol, flavonone, flavononol, isoflavone, anthocyanine, chalcone, and aurone; phenolic acid subgroup includes chlorogenic acid, gallic acid and ellagic acid. Polymer (tannin group) can be divided into two sub groups, condensed tannin and hydrolysable tannin. Hydrolysable tannin sub group can be divided to gallotannin and ellagtannin. Among these phenolic compounds,

Table 11.7 Flavonoid content of selected fruits and vegetables (mg/100 g edible portion)

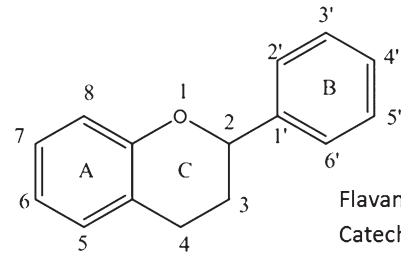
	Anthocyanidin[a]	Flavan-3-ols		Flavonols	
Produce	Cyanidin	Epicatechin	Catechin	Kaempferol	Quercetin
Apple, with skin	2.44	6.07	0.89	0.02	4.27
Apricot		5.47	4.79		2.08
Banana	7.39(Del)	0.02	6.10		
Beans, Kidney, canned		0.35	1.66		
Blackberry	90.31	4.66	37.06	0.06	1.76
Blueberry	61.35(Mal)	13.69	37.24	1.81	5.05
Cabbage, red	72.86				0.38
Carrot				0.10	0.31
Chard, Swiss			2.15	4.30	2.63
Cherry, sour	6.64	3.83	0.30		2.92
Cherry, sweet	75.18	6.97	1.31		2.64
Cranberry	41.81	4.37	0.39	0.09	15.09
Grape, red	34.71(Mal)	1.20	0.82		1.38
Onion	6.16			1.10	33.43
Peach	1.61	2.34	4.92	–	0.68
Pear	12.18	3.76	0.27		4.51
Plum	39.68	2.44	17.55	0.01	12.45
Strawberry	31.27(Pel)	0.12	3.32	0.46	1.14

Source: USDA (2006)
[a]Anthocyanidin: *Del* Delphinidin, *Mal* Malvidin, *Pel* Pelargonidin

of which approximately 8000 are known to occur in plants the flavonoids form the largest group with more than 5000 known structures. Phenolic compounds range from structures that are very lipophilic (e.g. Tangeretin) to those that are very water soluble (e.g. Quercetin 3-sulfate). The size of molecule varies greatly, ranging from monomer, catechol with molecular weight of 110 to the complex polymers which has molecular weight of over 1500.

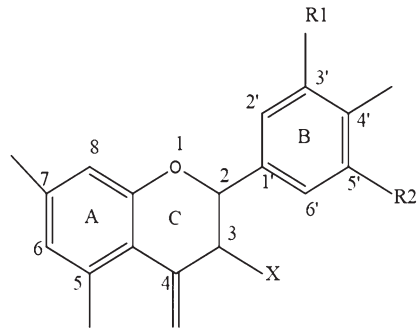


Flavonoids, diphenylpropanes (C6-C3-C6) constitute one of the most distinctive polyphenolic groups and occur ubiquitously in plant foods and are common component in fruits and vegetables. They occur generally as *O*-glycosides with sugars bound usually at the C3 position. Some flavonoids in common fruits and vegetable are known to have various biological effects *in vitro* and *in vivo* and their significance of health beneficial effects has been an important issue in the recent years.



Flavan-3-ol: A5, A7, B3, B4 =OH
Catechin, Epicatechinn, C3 =OH

Flavonoid Basic Structure (C6-C3-C6)



Flavonol X = OH
Quercetin, R1 = OH, R2 = H
Kaempferol R1 = H, R2 = H
Myrcetin R1 = OH, R2 = OH
Flavones
Apengenin R1 = H, R2 = H
Luteolin R1 = OH, R2 = H

Flavonol and Flavone Structure

Minerals

The total amount of mineral matter in fruits and vegetables varies in different products from as little as 0.1% in some varieties of yam to as much as 0.4% in kohlrabi. The most common mineral constituents in fruits and vegetables are potassium, calcium, magnesium, iron, phosphorus, sulfur and nitrogen, together with certain other elements such as sodium, aluminum and silicon which, though not essential to the plant, are often well-represented in the soil. Copper, manganese, zinc, boron, and molybdenum, all essential micronutrients, are also consistently present. The most abundant individual mineral element in fruits and vegetables is potassium, 60–600 mg. Spinach, potatoes, broccoli and cauliflowers are good sources of potassium among vegetables and bananas among fruits. Peas, beans, and dark green leafy vegetables (spinach and collard) are good sources of iron. Vegetables in general are richer in minerals than are fruits (Table 11.8).

The mineral elements can have an important influence on the quality of fruit and vegetable products: calcium can have a marked effect on texture. Metallic constituents also strongly influence color by their combination with organic components, while the trace metals are all constituents of prosthetic groups of tissue enzymes which control the metabolic activity of harvested products and can cause marked changes in quality during and subsequent to various processing procedures.

Table 11.8 Minerals in some fruits and vegetables (mg/100 g FW)

Fruit or vegetable	Ca	Fe	Mg	P	K	Na	Zn
Apple	6	0.12	5	11	107	1	0.04
Banana	5	0.26	27	22	358	1	0.15
Grape, muscadine	37	0.26	14	24	203	1	0.11
Orange, Navel	43	0.13	11	23	166	1	0.08
Peach	6	0.25	9	20	190	0	0.17
Plum	6	0.17	7	16	157	0	0.10
Watermelon	7	0.24	10	11	112	1	0.10
Broccoli	47	0.73	21	66	316	33	0.41
Cabbage	40	0.47	12	26	170	18	0.18
Cauliflower	22	0.42	15	44	299	30	0.27
Green Bean	37	1.03	25	38	211	6	0.24
Pea	43	2.08	24	53	200	4	0.27
Potato	12	0.78	23	57	421	6	0.29
Spinach	99	2.71	79	49	558	79	0.53
Tomato	10	0.27	11	24	237	5	0.17

Source: USDA., ARS (2011)

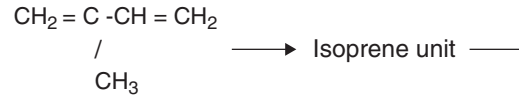
Many fruits and vegetables are important sources of vitamins (Table 11.9). They are the major source of vitamin A and C in our diet. Provitamin A carotenoids are found in many fruits and vegetables and one of the most abundant carotenoids is β-carotene, which exhibits the greatest amount of provitamin A activity. In general, yellow, orange, and red fruits and vegetables such as papaya, apricot, peaches, cantaloupes, tomatoes, carrots, sweet potatoes, winter squash, pumpkins, contain significant amounts of β-carotene (provitamin A carotenoid). Ascorbic acid occurs in large amounts in asparagus, papaya, oranges, cantaloupe, cauliflower, broccoli, Brussels sprouts, green pepper, grape fruit, lemon and strawberries. The vitamin content of a given species can vary considerably with variety, growing conditions, maturity, postharvest storage, and processing conditions.

Pigments

Although the total quantity of pigments in fruits and vegetables is very limited, but they are exceptionally important to consumers' pleasure. The main pigments of fruits and vegetables can be classified as: (1) the carotenoids, (2) the chlorophylls, (3) the anthocyanins, and (4) the anthoxanthins.

Carotenoids: The carotenoids are a group of yellow, orange, and orange-red fat soluble pigments composed of isoprene units and widely distributed in fruits and vegetables. They are present in the lipid material along with the chlorophylls. The green color of the chlorophyll masks the yellow to red color of the carotenes except in very young leaves.

Chemically, carotenoids are polyene hydrocarbons biosynthesized by only plants that formed from eight isoprene units (tetraterpenes):



Some carotenoids are derived by hydrogenation, dehydrogenation, and/or cyclization of the basic structure of the 40-C carotenoids. Carotenoids are divided into two main classes: carotenes and xanthophylls. Carotenes are pure polyene hydrocarbons and xanthophylls contain oxygen in the form of hydroxy, epoxy or oxo groups. The basic carbon structure for lycopene shows the symmetrical arrangement around the central pair of carbons. This structure can be cyclized to form β-carotene and the 15-15′ carbon pair forms the center of the molecule. Other carotenoids have different end groups with the

Table 11.9 Major vitamins in some fruits and vegetables (fresh weight)

Fruit or vegetable	Vitamin A (RAE, μg/100 g)	Vitamin C (mg/100 g)	Vitamin B6 (mg/100 g)	Thiamin (mg/100 g)	Niacin (mg/100 g)
Apple		4.6	0.041	0.17	0.091
Apricot	3	10.0	0.054	0.030	0.600
Banana	96	8.7	0.367	0.031	0.665
Bluberry	3	9.7	0.052	0.037	0.418
Cherry, sweet	3	7.0	0.049	0.027	0.154
Grapefruit	3	31.2	0.053	0.043	0.204
Grape, American	58	4.0	0.110	0.092	0.300
Melon, honeydew	3	18.0	0.088	0.038	0.418
Orange	11	53.2	0.060	0.087	0.282
Peach	16	6.6	0.025	0.024	0.806
Pear	1	4.3	0.029	0.012	0.161
Pineapple	3	47.8	0.112	0.079	0.500
Strawberry	1	58.8	0.047	0.024	0.386
Watermelon	28	8.1	0.045	0.033	0.178
Asparagus	38	5.6	0.091	0.143	0.978
Beans, Snap	35	12.2	0.141	0.082	0.743
Broccoli	31	89.2	0.175	0.071	0.639
Asparagus	38	5.6	0.091	0.143	0.978
Beans, Snap	35	12.2	0.141	0.082	0.743
Broccoli	31	89.2	0.175	0.071	0.639
Brussels sprout	38	85.0	0.219	0.139	0.745
Cabbage	5	36.6	0.124	0.061	0.234
Carrot	835	5.9	0.138	0.066	0.983
Cauliflower	0	48.2	0.184	0.05	0.507
Corn, sweet	9	6.8	0.093	0.155	1.770
Onion	0	7.4	0.12	0.046	0.116
Pea, green	38	40.0	0.169	0.266	2.090
Potato, russet	0	5.7	0.345	0.082	1.036
Spinach	469	28.1	0.195	0.078	0.724
Squash, winter	68	11	0.154	0.07	0.5
Sweet potato	709	2.4	0.209	0.078	0.557
Tomato	42	13.7	0.08	0.037	0.594
Cabbage	5	36.6	0.124	0.061	0.234

USDA., ARS (2011)

same central structure. About 60 different end groups are known, comprising about 500 known carotenoids.

Postharvest Deterioration

Today's consumers have unlimited access to an amazing range of fresh produce throughout the year. A major problem, however, has been maintaining the produce at high quality. Changes that occur in fruits and vegetables during the ripening process and after harvest influence their use as foods. Following harvest, the sources of nutrients and water that supplied the growing fruits and vegetables are no longer available. In order to sustain life processes, respiration continues to produce metabolic energy with the uptake of oxygen and loss of carbon dioxide and water. Consequently, postharvest deterioration accompanies with the use of the reserved materials accumulated during photosynthesis that are no longer replenished.

Therefore, in order to increase shelf-life of fruits and vegetables and reduce deterioration, increased attention has been given in recent years to the postharvest physiology of fresh fruits and vegetables. In order to conserve high quality of fruits and vegetables, we have to understand the basic biochemical reactions that are taking place in plant cells before and after harvest. Therefore, the nature and causes of losses, due to both wastage and reduced quality, between harvest and consumption should be studied.

The magnitude of postharvest losses in fresh fruits and vegetables is estimated 5–25% in developed countries and 25–80% in developing countries, depending upon the commodity, cultivar, and handling conditions. In tropical regions, which include a large proportion of the developing countries, these losses can assume considerable economic and social importance. To reduce these losses, producers and handlers must understand the biological and environmental factors involved in deterioration and use postharvest techniques that delay senescence and maintain the best possible quality. Thus the main role of food scientists or postharvest physiologists is to devise methods by which deterioration of produce is restricted as much as possible during the period between harvest and consumption.

Cellular Components and Physiology of Fruits and Vegetables

The cells of fruits and vegetables are typical plant cells. The main type of cell in the edible portion of most fruits and vegetables is the parenchyma cell. The walls of parenchyma cells in young plants are composed almost entirely bounded by a more or less rigid cell wall composed of cellulose, fiber and other polymers such as pectic substances, hemicelluloses and lignins. A layer of pectic substances forms the middle lamella and acts to bind adjacent cells together. As the plant grows older the nature of these cementing substances often changes, lignins and other compounds are deposited, and the cellulose layer of the cell wall thickens. Adjacent cells often have small communication channels, called plasmadesmata linking their cytoplasmic masses. The wall is semi-permeable to water and solutes. The material within the cell is protoplasm composed of a very large number of different molecules. An important organelle found in plant cells but not in animal cells is granular green plastids called chloroplast, where photosynthesis occurs. Another unique to plant cells is a large central vacuole, a compartment that stores water and a variety of chemicals. The cytoplasm contains several important organelles, such as nucleus, mitochondria, chloroplasts (chlorophylls), chromoplasts (carotenoids), and amyloplasts which are membrane-bound bodies with specialized functions. Important processes which occur in the cytoplasm include the breakdown of storage reserves of carbohydrate by glycolysis and protein synthesis. Other types of plant cells besides parenchyma cells are the conducting cells, the supporting cells, and the protective cells. The walls of these cells are composed primarily of cellulose and lignin.

The most important fact in the postharvest handling of fruits and vegetables is that harvested fruits and vegetables are living structures. One readily accepts that produce is a living, biological entity when it is attached to the growing parent plant in its agricultural environment. But even after harvest, the produce is still in living state as it continues to perform the metabolic reactions and maintain the physiological systems which were present when it was attached to the plant. An important feature of plants is that they respire by taking up oxygen and giving off carbon dioxide and heat. They also transpire, that is, lose water. Therefore, the produce is dependent entirely on its own food reserves, moisture content, and its environmental conditions. The intact plant cells must respond to various physical and chemical challenges from the environment. It is important to recognize that different commodities, species and cultivars differ markedly in response to the environmental conditions. Therefore, no single condition or treatment is ideal for extending the shelf-life of all fruits and vegetables. Some of the major factors that influence the respiration rate and deterioration will be

discussed. The texture of fruits and vegetables depends on the turgor of living cells as well as on the occurrence of supporting tissues and the cohesiveness of the cells. Turgor pressure is created by the cell contents and the partially elastic cell wall in a delicately balanced force. It maintains the cell at a normal volume yet allows the exchange of substances. When the balanced force is disrupted by wilt, cooking or processing, the cell walls dies and the vacuoles are no longer covered by a living membrane of protoplasm and eventually the texture of food becomes soft.

Table 11.10 Fruits classified according to respiratory behavior during ripening

Climacteric fruits		Nonclimacteric fruits	
Apple	Muskmelon	Blackberry	Olive
Apricot	Nectarine	Cherry	Orange
Avocado	Papaya	Cranberry	Pea
Banana	Passion fruit	Cucumber	Pepper
Blueberry	Peach	Date	Pineapple
Guava	Pear	Grape	Raspberry
Kiwifruit	Plum	Lemon	Strawberry
Mango	Tomato	Lime	Watermelon

Kader (2002)

Respiration

Respiration is the major process in living tissues and can be described as the oxidative breakdown of the more complex materials normally present in cells, such as starch, sugars, and organic acids, into simpler molecules, such as carbon dioxide and water, with the concurrent production of energy and other molecules which can be used by the cell for synthetic reactions. Numerous enzymes are associated with these reactions.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}$$

Respiration rate is an excellent indicator of metabolic activity of the tissue and thus is a useful guide to the potential storage life of the produce. The loss of stored reserves during respiration means the hastening of senescence as they are exhausted. Therefore, the rate of deterioration of post-harvested commodities is generally proportional to the respiration rate. A large group of fruits that include tomato, mango, banana, and apple, show a pronounced increase in respiration coincident with ripening. Such an increase in respiration is known as a "respiratory climacteric," and this group of fruits is known as the climacteric class of fruits (Table 11.10). The intensity and duration of the respiratory climacteric varies widely amongst fruit. The respiratory climacteric, as well as the complete ripening process, may proceed while the fruit is either attached to or detached from the plant. Those fruits, such as citrus, pineapple and strawberry, that do not exhibit a respiratory climacteric, are known as the non-climacteric class of fruits. Non-climacteric fruit exhibit most of the ripening changes at the plant, although these usually occur more slowly than those of the climacteric fruits. All vegetables are considered to have a non-climacteric type of respiratory pattern. A clear understanding of the control mechanism of fruit ripening has important commercial benefits.

Climacteric and non-climacteric fruits may be further differentiated by their response to applied ethylene and by their pattern of ethylene production during ripening. It has been established that all fruits produce minute quantities of ethylene during development. However, coincident with ripening, climacteric fruits produce much larger amounts of ethylene than non-climacteric fruits. This difference between the two classes of fruits is further exemplified by the internal ethylene concentration found at several stages of development and ripening. The internal ethylene concentration of climacteric fruits varies widely, but that of non-climacteric fruits changes little during development and ripening. Ethylene, applied at concentration as low as 0.1–1.0 μL/L for 1 day, is normally sufficient to hasten full ripening of climacteric fruits, but the magnitude of the climacteric is relatively independent of the concentration of applied ethylene. In contrast, applied ethylene merely increases the respiration of non-climacteric fruits, the magnitude of the increase being dependent on the concentration of ethylene.

Environmental Factors that Influence Deterioration

Temperature: Temperature is the environmental factor that most influences the deterioration rates of postharvest commodities. The rate of respiration reactions, within the physiological temperature range, increases exponentially with increase in temperature. For each increase of 10 °C above optimum, the rate of deterioration increases by two- to threefold. Therefore, exposure to undesirable temperatures results in many physiological disorders. Many enzymes are inactive beyond 35 °C and inactivated at above 40 °C. At above 35 °C, metabolism becomes abnormal and results in a breakdown of membrane integrity and structure, with rapid deterioration of the produce. The lower limit for normal metabolism is the freezing point of the tissue which is usually between 0 °C and −2 °C. Within these temperature limits, respiratory changes associated with ripening and senescence proceed, but at different rates. Therefore, temperature management is the most effective tool for extending the shelf life of fresh commodities. In general, lowering of temperature can retard the rate of respiration and undesirable physiological changes. However, exposure of the commodity to too low temperature results in chilling and freezing injury. Chilling injury occurs in some commodities (especially those of tropical or subtropical fruits) held at temperature above their freezing point and below 15 °C, depending on commodity.

Relative humidity: The rate of water loss from fruit and vegetables depends on the vapor pressure deficit between the commodity and the surrounding ambient air, which is influenced by temperature and relative humidity. Therefore, to control the rate of water loss from the produce, one should lower the temperature to reduce the vapor pressure difference between the produce and the air, or provide a barrier to water loss. An effective method for reducing water loss from fruits and vegetables is to increase the relative humidity of the air. The use of very high relative humidity, however, favors the growth of microorganisms.

Atmospheric condition: Since oxygen and carbon dioxide are directly involved with respiration of fruits and vegetables, the composition of gases in the storage atmosphere can affect the deterioration rates. Therefore, reduction of oxygen or elevation of carbon dioxide can either delay or accelerate the deterioration of fresh produce. The magnitude of these effects depends on the commodity, cultivar, physiological age, temperature, concentrations of oxygen and carbon dioxide, and duration of storage time. Today, commercial controlled atmosphere (CA) storage and modified atmosphere (MA) storage are frequently used to extend the shelf life of fruits and vegetables. Commercial use of CA storage is greatest worldwide on apples and pears.

Other factors: Ethylene (C_2H_4) is a plant hormone that regulates many aspects of growth, development, ripening and senescence, especially in climacteric fruits. Therefore, its effects can be desirable or undesirable. Ethylene can be used to promote faster and more uniform ripening of fruits picked at the early mature stage, while exposure to ethylene can be detrimental to the quality of vegetables. Exposure of some commodity, such as potato or endive, to light results in greening due to formation of chlorophylls.

Effects of Processing on Fruits and Vegetables

The basic purpose of processing of fruits and vegetables is to extend the shelf life and to maintain the edible quality of the products by curtailing the activity of microorganisms and the chemical changes that would otherwise adversely affect. However, the severity of common commercial processing methods for fruits and vegetables, such as dehydration, canning, or freezing, results in extensive changes on the quality characteristics of a given commodity. Some alteration may be desirable for inactivation of antinutritional factors or softening of hard tissue or creation of new flavor and may also be undesirable by loss of nutrients, color, texture, or flavor. Undesirable chemical changes include oxidative rancidity of fats, loss of ascorbic acid and other

vitamins through oxidation, degradation of pectin, and discoloration. The chemical changes that occur when fruits and vegetables are cooked, steamed, canned, dried or frozen are very complex. Numerous chemical reactions that associated with the profound changes in sensory and nutritional qualities have been studied, but many of the exact reaction mechanisms are still unknown. Various enzyme activities in fruits and vegetables are also important factors for the sensory and nutritional qualities. Cellular disruption during peeling, dicing, size reduction, etc. before the enzymes are inactivated contributes significantly to the chemical changes that occur. Blanching is the most common types of heat processes used for fruits and vegetables. Blanching is a brief heat treatment involving exposure of the tissues to hot water or steam for a few minutes at about 100 °C. Prior to freezing, or dehydration, blanching is done primarily to inactivate enzymes, whereas prior to heat sterilization blanching is done for several reasons, the most important is to remove air from tissue. Under certain conditions, blanching may serve to activate enzymes in such a way as to promote desirable changes. For example, blanching of some green vegetables can stabilize their bright color (activation of chlorophyllase). Similarly, the texture-firming effect of blanching on certain vegetables has been attributed to activation of pectin methylesterase, which in turn catalyzes the conversion of pectin to pectic acids. Thus, in any processing method, both the growth of undesirable organisms and chemical, physical, and biochemical reactions of the food itself must be considered. Some of the major chemical reactions associated with the sensory and nutritional qualities during processing are:

Carbohydrate Reactions

Thermal processing softens plant tissues through the action of heat on pectic substances. The large molecules of insoluble protopectins are hydrolyzed during heat processing to smaller soluble pectin and pectic acid. Total pectic substances as well as protopectin in the raw vegetables were decreased while the amounts of pectins and pectates were increased after steaming. This was due to solubilization of protopectin to pectins (Table 11.11). On the other hand mild heat treatments can activate endogenous pectin methylesterase and this will partially demethylate pectic substances allowing internal calcium to effect crosslinking of pectic polymers with a resulting increase in tissue firmness. Starch granules in the presence of water gelatinize, resulting in changes in texture and water-holding capacity of the starch. Aqueous suspensions of starch can undergo hydrolysis in the presence of acid or enzymes and this alters texture and sweetness. Sugars can undergo caramelization reactions producing a brown color and a typical burnt flavor, and can degrade into a variety of compounds that influence flavor (e.g. furfural and 5-hydroxymethyl furfural). Sugar in the presence of amino groups can engage in the Maillard reaction resulting in a brown color, distinctive flavor, textural changes, loss of protein nutritive value and perhaps the development of toxic compounds.

Protein Reactions

Moderate heating results in protein denaturation. The consequences can include inactivation of enzymes, inactivation of proteinaceous inhibitors of digestive enzymes (e.g. trypsin inhibitors in

Table 11.11 Effect of steaming on pectic substances of carrots and parsnips

Pectic material	Raw		Steamed 45 min	
	Carrots (%)	Parsnips (%)	Carrots (%)	Parsnips (%)
Total pectic substance	18.6	16.4	13.2	15.0
Protopectin	14.1	10.2	3.6	5.7
Pectin	3.7	4.7	8.8	7.9
Peptic acid/pectates	0.8	1.6	1.3	2.1

Simpson and Halliday (1941)

soy products), decreasing protein solubility, alteration of protein functionality (water—0.8—holding capacity and emulsifying capabilities) and alterations in texture. Severe heating of proteins in the presence (6) of water can result in protein crosslinking (formation of isopeptide 0.7), alterations in flavor and texture, and a decrease in protein digestibility. In acidic solution, some (7.9) protein hydrolysis can occur with effects on texture and flavor. Severe heating in the presence (2.1) of water and the absence of air can result in desulfuration with accompanying alterations in color and flavor. Oxidation of proteins can result in protein crosslinking, thiodisulfide interchange reactions and oxidative degradation products that influence the texture of products. In the presence of alkali, heating can result in denaturation, racemization and formation of lysinoalanine-type compounds. Changes in protein solubility, texture, and nutritive value can result. Heating in the presence of active carbonyls will result in the Maillard reaction and Strecker degradation which have pronounced effects in color (browning), flavor, protein nutritive value, and texture. Heating in the presence of lipids can result in the formation of lipid-protein complexes and an alteration of protein texture and functionality.

Lipid Reactions

Heated lipids can undergo several types of chemical reactions that influence the sensory properties, functionality, and nutritive value. Triacylglycerols can be hydrogenated in the presence of hydrogen at elevated temperatures and pressures. In the presence of enzymes or acid, water and heat, triacylglycerols can be hydrolyzed that affects flavor and increases their susceptibility to other degradative reactions. Heat and oxidizing conditions (oxygen and inorganic or organic catalysts) can result in oxidation of unsaturated lipids. This is a very common reaction for lipids leading to the formation of aldehydes, ketones, acids, hydrocarbons, dimers and polymers which in turn, have profound effects on the flavor, color, texture, and nutritive value of foods. In some cases the breakdown of fatty acids is initiated by such simple processing as cutting. This enables enzymes present in one area of vegetable to act on compounds of their areas of the same tissue. The intact tissue of cucumbers and tomatoes has very little flavor. By cutting the tissue, the enzyme (lipoxygenase) almost instantaneously develops flavoring compounds by breaking down primarily linoleic and linolenic acids present in the triacylglycerols. Secondary reactions, such as isomerization, hydrogenation to alcohol, oxidation to acids, esterification, aldol condensation and many others will follow. When heated in the presence of pigments, peroxidizing lipids result in decolorization; when heated in the presence of flavor, flavor changes occur; and when heated in the presence of vitamins (A, C, D, E, folate), loss of vitamin activity can occur.

Color Change

Several major natural pigments in fruits and vegetables degrade significantly when exposed to heat. One of the most important chemical reactions that influence the color quality of green vegetables during heat processing is a degradation of chlorophylls. Color changes are encountered most visibly in processing of green peas, green beans, kale, Brussels sprouts and spinach. Green vegetables contain volatile acids that are partially given off during heat processing due to cellular disruption and lower the pH of vegetable tissue. Since chlorophylls are very sensitive to any pH below 7, they lose magnesium in the acidic condition, changing color from green to olive drab (pheophytin). Conversion of chlorophylls to pheophytins, which is accompanied by a color change in spinach, beans, asparagus and peas is shown in Table 11.12. Chlorophyllase is mostly inactivated when vegetables are blanched, hence, chlorophyllides and pheophorbides are rarely detected. However, in the fermentation of cucumbers chlorophyllase is active. The result is a color change from dark green to olive-green, caused by large amount of pheophorbides. Okra and turnip greens showed considerable formation

Table 11.12 Pheophytin *a, b* and pyropheophytin *a, b* in commercial canned vegetables

	Pheophytin (μg/g DW)		Pyropheophytin (μg/g DW)	
	a	*b*	*a*	*b*
Spinach	830	200	4000	1400
Beans	340	120	260	95
Asparagus	180	51	110	30
Peas	34	13	33	12

Von Elbe and Schwartz (1996)

of chlorophylides and pheophorbides when blanched at 82 °C, but considerable less of these pytol-free derivatives when blanched at 100 °C. The difference indicates deactivation of chlorophyllase at elevated temperature. In snap beans, the pigments were unaffected by blanching temperature, because of the low initial enzyme activity. Considerable formation of phytol-free derivatives in unblanched frozen peas was observed, whereas none was detected in peas blanched before freezing. A change in color occurs during drying of vegetables, its extent being dependent on water activity. The conversion of chlorophylls to pheophytins continues in blanched vegetables even during frozen storage. In beans and Brussels sprouts, immediately after blanching (2 min at 100 °C), the pheophytin content amounts to 8–9%, while after storage for 12 months at −18 °C it increases to 68–83%. Pheophytin content rises from 0% to only 4–6% in paprika peppers and peas under the same conditions. Chlorophylls are further subject to both enzymatic and nonenzymatic oxidative changes. A number of studies contribute the bleaching of chlorophylls to the enzyme activities of lipase and lipoxygenase. Regreening of processed vegetable has been observed and attributed to the formation of metallochlorophyll complexes involving copper or zinc. A number of attempts have been made to preserve chlorophyll during green vegetable preservation, but none has been fully successful. Since acidic conditions favor pheophytin formation, many studies have involved pH control. The improved color retention obtained at higher pHs is soon lost during storage. Other investigators have attempted to minimize heat exposure by applying high-temperature short-time processing or a combination of HTST processing and pH control. Some improvement in color retention has been achieved by producing the more heat stable phytol-free pigments. However, results were inconsistent and were influenced by biological variability attributable to differences in growing conditions.

Anthyocyanins can undergo color changes during heating especially if the pH is altered or tin is present. Anthocyanin losses also occur enzymatically. Enzyme systems involved include glucosidases (anthocyanases), peroxidases and phenolases. Since these enzyme systems are present in many fruits and vegetables, inactivation is essential. Colorless proanthocyanidins with metal ions can result in pink discoloration of fruits, such as pear. Also nonenzymatic or Maillard browning may contribute to discoloration of fruits and vegetables during heat processing and subsequent storage when conditions are favorable for these reactions.

Flavor Change

Heating fruits and vegetables can cause dramatic changes in flavor. Most flavor changes can be attributed to the loss of certain compounds by volatilization or by formation of new substances via degradation or addition reactions. For example, the major carbonyl component of the fresh banana, 2-hexanal, is not detected in the heat processed puree. Volatile losses are also important to flavor change in other canned foods, including pear, cabbage, and onion. Volatile losses may also continue through chemical mechanisms during post-processing storage. For example, in plain tin cans this may occur at the tin/product interface by reduction of aldehydes and ketones to primary and secondary alcohols. New volatiles may also arise in canned foods. Formation of hydrogen sulfide and other volatile sulfur compounds in heated products, such as cabbage and corn, are examples of this kind. Methyl sulfide is generated during heat processing of tomatoes, and off-flavor in canned apple sauce has been attributed to the formation of caproic acid. Nonvolatile substances may also contribute to

flavor changes in heated foods. Off-flavors in some canned fruits and vegetables are attributed to the formation of ammonium pyrrolidonecarboxylate from the cyclization of glutamine. Moreover, failure to completely inactivate enzymes may also result in flavor changes during processing and subsequent storage. In some instances, enzymes are naturally heat resistant (peroxidase), and in others the enzyme acquires heat resistance because of the manner in which it is physically situated in the tissues. Examples of the latter are β-glucosidase in plum kernels and lipoxygenase in the cob of sweet corn. Peroxidase, which can catalyze production of off-flavors in vegetables, can partially "regenerate" of "reactivate" following HTST thermal processing.

Texture Change

As discussed previously, texture of fruits and vegetables is likely to soften as a result of heat treatment. This is due to various factors including the loss of turgor pressure, loss of extracellular and vascular air, and the denaturation or degradation of cell wall constituents and other polysaccharides. Failure to inactivate pectin-hydrolyzing enzymes early in the process can result in undesirable texture changes in certain products, such as canned tomatoes. Starch granules gelatinize during heating in an aqueous environment, and this reaction undoubtedly influences the texture of certain commodities. The deterioration of texture can be minimized by choosing less mature produce, lessening the severity of thermal processes, choosing cultivars that are less susceptible to softening, and by adding acids, pectic enzyme inhibitors, or calcium salts to the canning medium. Fruits heated in sucrose syrups tend to be firmer than those heated in the absence of this solute. Sucrose firms the texture of canned fruit by osmotic dehydration of the tissue, which in turn appears to facilitate cohesion of cell wall polysaccharides. While severe heat processing tends to soften plant tissues through the action of heat on pectic substances, mild heat treatments can be used to activate endogenous pectin methylesterase. This will partially demethylate pectic substances allowing internal calcium to effect crosslinking of pectic polymers with a resulting increase in tissue firmness.

Nutrient Loss

The destruction of nutrients during the thermal process is dependent on time/temperature treatment used as the basis of the process, rate of heat transfer into the product, and thermal stability of nutrients and their solubility in water (Tables 11.13 and 11.14). Increasing the rate of heat transfer into the product has been one of the primary research in commercial development. Leaching losses of water soluble vitamins and minerals can be substantial if plant products are exposed to hot water. Therefore, optimizing nutrient retention in commercial processing has been studied extensively. The use of high-temperature short-time (HTST) processes is particularly adaptable to aseptic processing to retain high percent of vitamins (Table 11.15). In the processing of fruits and vegetables, the HTST processes are most likely to retain more of the water soluble nutrients and to minimize the loss of heat labile vitamins such as C. The blanching of vegetables prior to canning or dehydration is important if losses of vitamin C and vitamin A through enzymatic activity are to be avoided.

Table 11.13 Losses (%) of nutrients in the canning process

	Vitamin					
Product	A	B_1	B_2	Niacin	B_6	C
Asparagus	43.3	66.7	55.0	46.6	64.0	54.5
Green beans	51.7	62.5	63.6	40.0	50.0	78.9
Carrots	9.1	66.7	60.0	33.3	80.0	75.0
Spinach	32.1	80.0	50.0	50.0	75.0	72.5

Harris and Karmas (1975)

Table 11.14 Retention of ascorbic acid in peas at various stages of processing and after cooking

	Fresh	Frozen	Canned	Air-dried
	Retention of ascorbic acid (%)			
Blanching	–	75	70	75
Processing	–	75	63	45
Thawing	–	71	–	–
Cooking	44	39	36	25

Harris and von Loesecke (1960)

Table 11.15 Effect of HTST processing on nutrient losses

Product	Thiamin loss (%)		Pyridoxin loss (%)	
	HTST	Conv.	HTST	Conv.
Strained lime beans	15.8	40.3	9.5	10.1
Tomato juice concentrate	0	2.8	0	0

Harris and Karmas (1975)

Table 11.16 Changes in concentration of β-carotene in cooked dehydrated carrots

Carrot	β-Carotene content (μg/g solids)
Fresh	980–1860
Explosive puff-dried	805–1060
Freeze-dried	870–1125
Conventional air-dried	636–987

Dellamonica and McDowell (1965)

One of the two most important vitamins in our diet derived from in fruits and vegetables is a group of provitamin A carotenoids and they present in the stable *trans*-configuration. However, processing and storage under acidic conditions, elevated temperatures, and/or light would favor isomerization and isomers are far less in biological activity. Therefore, extended holding at elevated temperatures or heat sterilization can have some effect on the total carotenoid content and vitamin A value. Changes in the b-carotene content of cooked dehydrated carrots (Table 11.16) illustrate typical extents of degradation during processing and typical exposure to oxygen. Other losses of carotenoids can occur because of enzymatic and nonenzymatic oxidation. Losses have been reduced in blanched, compared to unblanched tissue, suggesting enzymatic destruction. Destruction of carotenoids has been associated with lipoxygenase activity during the direct oxidation of fatty acids. Nonenzymatic oxidation of carotenoids with concurrent color loss is of major concern in dehydrated fruit and vegetable products. The relative stability of carotenoids against oxidation when present in water-containing foods suggests a protective effect of water, and therefore, water activity is related to carotenoid stability in dehydrated products.

Dehydration of Fruits and Vegetables

Commercial dehydration is carried out by using conveyor or tunnel dryer, spray or drum dryer, fluidized bed dryer, or freeze-dryer depending on the state of the commodity to a residual moisture content of 4–8%. Temperature of drying chambers varies depending on the dryers and commodities. Solar drying is still a common process in southern and tropical countries for dates, fig or raisins. Dehydration reduces the natural water content of fruits and vegetables below the level of critical for the growth of microorganisms (12–15%) without being detrimental to important nutrients, flavor and appearance. Freeze-drying is known to provide high quality products with good shape retention and porous structure that is readily rehydrated.

Since the rate of a chemical reaction in a food is a function of many factors, mainly reactant concentration, temperature, pH, oxidation-reduction potential and inhibitors and catalysts along with migration of solutes, the dehydration process is usually accompanied by significant changes. The concentration of important components such as protein and carbohydrates leads to accelerate their chemical reactions, like Maillard browning, resulting in a darker color and development of new flavor compounds. Oxidative reactions degrade proteins, lipids, vitamins, and original aroma compounds. Sulfite treatment is used to prevent both enzymatic and nonenzymatic browning reactions, stabilizes vitamin C and prevents microbial contamination during processing and storage. Dehydrated fruits and vegetables are light, air and moisture sensitive and therefore require careful packaging.

Canning of Fruits and Vegetables

Stone fruits, pears, pineapple are major canned fruits and strawberries are canned to a lesser extent. First, all fruits are sorted and washed. Apples and pears are peeled and sliced, cherries are freed from seeds and stems, plums, prunes, apricots and peaches are halved and the seeds are removed, strawberry calix is removed and red current stems are removed. Specialized equipment has been developed for these procedures. In order to prevent discoloration during processing, ascorbic acid is added. To enhance aroma and taste, sugar and citric acid solutions are added and the can is vacuum sealed at 77–95 °C for 4–6 min and heat sterilized under the required conditions for each commodity.

Blanching is the most common operation in the canning process of vegetables. It serves not only to inactivate the enzymes, but to remove the air present in plant tissue and to induce shrinkage or softening of the product (leafy vegetables, such as spinach). Brine (1–3% NaCl solution) often serves as filling liquid with citric acid (up to 0.05%), calcium slats for firming the plant tissue. Sterilization is carried out in rotation retorts and the time and temperature vary depending on the commodity, size of cans, and the state of the product. After the required sterilization, the cans are quickly cooled. In order to retain better quality, high temperatures and shorter times (HTST) sterilization process is commonly used today. As indicated before, the losses in sensory and nutritional qualities are expected. Nutrient losses in fruits and vegetables during canning vary greatly (from 0 to 91%) depending on the nutrient and product. Minimizing losses of sensory and nutritional qualities due to canning process have been the major tasks for food scientists for the last 6–70 years.

Freezing of Fruits and Vegetables

Fruits and vegetables are often frozen and stored as an end product or for further processing. Among fruits, apples, apricots, cherries, pineapples and strawberries are commonly frozen. Properly sized fruits pieces are rapidly chilled (below −30 °C) for 2–3 h to avoid migration of soluble solids, formation of large ice crystals and microbial growth. Some fruits are covered, prior to freezing, with a 30–50% sugar solution (and citric acid and ascorbic acid) to improve aroma, texture, and color of the fruits. Frozen fruit is stored at −18–25 °C which has shelf life of 2–3 years. Asparagus, beans, peas, Brussels sprout, broccoli, carrots, cauliflower, spinach, sweet corns, and potatoes are very suitable for freezing. They are blanched (boiling water or steam) for 2–5 min as in the case of canning. Immediately after blanching, the vegetables are cooled and frozen at −40 °C. Freezing is usually carried out using conventional freezing method in plate or air freezers then stored at −18–20 °C. Major losses of sensory and nutritional qualities of frozen fruits and vegetables loss sensory and nutritional qualities are usually taking place at the primary processing steps of washing and blanching, but they are preserved during freezing and thawing. However, freezing method, among different processing methods, is the most favorable method in retaining the original quality of fresh fruits and vegetables.

Lactic Acid Fermentation

Pickled vegetables by the lactic acid fermentation have a long history as one of the oldest food preservation methods. Cucumber, cabbage, green beans and others, are produced by spontaneous lactic acid fermentation at lower pH that inhibits the growth undesirable microorgnisms and stabilizes some nutrients and sensory qualities. Today, sauerkraut and kimichi manufactured by fermented cabbages with probiotic lactic acid bacteria are considered as probiotic foods. Vegetables are treated with 2.5–3% salt at about 20 °C for lactic acid fermentation. For sauerkraut, white cabbage heads are shredded, mixed with salt (at 1.8–2.5%), and packed in layers in barrels or large tanks. The lactic acid fermentation initiated by starter cultures at 18–24 °C and lasts for 3–6 weeks. During the first 48 h of anaerobic fermentation, the pH falls from around 6.2 to 3.7–4.2. The acid formed promotes the growth of

Leuconostoc mesenteroids and inhibits the growth of competing microorganisms. After fermentation is complete, the sauerkraut pH is about 3.6. The sauerkraut is usually packed in plastic bags, glass jar, or canned. The cans are filled at 70 °C and exhausted, sealed and sterilized at 95–100 °C. Vitamin C content of sauerkraut ranges 10–38 mg/100 g with titratable acid of 0.28–0.42% as acetic acid.

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Herbs and Spices

12

Zhuohong Xie and John W. Finley

Herbs and spices are important food ingredients. The human consumption of herbs and spices can date back to 5000 BC. The world production of spices is estimated to be 8,730,271 tons in 2013 (FAOSTAT). The major producer countries are India, China, Thailand and USA. Table 12.1 summarizes some of the more common spices, the portion of the plant and their region of cultivation. Herbs and spices are consumed as is or formulated into various food, beverage and dietary supplement products. Due to their characteristic chemical compounds, herbs and spices are used to flavor foods and beverages, to inhibit microbial growth and preserve food quality. Increasing evidence also suggest consumption of certain herbs and spices bring in potential health benefits. Although the definitions sometimes overlap, generally herbs are plant leaves or flowering parts either fresh or dried and spices are small pieces from roots, bark or seeds of plants. Most spices also contain essential oils which are normally recovered by steam distillation.

The flavor contribution of spices and herbs comes primarily from the aroma of the volatile compounds in the product. Most spices are fairly complex mixtures of compounds that define the distinctive character of the product. In some cases one compound may dominate the aroma profile for example cloves, cinnamon, anise and thyme.

The aroma compounds in herbs and spices are either phenolics or terpene based compounds. Table 12.2 summarizes the terpene and phenolic compounds which are basic to the aromas. Terpenes represent a very large family of molecules which are frequently utilized for defense by the plants. Terpenes are the sources of typical aromas from bark and needles of conifers, citrus products and flowers. The aromas have been described as pine-like, citrus, fresh, and floral. These compounds contribute to the overall flavor of a broad range of herbs and spices. They are frequently associated with fresh flavors. They tend to be volatile so they can be lost during cooking. The phenolic compounds tend to be more dominant in the flavor of the herb or spice. Examples of phenolics contributing dominant flavors are vanillin in vanilla, cinnamaldehyde in cinnamon and Eugenol in cloves.

Figure 12.1 illustrates some typical structures of phenolics and terpenes found in spices and herbs. It can be seen that the phenolic compounds contain a benzene ring appended with at least one hydroxyl group. Terpenes all contain a block or at least five carbons in a zigzag formation. Both phenolic compounds and terpenes include a wide range of compounds with many aroma characteristics.

Spices contain volatile oils, usually referred to as essential oils, which are isolated by steam distillation. The primary constituents in essential oils are mono- and sesquiterpenes, phenolics or phenol ethers (Fig. 12.2).

The volatile components in spices are frequently complex mixtures, however in some

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_12

Table 12.1 Common spices and region of origin

Common name	Latin name	Region of cultivation
Fruits		
Black Pepper	*Piper nigrum*	Tropical and sub-tropical regions
Vanilla	*Vanilla plantifolia, Vanilla fragans, Vanillathaitensis, Vanilla pompon*	Madigascar, Comore Island, Mexico, Uganda, Indonesia
Allspice	*Pimenta diocia*	Central America, Caribbean Islands
Paprika (Bell Pepper) Chili (Tobasco)	*Capsicum annuum* *Capsicum frutescens*	Widely distributed throughout the world
Bay tree fruits and leaves	*Crocus spinosa Laurus nobilis*	Mediterranean region
Juniper berries	*Juniperus communis*	Temperate climate
Aniseed	*Pimpinella anisum*	Mediterranean and southwest/Asia
Caraway	*Carum carvi*	Temperate climate
Coriander	*Coriandrum sativum*	Southern Europe and northern Africa to southwestern Asia
Dill	*Anethum graveolens*	Tolerates temperatures slightly below freezing
Seeds		
Fenugreek	*Trigonfoenum greacum*	Temperate and Mediterranean
Mustard White Black	*Sinapsis alba* *Brassica nigra*	Temperate regions
Nutmeg	*Myristica fragrans*	India, Indonesia, Siri Lanka
Cardamom	*Elettaria cardamomum*	India, Siri Lanks
Flowers		
Cloves	*Syzygium aromatiicum*	
Saffron	*Crocus sativus*	Mediterranean, India, Australia
Caper	*Capparis spinosa*	Mediterranean
Rhizomes		
Ginger	*Zingeiber Officianale*	South China, India, Japan. Caribbean, Africa
Turmeric	*Curcuma longa*	India, China, Indonesia
Bark		
Cinnamon	*Cinnamomum zeylanicum, C. aromaticum, C. burmanti*	China, Siri Lanka, Indonesia, Caribbean
Roots		
Horseradish	*Armoracia rusticana*	Temperate regions
Leaves		
Basil	*Ocimum basilcum*	Mediterranean, India
Parsley	*Petroselinum crispum*	Temperate regions
Savory	*Satureia hortensis*	Temperate regions
Tarragon	*Artemisia dracunculus*	Temperate regions
Marjorum	*Origanum majorana*	Mediterranean, temperate regions
Oregano	*Origanum heracleoticum, O. onites*	Temperate regions
Rosemary	*Rosmarinus officialis*	Mediterranean
Sage	*Salvia officialis*	Mediterranean
Chives	*Allium schoenoprasum*	Temperate regions
Thyme	*Thymus vulgaris*	Temperate regions

Table 12.2 Aromas contributed by phenolic and terpenes

Chemical compounds	Aroma descriptor
Phenolics	
Eugenol	Clove
Cinnamaldehyde	Cinnamon
Anethole	Anise
Vanillin	Vanilla
Thymol	Thyme
Cavacrol	Oregano
Estragole	Caraway
Terpenes	
Pinenes	Pine needles and bark
Limonene, terpinene, citral	Citrus fruits
Geraniol	Roses
Linalool	Lily of the valley
Cineole	Eucalyptus
Menthol and Menthone	Peppermint
L-Carvone	Spearmint
D-Carvone	Caraway

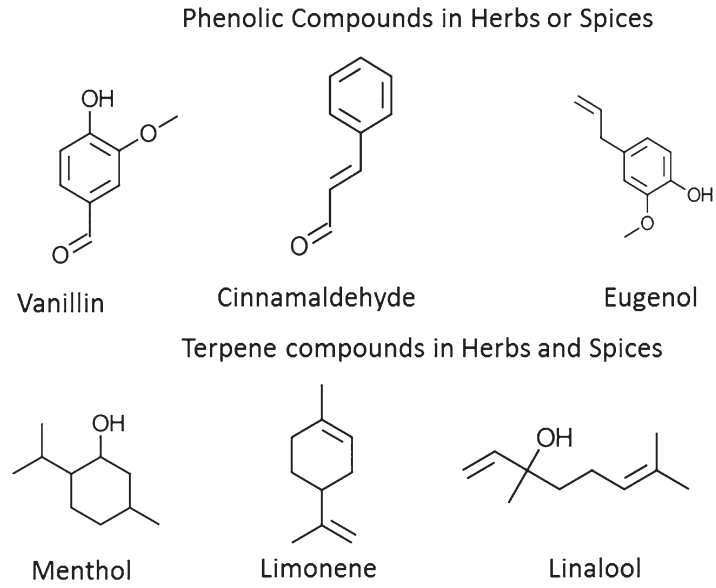


Fig. 12.1 Structures of phenolics and terpenes found in herbs and spices

cases the dominant volatiles define the aroma. For example eugenol in cloves, cinnamaldehyde in cinnamon and carvone in caraway. Table 12.3 summarizes the principal aroma compounds found in some of the major spices.

The aroma of many spices is the result of the major aroma producing compounds such as cinnamaldehyde in cinnamon, eugenol in cloves and (E)-anethole in caraway. Other spice aromas are much more complex.

The antimicrobial effects of herbs and spices are well recognized. Foodborne bacteria are sensitive to extracts from herbs and spices such as oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage, vanillin and mustard (Tajkarimi et al. 2010; Ceylan and Fung 2004). The essential oil fraction of herbs and spices are rich in antimicrobial compounds (Ceylan and Fung 2004). Phenols, alcohols, aldehydes, ketones, terpenes, ethers,

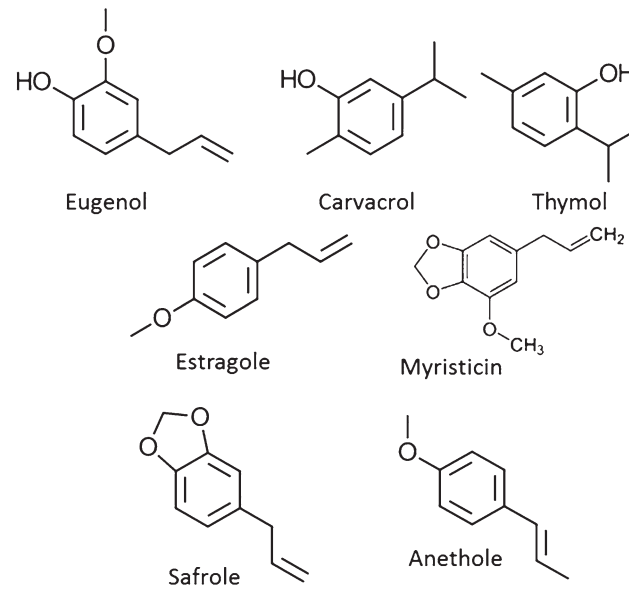


Fig. 12.2 Aromatic compounds found in essential oils

Table 12.3 Major aroma compounds found in spices and herbs

Spice/herb	Major aroma components
Black pepper	Germacrene d (11.01%), limonene (10.26%), β-pinene (10.02%), α-phellandrene (8.56%), β-caryophyllene, (7.29%), α-pinene (6.40%) and *cis*-β-ocimene (3.19%); *P. guineense* (black)—β-caryophyllene (57.59%), β-elemene (5.10%), bicyclogermacrene (5.05%) and α-humulene (4.86%)
White pepper	β-Caryophyllene (51.75%), *cis*-β-ocimene (6.61%), limonene (5.88%), β-pinene (4.56%), linalool (3.97%) and α-humulene (3.29%)
Vanilla	Vanillin, (r) (+)-*trans*—α-ionone, *p*-hydroxybenzylmethylether
Allspice	Eugenol, β-caryophyllene, methyleugenol, 1,8-cineole,α-phellandrene
Bay leaf	Cineole, α-pinene, β-pinene, α-phellandrene, linalool
Juniper berries	α-Pinene, myrcene, β-pinene, δ^3-carene, (e)-anethole
Aniseed	(e)-Anethole
Caraway	(s)(+)-Carvone, limonene
Coriander	(s)(+)-Linalool, (r)(−)-linalool, linalyl acetate, citral, 2-alkeales c_{10}–c_{14}
Dill (herb)	(s)-Carvone, (s)(+)-phellandrene, (3r,4s,8s)(+)-epoxy-p-menth-1-ene, myristican, limonene
Fenugreek	Linalool, 3-isobutyl-2-methoxypyrazine, 2-methoxy-3-isopropylpyrazine, 3-hydroxy-4,5-dimethyl-2(5H)-furanone
Nutmeg	α-Pinene, β-pinene, sabinene, limonene, safrole, myristicin, 1,8-cineole
Cardamom	1,8-Cineole, α-terpinyl acetate, limonene, sabinene
Clove	Eugenol, β-caryophyllene, eugenol acetate
Saffron	Safranal, 2,6,6-trimethyl-4-hydroxy-1-cyclohexene-1-formaldehyde
Ginger	(−)Zingiberene, β-bisabolene, (−)-sesquiphellandrone, (+)-ar-cucumene, citral, citronellyl acetate
Turmeric	Turmerone, ar-turmerone, zngiberene
Cinnamon	Cinnamaldehyde, eugenol, safrole, linaloor, camphor
Parsley	p-Mentha-1,3,8-triene, 2-sec-butyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, (z)-6-decanal, (e,e)-2,4-decadienal, myrcene
Marjoram	*cis*-Sabinehydrate, *trans*-sabaninehydrate, 1-terpinen-4-ol
Rosemary	1,8-Cineol, camphor, α-pinene, camphene
Oregano	Carvacrol, thymol
Sage	1,8-Cineol, camphor, α-pinene, thujone
Thyme	Thymol, p-cymene, carvacol, linalool

Adapted from Belitz et al. (2009)

Table 12.4 Major antimicrobial compounds found in spices and herbs

Spice/herb	Major antimicrobial compounds
Rosemary	Anethole, apigenin, ascorbic acid, borneol, bornyl acetate, caffeic acid, camphor, delta-3-carene, carveol, caryophyllene, chlorogenic acid, 1,8-cineole, p-cymene, genkwanin, geraniol, glycolic acid, limonene, linalool, methyl eugenol, niacin, pinene, rosmarinic acid, safrole, terpinene, thujone, ursolic acid
Cinnamon	Benzaldehyde, camphene, camphor, caryophyllene, 1,8-cineole, cinnamaldehyde, cuminaldehyde, p-cymene, eugenol, farnesol, furfural, guaiacol, limonene, linalool, methyl eugenol, methyl salicylate, myrcene, niacin, pinene, piperitone, safrole, terpineol
Thyme	Borneol, bornyl acetate, caffeic acid, camphene, delta-3-carene, beta-carotene, carvacrol, chlorogenic acid, 1,8-cineole, p-cymene, geraniaol, limonene, linalool, methionine, myrcene, niacin, pinene, rosmarinic acid, terpinen-4-ol, terpineol, thymol, tryptophan, ursolic acid
Oregano	Anethole, apigenin, borneol, cadinene, caffeic acid, camphor, delta-3-carene, beta-carotene, carvacrol, carvone, caryophyllene, 1,8-cineole, p-cymene, geraniol, kaempferol, limonene, linalool, luteoline, niacin, pinene, rosmarinic acid, terpinene, thymol, ursolic acid
Bay	Acetic acid, benzaldehyde, borneol, bornyl acetate, cadinene, delta-3-carene, camphene, caryophyllene, 1,8-cineole, costulonide, cubebin, p-cymene, eugenol, geraniol, limonene, linalool, methyl eugenol, myrcene, pinene, terpinen-4-ol, terpineol
Garlic	Ajoene, allicin, alliin, allistatin-I, allistatin-II, arginine, ascorbic acid, choline, citral, diallyl disulfide, diallyl disulfide, geraniaol, glutamic acid, linalool, niacin, trypthophan
Cloves	Anethole, benzaldehyde, carvone, caryophyllene, chavicol, cinnamaldehyde, ellagic acid, eugenol, eugenyl acetate, furfural, gallic acid, kaempferol, linalool, methyl eugenol
Allspice	Anethole, ascorbic acid, cadinene, delta-3-carene, beta-carotene, caryophyllene, copaene, p-cymene, eugenol, linalool, methyl eugenol, myrcene, pinene, selinene, terpinen-4-ol, terpinene
Sage	Apigenin, ascorbic acid, borneol, bornyl acetate, camphor, beta-carotene, caryophyllene, chlorogenic acid, 1,8-cineole, limonene, linalool, myrcene, niacin, pinene, rosmarinic acid, terpinen-4-ol, terpinene, thujone, ursolic acid
Vanilla	Acetaldehyde, acetic acid, anisaldehyde, benzaldehyde, benzoic acid, cresol, eugenol, furfural, guaiacol, vanillic acid, vanillin, vanillyl alcohol

Adapted from Duke (1994)

hydrocarbons, alkaloids and peptides in herbs and spices are the effective antimicrobial components (Ciocan and Bara 2007; Ceylan and Fung 2004). The antimicrobial compounds in common herbs and spices are summarized in Table 12.4.

Oxidation is one of the reasons for deterioration of food quality. Free radicals form chain reactions in the food matrix, causing rancidity and unfavorably change in color and flavor. The chained reaction can be stopped by radical scavenging agents, also known as antioxidants. Strong antioxidants, including certain vitamins, phenols (flavonoids), terpenoids, carotenoids, phytoestrogens, minerals, etc. are found in many herbs and spices (Suhaj 2006). For example, rosemary extract containing diterpenes and carnosic acid can be used as a stabilizer of fatty oil and antioxidant in sausages. The antioxidant compounds in common herbs and spices are listed in Table 12.5. It is noteworthy that the category of aroma compounds and antimicrobial compounds sometimes overlap, meaning that the same compound may have both properties.

Recent scientific data support that chemical compounds in herbs and spices may provide health benefits including antioxidant and anti-inflammatory effects, reducing risks of cardiovascular diseases and diabetes, improving the overall health, cognition, GI health etc. (Butt et al. 2013; Suhaj 2006; Gruenwald et al. 2010; Jamal et al. 2006; Singletary 2010). The health benefits will be elaborated in the following individual herb and spice section.

Table 12.5 Major antioxidant compounds found in spices and herbs

Spice/herb	Major antioxidant compounds
Black pepper	Ascorbic acid, beta-carotene, camphene, carvacrol, eugenol, gamma-terpinene, lauric acid, linalyl acetate, methyl eugenol, myrcene, myristic acid, myristicin, palmitic acid, piperine, terpinen-4-ol, ubiquinone
Caraway	Beta-carotene, camphene, camphene, carvacrol, gamma-terpinene, gamma-terpinene, lauric acid, myrcene, myrcene, myristic acid, myristicin, myristicin, palmitic acid, quercetin, tannin, terpinen-4-ol
Chili pepper	Alanine, ascorbic acid, beta carotene, caffeic acid, campesterol, capsaicin, capsanthin, chlorogenic acid, hesperidin, histidine, kaempferol, lauric acid, lutein, methionine, myrcene, myristic acid, myristic acid, p-coumaric acid, palmitic acid, pentadecanoic acid, quercetin, scopoletin, stigmasterol, terpinen-4-ol, tocopherol, tryptophan
Coriander	Apigenin, ascorbic acid, beta-carotene, beta-carotene, beta-sitosterol, caffeic acid, camphene, chlorogenic acid, gamma-terpinene, isoquercitrin, myrcene, myristic acid, myristicin, p-hydroxybenzoic acid, p-hydroxybenzoic acid, palmitic acid, protocatechuic acid, protocatechuic acid, quercetin, rhamnetin, rutin, scopoletin, tannin, terpinen-4-ol, *trans*-anethole, vanillic acid, vanillic acid
Dill	Alpha-tocopherol, anethole, ascorbic acid, beta-sitosterol, caffeic acid, camphene, carvacrol, chlorogenic acid, eugenol, ferulic acid, gamma-terpinene, histidine, isoeugenol, isorhamnetin, kaempferol, lauric acid, methionine, myrcene, myristic acid, myristicin, palmitic acid, quercetin, scopoletin, selenium, stigmasterol, terpinen-4-ol, *trans*-anethole, vicenin
Ginger	6-Gingerol, 6-shogaol, alanine, ascorbic acid, beta-carotene, beta-sitosterol, caffeic acid, camphene, capsaicin, chlorogenic acid, curcumin, delphinidin, ferulic acid, gamma-terpinene, kaempferol, lauric acid, methionine, myrcene, myricetin, myristic acid, p-coumaric acid p-hydroxybenzoic acid, palmitic acid, quercetin, selenium, shikimic acid, sucrose, terpinen-4-ol, tryptophan, vanillic acid, vanillin
Nutmeg	Camphene, cyanidin, eugenol, gamma-terpinene, isoeugenol, kaempferol, lauric acid, methyl eugenol, myrcene, myristic acid, myristicin, myristicin, oleanolic acid, palmitic acid, quercetin, terpinen-4-ol
Oregano	camphene, carvacrol, gamma-terpinene, linalyl acetate, myrcene, terpinen-4-ol, thymol
Red (sweet) pepper	Alanine, alpha-tocopherol, ascorbic acid, beta-carotene, beta-sitosterol, caffeic acid, campesterol, camphene, capsaicin, capsanthin, chlorogenic acid, eugenol, gamma-terpinene, hesperidin, histidine, lupeol, lutein, methionine, myrcene, myristic acid, p-coumaric acid, palmitic acid, pentadecanoic acid, scopoletin, selenium, stigmasterol, terpinen-4-ol, tocopherol, tryptophan
Rosemary	apigenin, ascorbic acid, beta-carotene, beta-sitosterol, caffeic acid, camphene, camphene, camphene, carnosic acid, carnosol, carvacrol, chlorogenic acid, gamma-terpinene, hesperidin, hispidulin, isorosmanol, labiatic acid, luteolin, luteolin-3′-o-(3″-o-acetyl)-beta-D-glucuronide, luteolin-3′-o-(4″-o-acetyl)-beta-D-glucuronide, methyl eugenol, myrcene, oleanolic acid, rosmadial, rosmanol, rosmaridiphenol, rosmarinic acid, rosmariquinone, squalene, tannin, terpinen-4-ol, thymol, *trans*-anethole, ursolic acid
Sage	Alanine, apigenin, ascorbic acid, beta-carotene, beta-sitosterol, caffeic acid, campesterol, camphene, carnosic acid, carnosol, carnosol, carnosolic acid, catechin, chlorogenic acid, cholesterol, chrysoeriol, ferulic acid, fumaric acid, gallic acid, gamma-terpinene, hispidulin, labiatic acid, linalyl acetate, luteolin, myrcene, oleanolic acid, p-coumaric acid, palmitic acid, rosmanol, rosmarinic acid, salicylic acid, selenium, stigmasterol, terpinen-4-ol, thymol essential oil, ursolic acid, uvaol, vanillic acid
Thyme	4-Terpineol, alanine, anethole essential oil, apigenin, ascorbic acid, beta-carotene, caffeic acid, camphene, carvacrol, chlorogenic acid, chrysoeriol, eriodictyol, eugenol, ferulic acid, gallic acid, gamma-terpinene, isochlorogenic acid, isoeugenol, isothymonin, kaempferol, labiatic acid, lauric acid, linalyl acetate, luteolin, methionine, myrcene, myristic acid, naringenin, oleanolic acid, p-coumaric acid, p-hydroxybenzoic acid, palmitic acid, rosmarinic acid, selenium, tannin, thymol, tryptophan, ursolic acid, vanillic acid
Turmeric	Ascorbic acid, beta-carotene, caffeic acid, curcumin, eugenol essential oil, p-coumaric acid, protocatechuic acid, syringic acid, vanillic acid

Adapted from Suhaj (2006)

Black Pepper

Both black and white pepper are widely consumed. Black pepper is harvested before it is fully ripe the flesh is removed and the seed is dried. White pepper, which has a milder flavor, is from the seed or ripe fruit. The major aromatic compound in black pepper is (−)-rotundone. The main differences between black and white pepper is the concentrations in the product. The losses of aroma compounds account for the instability of pepper after it is ground. Therefore, fresh ground pepper provides better flavor in most food applications.

Blackening of fresh pepper is an oxidation of (3,4-dihydroxy phenyl) ethanol glycoside by an o-diphenol oxidase (PPO) which is present in the fruit. Bandyopadhyay et al. (1990) reported during the conversion of green pepper to black pepper 75% of the total pheolic content is lost. Included is a complete loss of o-diphenol oxidase oxidizable phenolic compounds. The major substrate for o-diphenol oxidase is 3,4-dihydroxy-6-(*N*-ethylamino) benzamide. Early work established the presence of α-pinene, β-pinene, 1-β-phellandrene, dllimonene, piperonal, and dihydrocarveol.

The volatile oils in black pepper constitute 2–5% of the dry berries. Pepper oil is traditionally prepared by steam distillation of the black peppercorns. Liquid carbon dioxide is now emerging as a useful technology to prepare essential oils including pepper oil. Liquid carbon dioxide was described by Ferreira et al. (1999). In studies on black pepper berries from India and Malaysia, the presence of optically active monoterpenes, indicated (±)-linalool, (+)-α-phellandrene, (−)-limonene, myrcene, (−)-α-pinene, 3-methylbutanal and methylpropanal were the most important aroma compounds in black pepper. Additionally, 2-isopropyl-3-methoxypyrazine and 2,3-diethyl-5-methylpyrazine identified as causing a moldy or musty off aroma in a black pepper sample from Malaysia (Jagella and Grosch 1999a).

Piperine is the most abundant constituent of pepper oleoresin (Borges and Pino 1993). The pungency of black pepper *(P. nigrum* L.) was attributed to the presence of piperine, the structure is shown in Fig. 12.4. After removal of piperine form the pepper resin the oleoresin was named chavicine (Govindarajan 1977). Chavicine was thought to possess greater bite the tongue than crystalline piperine, however it was later shown that piperine in solution was very pungent. Later research demonstrated that piperine was the major pungent principle and chavicine is a mixture of piperine and several minor alkaloids. Five additional pungent alkaloids have been identified in pepper extracts. They are piperettine, piperylin, piperolein, A and B and piperanine (Zachariah et al. 2008).

Structures of some of the major components in black pepper oil are found in Figs. 12.3 and 12.4.

The primary and most important in pepper oleoresin is piperine. Black pepper oil contributes the aroma, oleoresin constitutes the components that complete the overall taste with the alkaloidpiperine imparts pungency. β-Carophyllene, is the most abundant sesquiterpene hydrocarbon present in pepper oil. Other sesquiterpene hydrocarbons are β-bisabolene, α- and β-cadinenes, calamenene, α-copaene, α and β-cubebenes, *ar*-curcumene, β- and δ-elemenes, β-farnesene, α-guaiene, α- and γ-humulenes, isocaryophyllene, γ-muurolene, α-santalene, α- and β-selinenes, ledene, sesquisabinene and zingiberene (Zachariah and Parthasarathy 2008).

Beyond contribution to flavor, piperine is also the major ingredient related to black pepper's potential health benefits. Piperine in black pepper may be responsible for antioxidant, antimicrobial, anti-inflammatory, gastro-protective, antidepressant and anti-cancer activities of the spice (Li 2006; Butt et al. 2013). Most of these, however, are results from in vitro and animal studies; thus, further clinical research is warranted to explore the efficacy and safety on the health promoting use of black pepper.

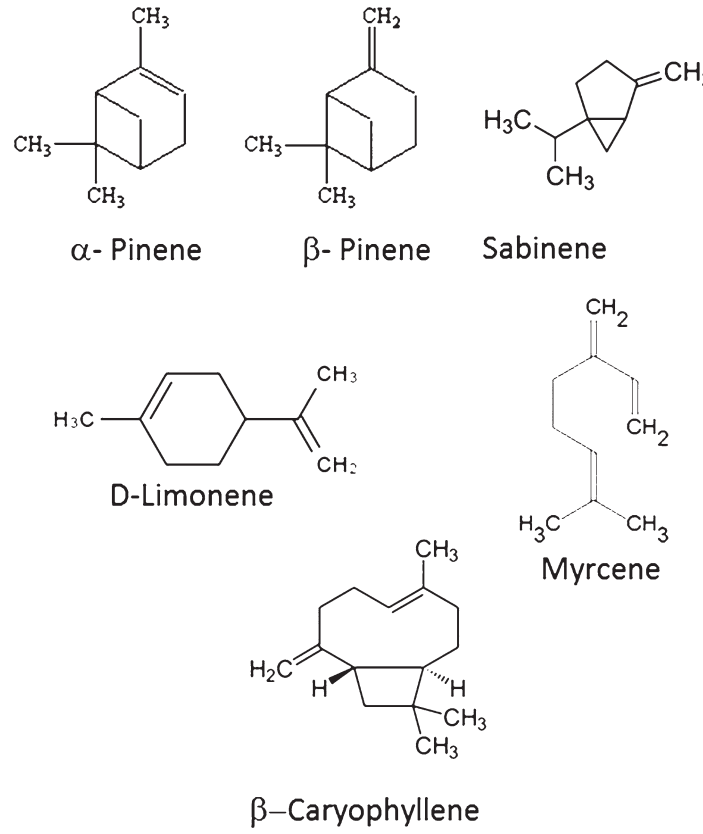


Fig. 12.3 Major aroma compounds in black pepper

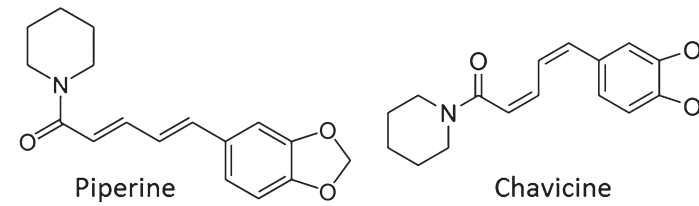


Fig. 12.4 Piperine and chavicine are the primary pungent compounds in black and white pepper

Vanilla

Like Cocoa, vanilla originated in Central America, where the Aztecs used it to flavor cocoa well before the discovery of the New World. The vanilla plant is a member of the orchidaceae family. The cultivated species of vanilla include *Vanilla fragans, Vanilla pompona* and *Vanilla tahitensis*. The vanilla bush is a tropical plant that requires a warm and humid climate. The temperature must be as even as possible. The major vanilla-producing countries are Madagascar, Indonesia, China, and Turkey. Madagascar which cultivates 64,000 ha, is the largest producer and some argue has the highest quality vanilla (Table 12.6). Vanilla is especially popular in ice creams, beverages, desserts, dairy products, chocolate, confectionery products and pastry.

Table 12.6 World country vanilla production

Country	Production (tons)	%
Madagascar	3719	48
Indonesia	2000	26
Papua New Guinea	510	6.6
Mexico	420	5.4
China	286	3.7
Turkey	280	3.6
Uganda	218	2.8
Tonga	186	2.4

2014 Top vanilla producers (Source: FAOSTAT 2017)

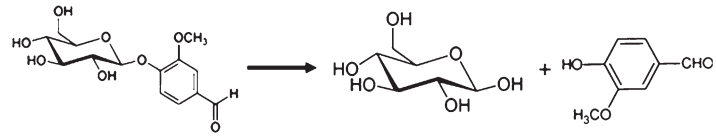


Fig. 12.5 Enzymatic hydrolysis of vanillin glucoside to produce vanillin

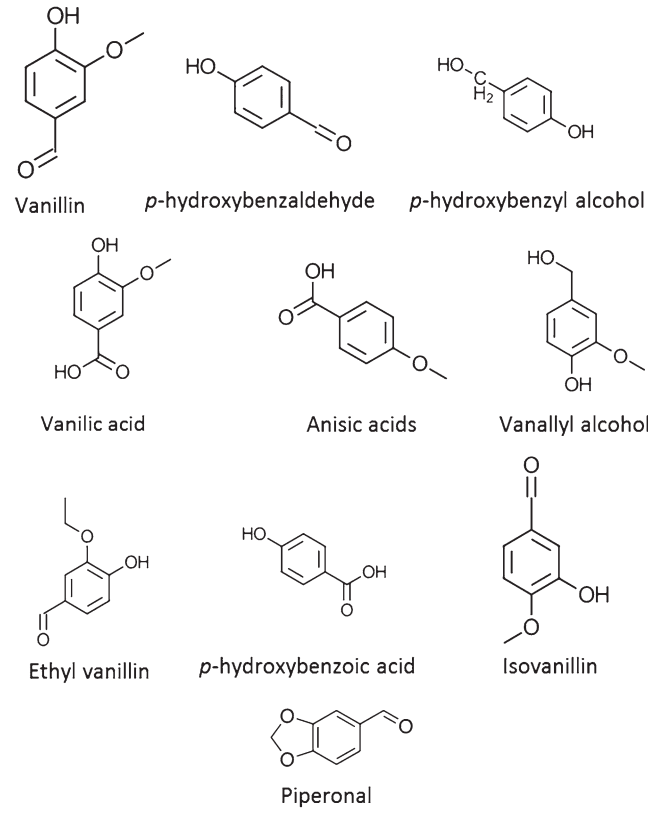


Fig. 12.6 Some major aroma compounds in vanilla

Vanilla is unique among spices because the processing is considerably more involved to produce the culinary form. Fresh vanilla pods have little or no taste. Vanillin which is the primary aroma/flavor constituent is bound as a glycoside which is enzymatically hydrolyzed to release the vanillin. The enzymatic reaction is induced by a sequence of blanching or steaming. During the curing process, the vanillin glycoside is hydrolyzed to yield vanillin and glucose and some other minor aromatic substances as shown in Fig. 12.5

Vanilla fruit grows quickly on the vine and matures for harvest in about 6 months. Vanilla fruits ripen at widely differing rates, thus daily harvest is required to ensure the optimum flavor from every fruit. Individual pods are hand-picked as it begins to split on the end. Overripe fruits are likely to split, causing a reduction in market value. The commercial value of vanilla beans is fixed based on the length and appearance of the pod.

Fruit is more than 15 cm (5.9 in) in length, it belongs to first-quality product. The largest fruits (greater than 16–21 cm) are reserved for the gourmet vanilla market. Fruits between 10 and 15 cm long, pods are under the second-quality category, and fruits less than 10 cm in length are under the third-quality category.

There are four basic steps in curing vanilla beans killing, sweating, slow-drying, and conditioning of the beans (Havkin-Frenkel et al. 2004, 2005).

In order to initiate the enzymatic hydrolysis of the vanillin glycosides the vegetative cells of the vanilla bean must be disrupted. This process called killing disrupts the cells and tissue of the fruits, imitating the enzymatic reactions responsible for releasing the aromatic compounds. Killing can be accomplished by heating in hot water, freezing, or scratching, heating in an oven or exposing the beans to direct sunlight. The different methods give different profiles of enzymatic activity allowing the glycosidase enzymes to act on the glucovanillin to release the free vanillin (Frenkel et al. 2010; Arana 1944).

Hot-water killing is accomplished by dipping the beans in hot water 63–65 °C (145–149 °F) for 3 min, or at 80 °C (176 °F) for 10 s. Fruits can be scratched lengthwise to disrupt the cells structure of the fruits (Arana 1944). Fruits can be frozen and thawed to initiate the release of enzyme and substrate however they must be thawed for the sweating stage. Frozen or quick-frozen fruits must be thawed again for the subsequent sweating stage. Fruits can also be tied bundles and rolled in blankets and heated in and oven at 60 °C for 36–48 h. The Aztecs exposed the fruits to sunlight until they turned dark brown to imitate the enzymatic reaction (Frenkel et al. 2010).

Sweating is a combination of hydrolytic and oxidative processes. Sweating is the process of holding the fruits, for 7–10 days at temperature of 45–65 °C with high humidity. At the end of the sweating process the fruits are brown and have developed the characteristic vanilla flavor and aroma. At the end of sweating they contain 60–70% moisture content by weight (Frenkel et al. 2010).

The drying process reduces the beans to 25–30% moisture which is sufficient to prevent rotting and stabilizes the aroma compounds in the pods. Traditionally the drying is accomplished by exposing the beans to intermittent shade and sunlight. Drying is the most difficult stage to control because it can result in uneven drying which can lead to loss is in vanillin (Frenkel et al. 2010).

Conditioning is accomplished by storing the pods for 5–6 months in closed boxes. During this stage the fragrance develops. The cured vanilla fruits contain an average of 2.5% vanillin.

Vanilla is one of the more complex aromas containing over 170 compounds. Vanillin is the primary aroma material thus "imitation" vanilla produced enzymatically is used in many food applications. Natural vanillin is produced from the enzymatic hydrolysis of a glycoside during fermentation of the fruit. Two other important contributors to the aroma of fresh vanilla are (R) (+)-*trans*-α-ionone and *p*-hydroxybenzylmethylether.

Other aroma compounds in vanilla have been reported including aromatic carbonyls, aromatic alcohols, aromatic acids, aromatic esters, phenols and phenol ethers, aliphatic alcohols, carbonyls, acids, esters and lactones. The levels of vanillin, *p*-hydroxybenzaldehyde and their respective acids (vanillic acid and *p*-hydroxybenzoic acid), are used as indicators of vanilla bean quality in commercial operations (Klimes and Lamparsky 1976; Adedeji et al. 1993; Ranadive 1994; Azeez 2008). The major aroma compounds in vanilla are shown in Fig. 12.6.

Chemical composition of processed vanilla Composition of processed vanilla beans variable and complex due to a number of variables such as species, growth conditions, soil composition, fruit maturity and mainly, the type of processing. All these variables define the relative content of the chemical constituents in the processed beans, which makes it difficult to define their typical composition. Perez-Silva et al. (2006) studied the volatile compounds in vanilla beans combining GC/MS analysis with GC-olifactory analysis to characterize the aroma contributors in vanilla beans. Table 12.7 summarizes the results of the study.

Vanillin can be synthesized by a number of methods and is frequently used as a replacement for the much more expensive natural vanilla. The 'classical' synthesis of vanillin from eugenol or isoeugenol was developed in 1896 and it remained the preferred method for about 50 years. Vanillin is now prepared industrially in large amounts by the Reimer–Tiemann reaction, starting with guaiacol, from which it is formed along with o-vanillin. A newer and more common means to produce vanillin is from the reaction of guracol and glyoxylic acid as shown in Fig. 12.7.

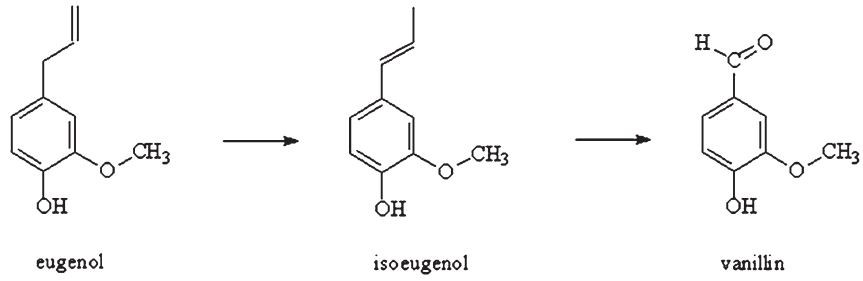
Table 12.7 Aroma compounds from vanilla beans and odor quality (from Perez-Silva et al. 2006)

Compounds	ppm	Odor quality	Intensity[a]
Phenols			
Guaiacol	9.3	Chemical, sweet spicy	+++
4-Methylguaiacol	3.8	Sweet, woody	+++
p-Cresol	2.6	Balsamic, woody, spicy	++
4-Vinylguaiacol	1.2	Chemical, phenolic	+
4-Vinylphenol	1.8	Sweet, woody	++
Vanillin	19,118	Vanilla, sweet	+++
Acetovanillone	13.7	Vanilla, sweet, honey	+++
Vanillyl alcohol	83.8	Vanilla like	+++
p-Hydroxybenzaldehyde	873	Vanilla like, biscuit	++
p-Hydroxybenzyl alcohol	65.1	Vanilla like, sweet	++
Aliphatic acids			
Acetic acid	124	Sour, vinegar	++
Isobutyric acid	1.7	Buttery	++
Butyric acid	<1	Buttery, oily	+
Isovaleric acid	3.8	Buttery, oily	++
Valeric acid	1.5	Cheese	+++
Alcohols			
2,3-Buranediol (Isomer 2)	8.0	Floral, oily	+
Anisyl alcohol	2.4	Herbal	++
Aldehydes			
2-Heptenal	2.1	Green, oily	+
E-2-decenal	1.8	Herblike, floral	++
(E,Z)-2,4-decadienol	1.4	Herb-like, fresh	++
(E,E)-2,4-decadienol	1.2	Fatty, wood	++
Esters			
Methyl salicylate	<1	Chalk	+++
Methyl cinnamate	1.1	Sweet	++
Ethyl linoleate	13.5	Sweet	++
Ketone			
3-Hydroxy-2-butanone	14.6	Buttery	+
Unknown[b]	6.2	Vanilla-like, chemical	+++

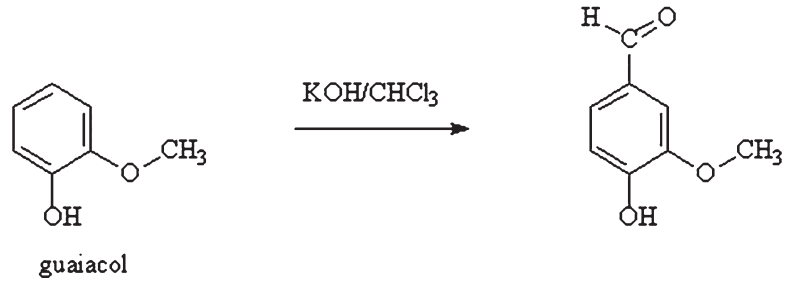
Aroma-active compounds detected by GC-O analysis of representative aroma extract from vanilla beans
[a](+) Weak, (++) medium, (+++) strong
[b]Mass fragmentation 91(90), 74(37), 69(34),89(25), 57(24), and RI 2528

Vanillin is also recovered as a by-product of paper pulp manufacture. Synthetic vanillin is used in both food and non-food applications, in fragrances and as a flavoring in pharmaceutical preparations.

In culinary applications vanilla flavoring in food can be achieved by adding vanilla extract or by adding vanilla beans to a liquid preparation. Natural vanilla gives a brown or yellow color to preparations, depending on the concentration. Good-quality vanilla has a strong aromatic flavor, but food with small amounts of low-quality vanilla or artificial vanilla-like flavorings are far more common.

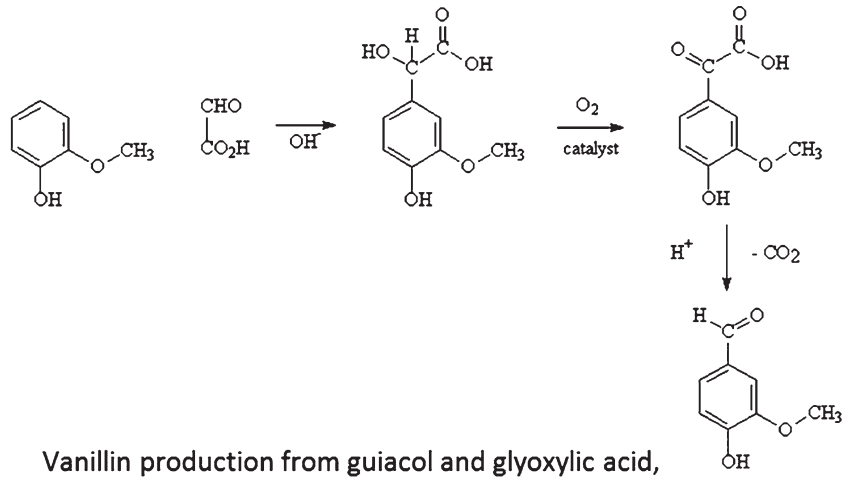


Classical Vanillin Synthesis from eugenol.



guaiacol

Vanillin production from guaicol by the Reimer-Tiemann Reaction



Vanillin production from guiacol and glyoxylic acid,

Fig. 12.7 Chemical approaches for the production of vanillin

Cardamom

Cardamom is a spice used in South and Southeast Asia, Middle East and Nordic countries. It is made from seeds of two plants (*Elettaria cardamomum* and *Amomum subulatum*). The major production countries are Guatemala, India and Sri Lanka. Cardamom is considered one of the most expensive spices after saffron and vanilla. The volatile oil components of cardamom first reported in detail by Nigam et al. (1965) and further summarized by Guenther (1975). The oil predominantly composed of oxygenated compounds, all which contribute to the aroma. Many of the alcohols, esters and aldehydes are found in many spice oils. The predominant volatiles in cardamom are 1,8-cineole and the esters,

Table 12.8 Main volatile components in cardamom oil

Component	Total oil (%)
α-Pinene	1.5
β-Pinene	0.2
Sabinene	2.8
Myrcene	1.6
α-Phellandrene	0.2
Limonene	11.6
1,8-Cineole	36.3
γ-Terpinene	0.7
p-Cymene	0.1
Terpinolene	0.5
Linalool	3.0
Linalyl acetate	2.5
Terpinen-4-ol	0.9
α-Terpineol	2.6
α-Terninyl acetate	31.3
Citronellol	0.3
Nerol	0.5
Geraniol	0.5
Methyl eugenol	0.2
Trans-Nerolidol	2.7

Source: Lawrence (1978); Govindarajan et al. (1982)

α-terpinyl and linalyl acetates (Lewis et al. 1966; Salzer 1975; Korikanthimath et al. 2002). The major components in cardamom oil are listed in Table 12.8. Structures of the main components are found in Fig. 12.8. Preliminary studies have found consumption of cardamom may have antioxidant (Kikuzaki et al. 2001), gut stimulatory and inhibitory, gastroprotective (Jamal et al. 2006), diuretic and sedative effects (Gilani et al. 2008), as well as alleviation of diarrhea, constipation, and high blood pressure (Gilani et al. 2008).

Ginger

Ginger is the rhizome of *Zingiber officinale* Roscoe and is one of the most widely used spices in a wide range of condiment for various foods and beverages. *Z. officinale* Roscoe, which is used for commercial ginger, and is grown extensively in many tropical countries. Like pepper (Piper nigrum) and the fruits of the Capsicum species, ginger is characterized by two classes of constituents: the odor (steam volatile) and non-volatile components responsible for the pungency.

Ginger oleoresin can be prepared from dried ginger by extraction using a variety of organic solvents. The oleoresin contains the major organoleptically important volatile oil and pungent principles. In addition the oleoresin includes triglycerides and a small amount of free fatty acids. Preparation and storage of the dried spice and oleoresin influences the organoleptic properties of these products. During storage of dried spice and particularly ground spice significant amounts of the volatile components in the oil are lost to evaporation. The oleoresin is prone to loss of pungency during as a result of degradation of the pungent gingerols. Heating ginger or the oleoresin from ginger results in the loss of both volatile and pungent factors.

The fatty oil of ginger ranges from 2 to 12% in dried gingers. Ginger fatty oil contain saturated and unsaturated fatty acids in a ratio of 46:53. The major fatty acids in ginger lipids are palmitic, oleic and linoleic (Zachariah 2008). The total lipid content varies widely (5.8–15%) among ginger varieties (Govindarajan 1982). The typical fatty acid profile of ginger lipid is shown in Table 12.9.

The composition of ginger oleoresin has a wide range depending on the variety and extraction conditions. Most commercial dried gingers contain 3.5–10% oleoresins and 15–30% volatile oils (Govindarajan 1982).

The non-volatile portion referred to as gingerols contain a 1-(4′-hydroxy-3′-methoxyphenyl)-5-hydroxyzlkan-3-one structure. The non-volatile pungent constituents in ginger include gingerols, shogaols, zingerone and paradols. All of these groups of compounds except zingerone contain hydrocarbon chains of 4–8 -CH_2- groups. The pungent non-volatile components in ginger are illustrated in Fig. 12.9.

The volatile composition of ginger oil is very complex. Miyazawa and Kameoka (1988) identified 72 components in the volatile oil extracted from the air-dried rhizomes.

The main components were zingiberene (21.8%), geranial (9.9%), geraniol (9.4%), β-bisabolene (7.9%), nerol (7.1%), 1,8-cineol

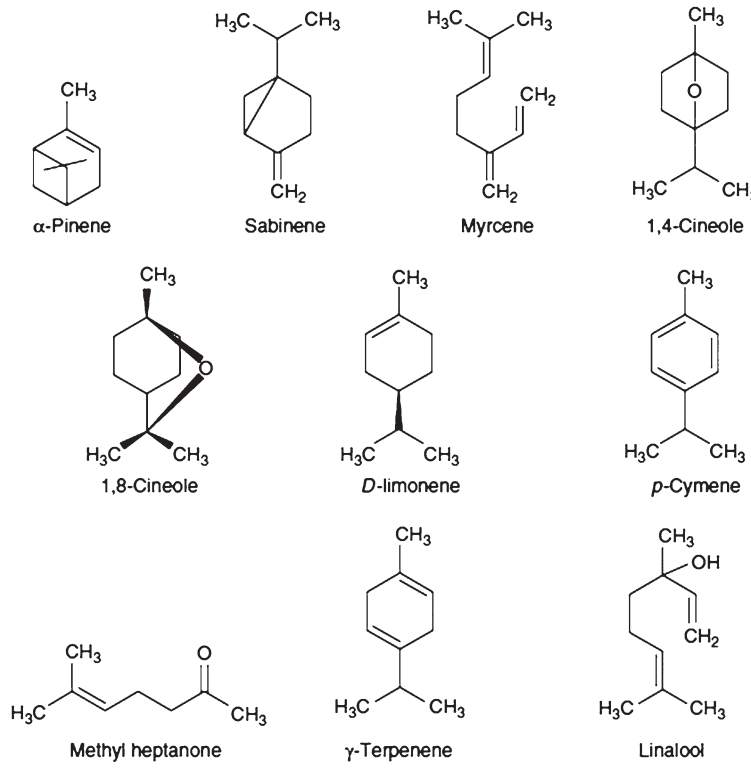


Fig. 12.8 Major components of cardamon oil (Chempakam and Sindhu 2008)

Table 12.9 Fatty acid composition of ginger lipids

Fatty acid	Fatty acid (%)
Caprylic acid	1.4
Capric acid	4.1
Lauric acid	7.6
Myristic acid	3.5
Pentadecanoic acid	0.4
Palmitic acid	23.2
Heptadecanoic acid	1.3
Stearic acid	3.3
Oleic acid	22.9
Linoleic acid	23.2
Linolenic acid	6.6
Arachidic acid	1.1

(6.2%), terpineol (5.6%), borneol (5.4%), phellandrene (3.1%), linalool (1.7%), methyl nonyl ketone (1.6%) and camphene (1.4%); the other components accounted for ~1% each of the volatile oil. The structures of the primary volatile compounds in ginger are shown in Fig. 12.10.

The impact of composition of ginger on ginger oil quality was summarized by Govindarajan (1982). It was proposed that Citral and citronellyl acetate were the most important attributes of aroma. Freshly prepared oil is rich in Zingiberene and β-sesquiphellandrene, and *ar*-Curcumene, increases during storage, and can be used as an indicator of aoil age and processing conditions. The ratio of zingiberene and β-sesquiphellandrene to *ar*-curcumene = 2:3 as a stable marker for identifying pure ginger oil. The lemony note from the citrals combined with the α-terpineol, β-sesquiphellandrene and *ar*-curcumene combine to form the basis of the characteristic ginger flavor.

Nerolidol contributes to the woody note; and *cis*- and *trans*-β-sesquiphellandrol were also contributors to the ginger flavor (Govindarajan 1982). Sesquiterpenes, particularly zingiberene, was identified as a major characteristic of ginger.

Ginger has been used as folk medicine since ancient time. With help from recent advances in analytical chemistry and nutrition, the bioactive compounds in ginger including gingerols,

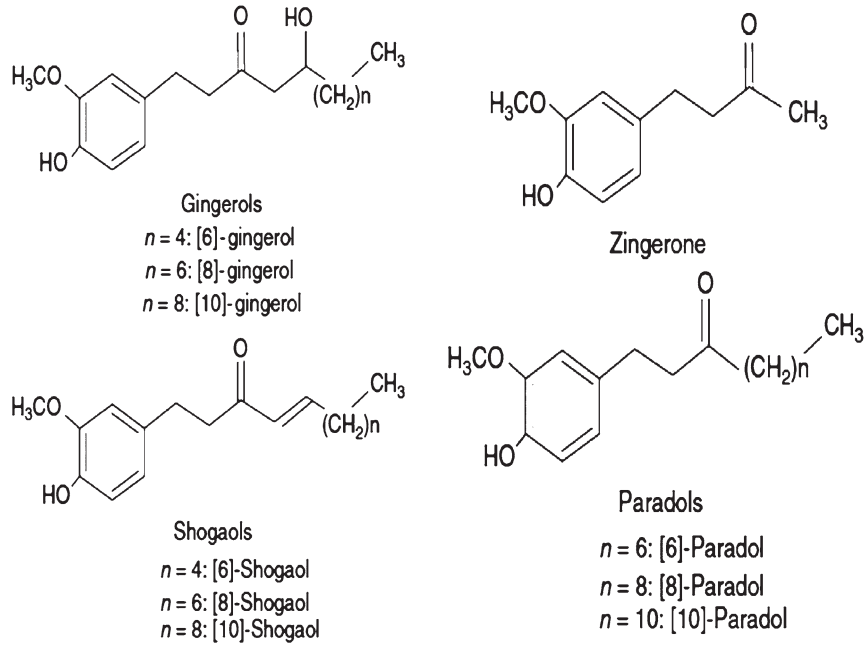
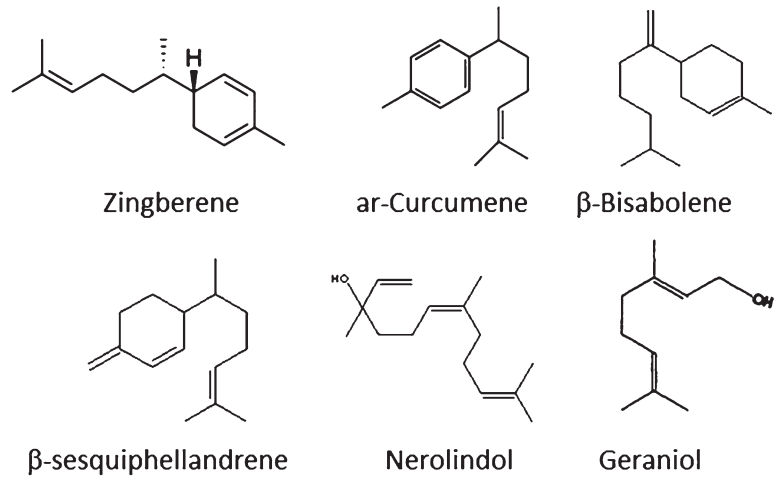


Fig. 12.9 Non volatile components of ginger oil

Fig. 12.10 Primary volatile compounds in ginger



β-carotene, capsaicin, caffeic acid, curcumin and salicylate have been identified (Tapsell et al. 2006). In vitro and animal studies suggest ginger has antioxidant, anti-inflammatory, and anti-platelet properties, as well as lipid and blood pressure lowering activities (Singletary 2010). In addition, a systematic review summarized 12 random clinical trials and concluded that ginger consumption is able to reduce pregnancy-associated nausea symptoms (Viljoen et al. 2014).

Turmeric

Turmeric, *Curcuma longa* L. (Zingiberaceae) is a spice cultivated in warm, rainy regions like India, China, Indonesia, Jamaica and Peru (Govindarajan 1980). The spice belongs to the genus *Curcuma*, which includes several plant species with underground rhizomes and roots. About 40 species of the genus are indigenous to India

(Velayudhan et al. 1999). The use of turmeric dates back nearly 4000 years to the Vedic culture in India, where it was used as a culinary spice but also had some religious significance.

Turmeric is used as a food additive to improve the palatability, storage and preservation of food. For example, turmeric is used in a wide range of curry powders and mustards. In Asian cuisines, dry or fresh turmeric, or ground turmeric, are used for vegetable and meat dishes and soup-like dishes (Govindarajan 1980). Turmeric oleoresin extract is used in brine pickle (Eiserlie 1966; Cripps 1967) and mayonnaise and relish formulations. It can be included in non-alcoholic beverages, or for garnishing and in some ice creams (Perotti 1975). Turmeric has in intense red orange color and it is commonly used as a natural food color (Govindarajan 1980).

The processing and storage condition can affect the quality of turmeric. Both dried rhizomes and leaves are used as raw materials to extract the volatile oil. Dried rhizomes contain 5–6% and leaves contain about 1.0–1.5% oil which is extracted by steam distillation. A large portion of volatile oil can be lost during processing (Chempakam et al. 2008a). The curcuminoids (a general terms for curcumin, demethoxycurcumin and bisdemethoxycurcumin) deteriorate on exposure to light and are also prone to oxidation which caused discoloration (Buescher and Yang 2000).

The primary component contributing to the unique aroma of turmeric is *ar*-turmerone. The primary compounds responsible for the color in the rhizomes are curcumin (1,7-*bis* (4-hydroxy-3-methoxy prenyl)-1, 6-heptadiene-3, 5-dione) and two related demethoxy compounds, demethoxy curcumin and *bis*-demethoxycurcumin (Fig. 12.11). Curcumin is insoluble in water but highly soluble in ethanol and acetone.

The oleoresin from turmeric is primarily used as a food color, and secondarily, to contribute a characteristic mild spicy aroma compatible with mustard, pickles, relish formulae, etc. Commercial oleoresin from turmeric has a curcuminoid content of 4.5–5.0%. It is highly viscous with a dark brownish-orange color. The product contains 30–40% curcumin, 15–20%

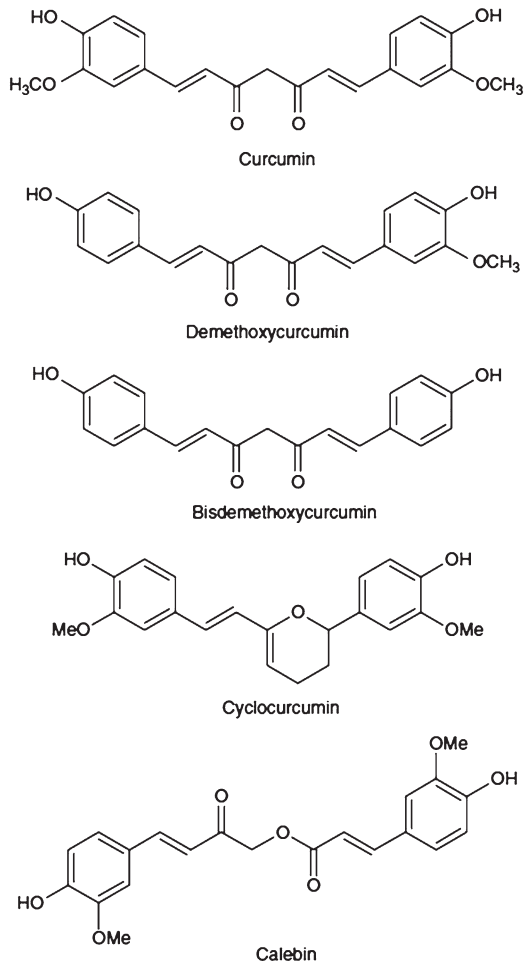


Fig. 12.11 Curcuminoids found in turmeric

volatile oil and delivers a fresh, clean, mildly pungent, woody-pungent, woody-spicy aroma of turmeric (Chempakam et al. 2008b).

Turmeric has gained lots of popularity recently due to increasing evidence on health benefits as well as promotion from celebrities and top chefs. In most studies, the active component of turmeric is considered to be curcumin. Consumption of curcumin showed lipid lowering effects in patients with type-2 diabetes and metabolic syndrome (Neerati et al. 2014; Yang et al. 2014). Curcuminoids may reduce inflammation in patients with cardiovascular diseases indicated by lowering C-reactive protein in blood (Sahebkar 2014). Curcumin was found to have anticancer activities in numerous studies (Hallman et al.

2017; Yue et al. 2016). A review also summarized that turmeric/curcumin products both oral and topical may improve skin health (Vaughn et al. 2016). Conclusive evidence on these health benefits are warranted.

Cinnamon

Cinnamon and its close relative, cassia, are among the earliest, most popular spices used by mankind. The genus *Cinnamomum* (family: Lauraceae) consists of 250 species of trees and shrubs distributed in Southeast Asia, China and Australia. True cinnamon, *Cinnamomum verum* syn. *C. zeylanicum*, is a native of Sri Lanka and South India. Cassia cinnamon is derived from different sources, including Chinese cassia (*C. cassia* syn. *C. aromatica*) from China and Vietnam, Indonesian cassia (*C. burmannii*) from Sumatra and the Java region and Indian cassia (*C. tamala*) from the north-eastern region of India and Myanmar (Baruah and Nath 2004; Leela 2008). Sri Lanka is the major cinnamon producing country with 60% of the world cinnamon trade. The dried inner bark of the cinnamon tree is used as a spice. Cinnamon oleoresin is obtained by solvent extraction of the bark, is used mainly for flavoring food products such as cakes and confectionary products. The volatile oil and oleoresin from cassia are also used for flavoring soft drinks and other beverages (Leela 2008). The genus Cinnamomum comprises several hundred species which occur naturally in Asia and Australia. They are evergreen trees and shrubs and most species are aromatic. *C. verum*, the source of cinnamon bark and leaf oils, is a tree indigenous to Sri Lanka, although most oil now comes from cultivated areas (Leela 2008). The major varieties and growing regions are listed in Table 12.10.

Cinnamon bark oil delivers a delicate aroma of the spice and a sweet and pungent taste. Its major constituent in cinnamon bark is cinnamaldehyde, however it is the minor components impart the characteristic odor and flavor that makes *C. zeylanicum* unique. The cinnamon bark oil is used in food flavoring such as meat and fast food seasonings, sauces and pickles, baked goods, confectionery, cola-type drinks, tobacco flavors and in dental and pharmaceutical preparations. Perfumery applications are limited because the oil has some skin-sensitizing properties. Cinnamon leaf oil has a warm, spicy aroma, but the aroma can be harsh and it lacks the body of bark oil. Leaf oils major constituent is eugenol rather than cinnamaldehyde. Leaf oil is used in seasonings and savory snacks, as a low cost fragrance in soaps and insecticides. Table 12.11 lists the profiles of the volatile compounds found in *C. zeylanicum* leaves and bark.

Table 12.10 Botanical sources of cinnamon and cassia

Cinnamon variety	Region of origin/production
Cinnamomum verum Presl (syn. *C. zeylanicum* Nees)	True or Ceylon cinnamon
C. cassia Presl	Cassia, Chinese cinnamon, "Cassia lignea"
C. burmannii Blume	Indonesian cassia
C. loureirii Nees	Vietnamese cassia
C. tamala (Buch.-Ham.) Nees & Eberm	Indian cassia

From Leela (2008)

Cinnamomum verum (C. zeylanicum) is used to produce both leaf and bark oils for flavoring and perfumes. The major component of bark oil is cinnamaldehyde and of leaf oil is eugenol. In cinnamon the volatile components are found in other parts of the plant including root bark, fruits, flowers, twigs and branches. The most important source is the bark which varies from 0.4 to 2.8% (Angmor et al. 1972; Wijesekera 1978; Krishnamoorthy et al. 1996). The oil from the stem bark of a commercial cinnamon sample contained 75% cinnamaldehyde, 5% cinnamyl acetate (Gruenwald et al. 2010), 3.3% caryophyllene, 2.4% linalool and 2.2% eugenol (Senanayake et al. 1978).

Cassia oil is distilled from a mixture of leaves, twigs and fragments of bark. Cinnamaldehyde is the major constituent and it is used mainly for flavoring cola-type drinks, with smaller amounts used in bakery products, sauces, confectionery and liqueurs. Like cinnamon bark oil, its use as a fragrance is limited by its skin sensitizing properties.

Table 12.11 The primary volatile components in *C. zeylanicum* leaf and bark extracts

Leaf			Bark		
	Volatile oil	Oleoresin		Volatile oil	Oleoresin
	%	%		%	%
α-Thujene	0.1		α-Pinene	tr	
α-Pinene	0.5		Camphene	tr	
β-Pinene	tr		Sabinene	tr	
Myrcene	tr		β-Pinene	tr	
α-Phellandrene	1.9		Limonene	tr	
p-Mentha-1(7),8-diene	tr		1,8-Cineole	tr	
p-Cymene	0.7		Camphor	tr	
1,8-Cineole	0.7		Z-cinnamaldehyde	tr	1.5
Terpinolene	tr		E-cinnamaldehyde	97.7	50.0
α-Terpineol	tr		α-Copaene	0.8	4.6
α-Cubebene	tr		α-Amorphene	0.5	
Eugenol	87.3	87.2	δ-Cadinene	0.9	7.8
β-Caryophyllene	1.9	1.4	Terpinen-4-ol		0.1
Aromadendrene	1.1	0.8	β-Caryophyllene		1.0
α-Amorphene	tr	0.4	Coumarin		16.6
Germacrene-D	0.6	0.2	α-Muurolene		4.4
Bicyclogermacrene	3.6	1.7	β-Bisabolene		1.4
δ-Cadinene	0.4	0.6	Cadina-1(2), 4-diene		1.8
Spathulenol	0.5	1.7	Ortho-methoxy cinnamaldehyde		1.5
Sabinene		tr	Cubenol		0.5
γ-Terpinene		tr	1-Heptadecene		0.2
Terpinen-4-ol		tr	1-Nonadecene		0.4
δ-Elemene		1.0	Tetracosane		0.1
Viridiflorol		0.3	Octacosane		0.1
Methoxy-eugenol		0.1	Nonacosane		0.2
Isospathulenol		0.3			
Neophytadiene		0.3			
Docosane		0.1			
Nonacosane		0.1			
Vitamin-E		0.2			
Total	99.4	97.1	Total	100	92.3

Adapted from Singh et al. (2007)

The volatile distribution of *C. Cassia* differs significantly form *C. zeylanicum.* Table 12.11 compares the bark and leaf volatiles from *C. zeylanicum*. The dried inner bark of cinnamon and cassia contains volatile oil, fixed oil, tannin, resin proteins, cellulose, pentosans, mucilage, starch, calcium oxalate and mineral elements. Cinnamon delivers a spicy aroma from its volatile oil which is composed of a mixture of monoterpenes, sesquiterpenes and phenylpropenes. The major volatile components in cinnamon are illustrated in Fig. 12.12.

The primary components influencing cinnamon quality are associated with the volatile components, cinnamon also contains several diterpenes. The diterpenes include cinncassiols A, B, C1 and their glucosides, cinncassiols C2 and C3, cinncassiols D1, D2 and D3 and their glucosides, cinncassiol E, cinnzeylanol, cinnzeylanin, anhydrocinnzeylanol and anhydrocinnzeylanin. Cinnamon also contains several benzyl isoquinoline alkaloids, flavanol glucosides, coumarin, *b*-sitosterol, cinnamic acid, protocatechuic acid, vanillic acid and syringic acid.

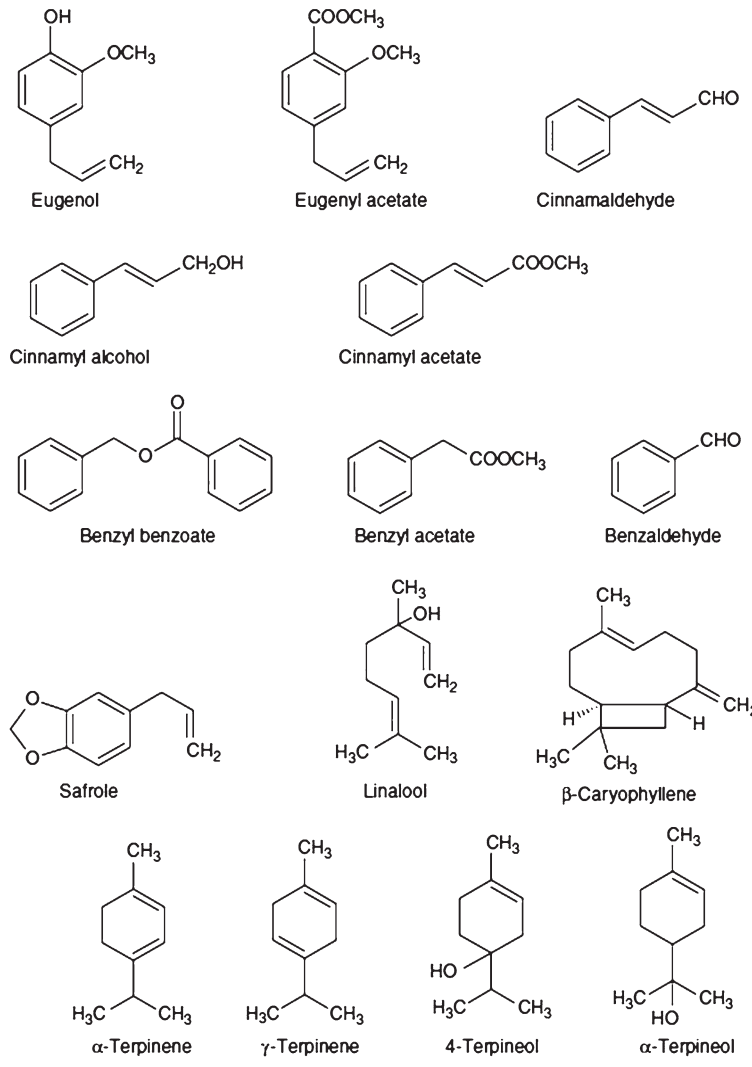


Fig. 12.12 The major volatile components in cinnamon (Leela 2008)

Some important non-volatile components reported in *Cinnamomum* and *Cassia* are listed in Table 12.12 and the structures are shown in Fig. 12.13.

Cinnamon is one of the oldest herbal medicines, which has been recorded in Chinese publications 4000 years before (Qin et al. 2003). Cinnamon has been used to treat dyspepsia, gastritis, blood circulation disturbance and inflammatory diseases in many countries since ancient times (Yu et al. 2007). The significant anti-allergic, anti-ulcerogenic, antipyretic, anaesthetic and analgesic activities have been confirmed previously (Kurokawa et al. 1998; Lee and Ahn 1998). The in vitro investigation of cinnamon has revealed that its extract mimics the function of insulin, which potentiates insulin action in isolated adipocytes (Broadhurst et al. 2000). Cinnamon extract has been shown to improve the insulin receptor function (Jarvill-Taylor et al. 2001; Leela 2008). Current in vitro and in vivo evidence suggests that cinnamon has anti-inflammatory, antimicrobial, antioxidant, antitumor, cardiovascular, cholesterol lowering, and immunomodulatory effects (Scaglione et al. 1989). In vitro studies have demonstrated that cinnamon acts as an insulin mimetic, to potentiate insulin activity or to stimulate cellular glucose

Table 12.12 Non-volatile components in cinnamon and cassia

Compound	Plant part	References
Lyoniresinol 3 α-O-β-glucopyranoside	*C. cassia* stem bark	Miyamura et al. (1983)
2,4,5-Trimethoxy phenol β-D apiofuranosyl-(1-6)-β-glucopyranoside	*C. cassia* stem bark	Miyamura et al. (1983)
Syringaresinol	*C. cassia* stem bark	Miyamura et al. (1983)
5,7,3′-Trimetyl (−) epicatechin	*C. cassia* stem bark	Miyamura et al. (1983)
5,7-Dimethyl-3′-,4′-di-O-methylene (±) epicatechin	*C. cassia* stem bark	Miyamura et al. (1983)
Cinnamic aldehyde cyclic glycerol-1,3-acetol (9,2′ *trans*)	*C. cassia* stem bark	Miyamura et al. (1983)
Cinnamic aldehyde cyclic glycerol-1,3-acetol (9,2′ *cis*)	*C. cassia* stem bark	Miyamura et al. (1983)
Cimmacassiol D_4	*C. cassia* stem bark	Nohara et al. (1982)
Cimmacassiol D_4 -glucoside	*C. cassia* stem bark	Nohara et al. (1982)
2′-Hydroxy cinnamaldehyde	*C. cassia* stem bark	Kwon et al. (1996)
3-(2-Hydroxy phenyl)-propanoic acid	*C. cassia* stem bark	Tanaka et al. (1989)
Cinncassiol E	*C. cassia*	Nohara et al. (1985)

From Leela (2008)

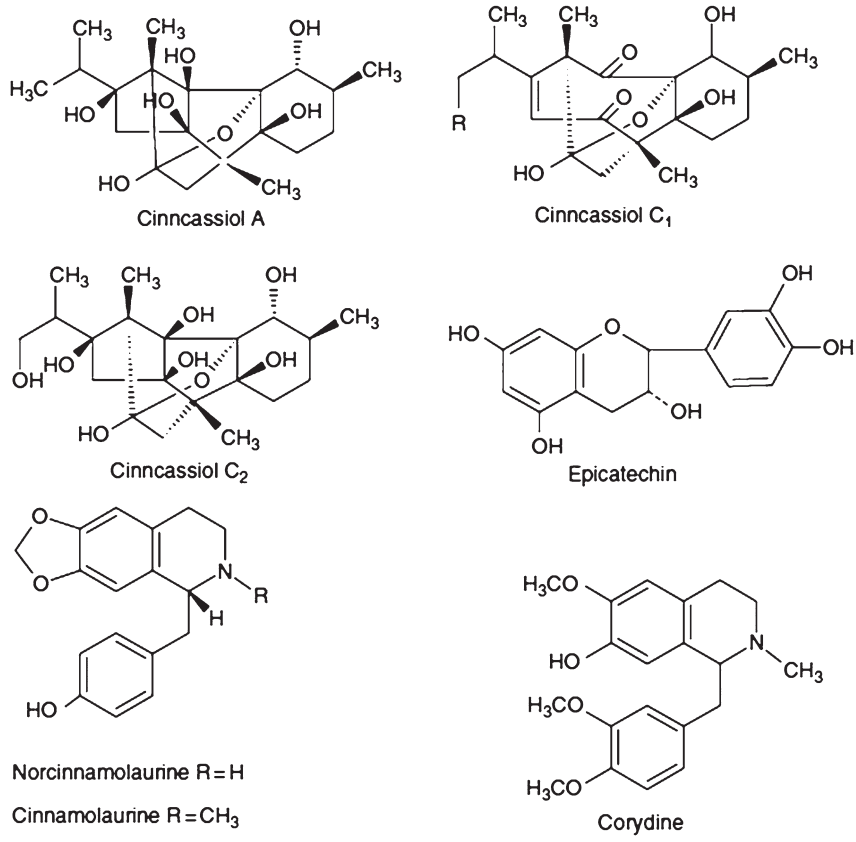


Fig. 12.13 Major non-volatile components in cinnamon

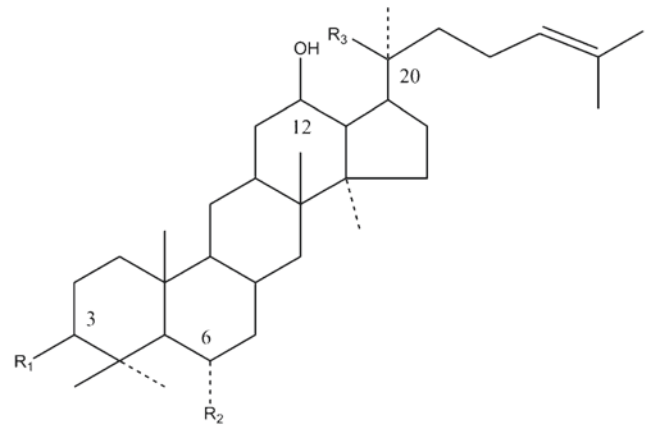
metabolism. Animal studies have demonstrated strong hypoglycemic properties. The use of cinnamon as an adjunct to the treatment of type 2 diabetes mellitus is the most promising area, but further research is needed before definitive recommendations can be made.

Ginseng

Ginseng is a traditional herb that has been used for a 1000 years by ancient East Asian. It was considered to be a panacea thus having the genus name *Panax*. There are 11 species in the genus *Panax* considered as ginseng. The edible part is the fleshy root of ginseng plant. Ginseng is grown in Asia and North America with China, South Korea, Canada, and the US being the four major producing countries, accounting for 99% of the annual 80,080 tons' production (Baeg and So 2013). As a functional food ingredient, ginseng has been found in over 11,000 retail products (Innova Market Insights 2017). Ginseng has been added into tea, energy drink, soup, candies, as well as dietary supplements in food and beverage.

The major bioactives in ginseng are ginsenosides, belonging to triterpene saponins. Most ginsenosides consist of a dammarane aglycone with different sugar moieties. The common ginsenosides are shown in Fig. 12.14. The composition of ginsenosides are affected by species, age, growing condition, harvesting method, as well as storage condition (Leung and Wong 2010).

The therapeutic potentials of ginseng and ginsenosides have been studied extensively. Consumption of ginseng has positive effects on cardiovascular diseases (Lee and Kim 2014), immune modulation (Scaglione et al. 1990), enhancement on Cognitive function (Vogler et al. 1999). The mechanism on efficacy of ginseng on cardiovascular diseases may include: inhibition of free radical production, stimulation of NO production, improvement in blood circulation, adjustment of vasomotor function, and improvement in the lipid profile (Lee and Kim 2014).

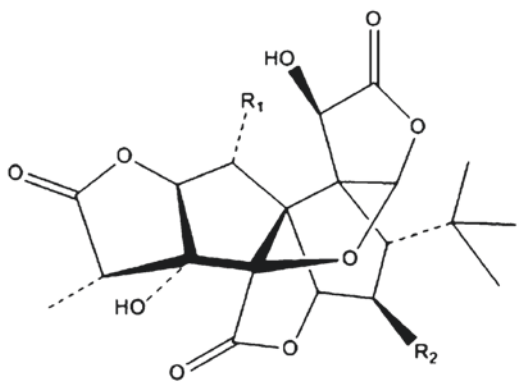


Ginsenoside	R_1	R_2	R_3
Rb_1	-O-Glc-Glc	-H	-O-Glc-Glc
Rc	-O-Glc-Glc	-H	-O-Glc-Ara (pyr)
Re	-OH	-O-Glc-Rha	-O-Glc
Rf	-OH	-O-Glc-Glc	-OH
Rg_1	-OH	-O-Glc	-O-Glc

Fig. 12.14 Structures of common ginsenosides. Abbreviation: *Glc* glucopyranoside, *Ara(pyr)* arabinopyranoside, *Rha* rhamnopyranoside. Adapted from Choi et al. (2002)

Ginkgo

Ginkgo (*Ginkgo biloba*) is a woody plant that has been grown widely in the world. Similar to ginseng, ginkgo leaf is considered a medicinal herb that has a rich history of consumption in China. Ginkgo has a variety of bioactives categorized into flavonoids (glycosides of kaempferol, quercetin, and isorhamnetin) and terpenoids (ginkgolide A, B, C, and J) (Kleijnen and Knipschild 1992). Figure 12.15 showed the structure of common ginkgolides. Ginkgo is usually made into extracts and is mainly added into dietary supplements or as prescribed herbal drugs. Standardized extracts from the leaves of ginkgo have a set ratio of bioactives. For example, EGb761 contains 24% of flavonoids and 6% of terpenoids. The extract EGb761 are commonly prescribed for dementia and cognitive impairment (O'Hara et al. 1998). A review of nine clinical trial found a 240 mg dose of EGb761 was able to improve cognition for the whole group of patients with Alzheimer's disease, vascular or mixed dementia, as well as for the Alzheimer's disease subgroup (Weinmann et al. 2010).



Ginkgolide	R_1	R_2
A	-H	-H
B	-OH	-H
C	-OH	-OH
J	-H	-OH

Fig. 12.15 Structures of terpenoids (ginkgolides) in Ginkgo

Food Fraud Risks

Herbs and spices are usually high value crops that require delicate care and intensive labor. They are mostly sold in powder, minced, oleoresin and essential oil forms. The combining factors above have made herbs and spices products susceptible to Economically Motivated Adulteration (EMA). Fraudulent adulteration of spices and herbs with added colors have been seen in international trade and retail markets (USP 2017). Market surveillance has showed 25% of the oreganos in the markets are adulterated with other plants (BBC 2015). Tested by Taiwan FDA, lead chromate has been found in one turmeric product at lead level as high as 1600 ppm (Liao 2016). Sudan dyes have been identified in paprika powders and oleoresins (USP 2017). With the enforcement of FSMA act, preventive controls have to be taken to ensure product quality and safety against EMA. The mitigation of risks would require a comprehensive supply chain control, a good QA testing system, as well as information tools such as database capturing fraudulent activities to be in place.

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Beer and Wine

13

John W. Finley

Alcoholic beverages are produced by fermentation of sugars to ethanol. Starting materials range from simple sugars to complex carbohydrates that are reduced to simple sugars by hydrolytic cleavage of starches and dextrins. Beer and wind represent direct products from fermentation whereas vodka, rum, whiskey and other distilled spirits and a distillation step.

Fermented beverages have been produced by many cultures since some of the earliest days of civilization. Mead, was produced in Asia from honey during the Vedic period (around 1700–1100 BC), and later mead was produced by the Greeks, Celts, Saxons, and Vikings. In Egypt, Babylon, Rome, and China, wine was made from grapes and beer from malted barley. In Godoy et al. (2003).

Most substances that contain fermentable sugars can undergo spontaneous fermentation by wild yeasts in the air. Beer-like beverages were independently invented among various cultures throughout the world. Analysis of residues in ancient pottery jars suggests that both beer and wine were produced about 7000 years ago in what is today Iran. In Mesopotamia, the oldest evidence of beer is believed to be a 6000-year-old. Wine's first appearance is from around 6000 BC in Georgia. The earliest firm evidence of wine production dates back to 5400 BC in Iran. In China chemical analysis of Neolithic jars confirmed that a fermented drink made of grapes, hawthorn berries, honey, and rice was being produced in 7000–6650 BC. A Sumerian poem honoring Ninkasi, the patron goddess of brewing, contains the oldest surviving beer recipe describing the production of beer from barley 3900 years ago. Beer became vital to all the grain-growing civilizations of classical Western antiquity, including Egypt. Knowledge of brewing was passed on to the Greeks who then passed the knowledge on to the Romans. Early Romans consumed beer, but during Republican times wine displaced beer as the preferred alcoholic beverage (Alba-Lois and Segal-Kischinevzky 2010).

Sumerian and Egyptian texts dating from about 2100 BC described medical uses of alcoholic beverages. The Hebrew Bible recommends giving alcoholic drinks to those who are dying or depressed, so that they can forget their misery (Proverbs 31:6–7).

During the Middle Ages, in Europe beer, often of very low strength, was an everyday drink for all classes and ages of people.

In South America produced a beer-like beverage was produced from cassava or maize. The maize or cassava had to be chewed before fermentation to provide amylase which converted the starches to fermentable sugars. In ancient Japan rice was chewed to hydrolyze thee rice starch in sake production.

Louis Pasteur determined that the fermentation process result from the action of living yeast converting transforming glucose into ethanol. He also demonstrated that only microorganisms are

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_13

capable of converting sugars into alcohol from grape juice, and that the process occurs in the absence of oxygen (Barnett 2000; Pasteur 1876).

Today, beer brewing and wine making are agricultural industries which evolved from ancient and empirical knowledge which emerged from many different cultures around the world. The ancient art of fermentation has been combined with basic scientific knowledge and applied toward modern production processes (Hornsey 2003).

Fermentation has been used to preserve and produce food and beverages since the Neolithic age. Lactic acid fermentation has been used to preserve many foods including cheeses, yogurt, pickles and kimchi as well as for producing alcoholic beverages such as beer and wine and beer.

Wine was consumed in Classical Greece at breakfast or at symposia, and in the 1st century BC it was part of the diet of most Roman citizens. Both the Greeks and the Romans generally drank diluted wine (the strength varying from one part wine and one part water, to one part wine and four parts water).

In Europe during the Middle Ages, beer, often of very low strength, was an everyday drink for all classes and ages of people. A document from that time mentions nuns having an allowance of six pints of ale each day. Cider and pomace wine were also widely available; grape wine was the prerogative of the higher classes.

Hard liquor, particularly brandy and rum, were provided for sailors during the long sea voyages of the Age of Exploration, when European powers plied the seas during the fifteenth, sixteenth, and early seventeenth centuries. Great Britain's longtime superiority at sea may also owe a debt to its navy's drink of rum-based choice, grog, which was made a compulsory beverage for sailors in the late eighteenth century.

Rum played a crucial part of the triangular trade between Britain, Africa, and the North American colonies that once dominated the Atlantic economy.

Alcoholic Fermentation

Ethanol contained in alcoholic beverages is produced by yeast fermentation. Wine is produced by fermentation of the natural sugars present in grapes; cider and perry are produced by similar fermentation of natural sugar in apples and pears, respectively; and other fruit wines are produced from the fermentation of the sugars in any other kinds of fruit. Mead is produced by fermentation of the natural sugars present in honey. Beer, whiskey, and vodka are produced by fermentation of grain starches that have been converted to sugar by the enzyme amylase, which is present in grain kernels that have been malted. Other sources of starch (e.g. potatoes and unmalted grain) may be added to the mixture where amylase is present to hydrolyze the starches to sugars. Whiskey and vodka are also distilled; gin and related beverages are produced by the addition of flavoring agents to a vodka-like feedstock during distillation.

Rice wines (including sake) are produced by the fermentation of grain starches converted to sugar by the mold *Aspergillus oryzae*. *Baijiu*, *soju*, and *shōchū* are distilled from the product of the fermentation. Rum and some other beverages are produced by fermentation and distillation of sugarcane. Rum is usually produced from the sugarcane product molasses.

Fermentation is the metabolic process that converts sugar to acids, gases or alcohol. The simplest representation summarizing the fermentation process is shown in Fig. 13.1.

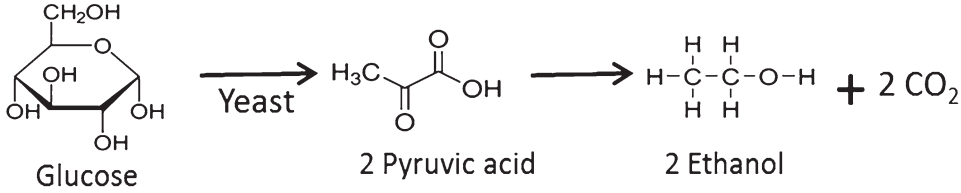


Fig. 13.1 Glucose is fermented to pyruvic acid and then converted to ethanol and carbon dioxide

The fermentation by yeast takes place in an anaerobic environment (when the electron transport chain is unusable) and becomes the cell's primary means of ATP (energy) production. The process converts the pyruvate produced by glycolysis and NADH and pyruvate to yield ethanol. In a process called oxidative phosphorylation NADH and pyruvate are used to generate ATP.

The overall process is called the Embden–Meyerhoff–Parnas pathway which describes the conversion of glycose or xylose for energy production and production of ethanol and CO_2 as illustrated in Fig. 13.2.

Glycolysis is the first step which converts glucose to pyruvate and results production of 2 NADH and 2 ATP molecules in the process.

$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightarrow 2\ CH_3COCOO^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2$ (Pyruvate)

Glycolysis is a sequence of ten enzyme-catalyzed reactions. Intermediates enter the pathway at various points in the pathway. For example, most monosaccharides, such as fructose and galactose, can be converted to one of the glycolysis intermediates. Glycolysis is an oxygen independent metabolic pathway that does not use molecular oxygen for any of its reactions. Glycolysis occurs in cytosol of the cell in most organisms. The most common type of glycolysis is the *Embden–Meyerhof–Parnas (EMP pathway)*.

Pyruvate is metabolized in processes like the Krebs cycle to produce energy under aerobic conditions. The products of this type of metabolism are ATP, H_2O, and CO_2. When abundant supplies of sugars present under anaerobic conditions which occur in bread fermentation, beer wort and wine production yeast undergo alcoholic fermentation. This type of metabolism yields much smaller amounts of energy compared to aerobic respiration.

The alcoholic fermentation utilizes the two pyruvates produced from glycolysis of glucose. The pyruvate molecules are decarboxylated by pyruvate decarboxylase to form acetaldehydes and CO_2. At the active sites on pyruvate decarboxylase pyruvate reacts with cofactors thiamine pyrophosphate (TPP) and magnesium to release carbon dioxide. The final step to form ethanol is the addition of a hydrogen ion to the acetaldehyde. The hydrogen ion comes from the NADH produced during glycolysis. The ethanol in beer and wine production prevents the growth of other microbes.

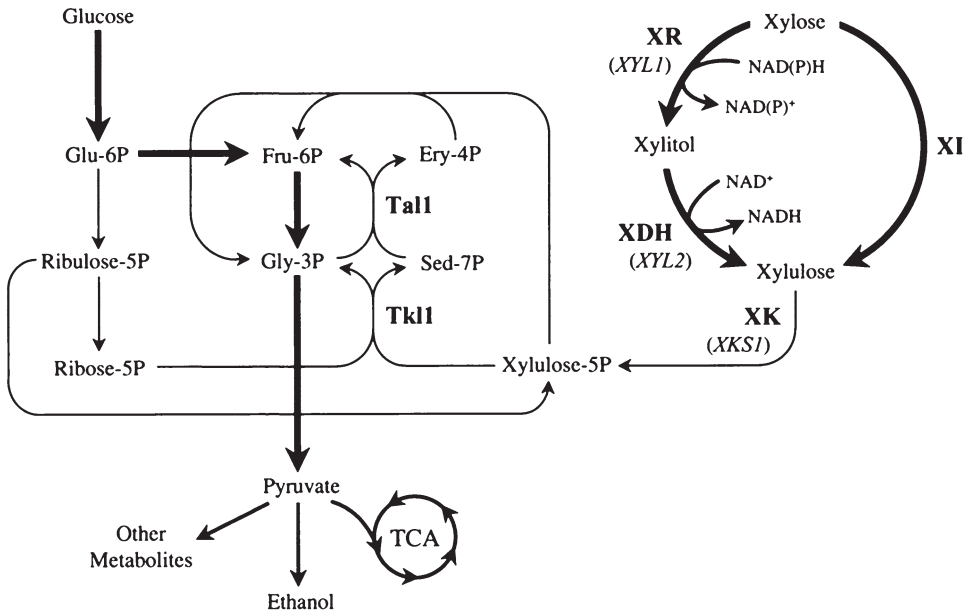


Fig. 13.2 Embden–Meyerhoff–Parnas pathway for production of pyruvate which enters the TCA cycle and ethanol

Saccharomyces cerevisiae is the yeast generally used in the beer and wine making process. The strains of yeast used in the beer making process ferments the different types of sugars found in the wort, pre-fermented beer, to produce ethanol. *Saccharomyces cerevisiae* has been has been in use for thousands of years. Beer technology was utilized by from Germanic and Celtic tribes around the first century AD. The oldest known example of yeast used in fermentation of a beverage was found in China around 7000 BC. *Saccharomyces cerevisiae* can be found all around the world on the surface of fruits and plants, in the soil, the gastrointestinal tract of animals, and the skin surface of animals.

Beer

Beer is produce by fermentation of malted barley (with or without other added starch or sugar sources), water hops yeast and water. Additions to the malt, called adjuncts are barley, wheat, rice or corn starches, their hydrolysis products resulting in fermentable sugars. Additional enzymes from microbial sources can be added to enhance carbohydrate hydrolysis or to clarify beer. He aroma and flavor of beer comes from the bitter taste of hops, kiln dried products from the barley malt and aroma compounds resulting from the fermentation. The fermentation process is based on the Embden–Meyerhoff–Parnas pathway for glycolysis and alcoholic fermentation.

The production of beer includes the malting of barley, the preparation of wort fermentation and delivery. The process is outlined in Fig. 13.3.

All beers are brewed using a process based the fermentation of malted grain. Many types of beers are produced resulting from variation in starting materials and regional preferences. Barley, wheat or sometimes rye are the preferred starting materials.

Malt is produced by germinating the grain to breakdown starches to fermentable sugars, after which the grain is dried in a kiln and sometimes roasted. The germination process activates several enzymes, particularly a-amylase and b-amylase, which hydrolyze the starch in the grain into sugar.

Malting commences with steeping of barley in water at 14–18 °C for up to 48 h, until it reaches a moisture content of 42–46%. This is usually achieved in a three-stage process, with the steeps being interspersed with ‘air rests’ that allow the barley to get some oxygen (to ‘breathe’).

Raising the moisture content allows the grain to germinate, a process that usually takes 3–5 days at 16–20 °C. In germination, the enzymes break down the cell walls and some of the protein in the starchy endosperm, which is the grain’s food reserve, rendering the grain friable. Amylases are produced in germination and these are important for the mashing process in the brewery.

Progressively increasing the temperature during kilning arrests germination, and regimes with progressively increasing temperatures over the range 50 to perhaps 110 °C are used to allow drying to <5% moisture, whilst preserving heat-sensitive enzymes. The more intense the kilning process, the darker the malt and the more roasted and burnt are its flavor characteristics.

Brewing is typically divided into seven steps: Mashing, Lautering, Boiling, Fermenting, Conditioning, Filtering, and Packaging.

Mashing is the process of mixing malted and milled grain with water, and heating this mixture up with holding or rests at certain temperatures to allow enzymes in the malt to break down the starch in the grain into sugars, typically maltose.

The malt is crushed in a “malt mill” to reduce the particle size of the grain kernels, increase their surface area, and separate the fermentable portions from the husks. The resulting grist is mixed with heated water in a vat called a “brewing kettle” for a process known as “mashing”. During this process, natural enzymes within the malt break down much of the starch into sugars which play a vital part in the fermentation process. Mashing usually takes 1–2 h, and during this time various temperature profiles activate different enzymes depending upon the type of malt being used. Hydrolysis of the starchy raw material by the amylase enzymes convert the starches into fermentable sugars such as glucose and maltose.

Lautering is the separation of the fermentable extracts from the grain and removing the spent grain. The process is conducted in either a lauter

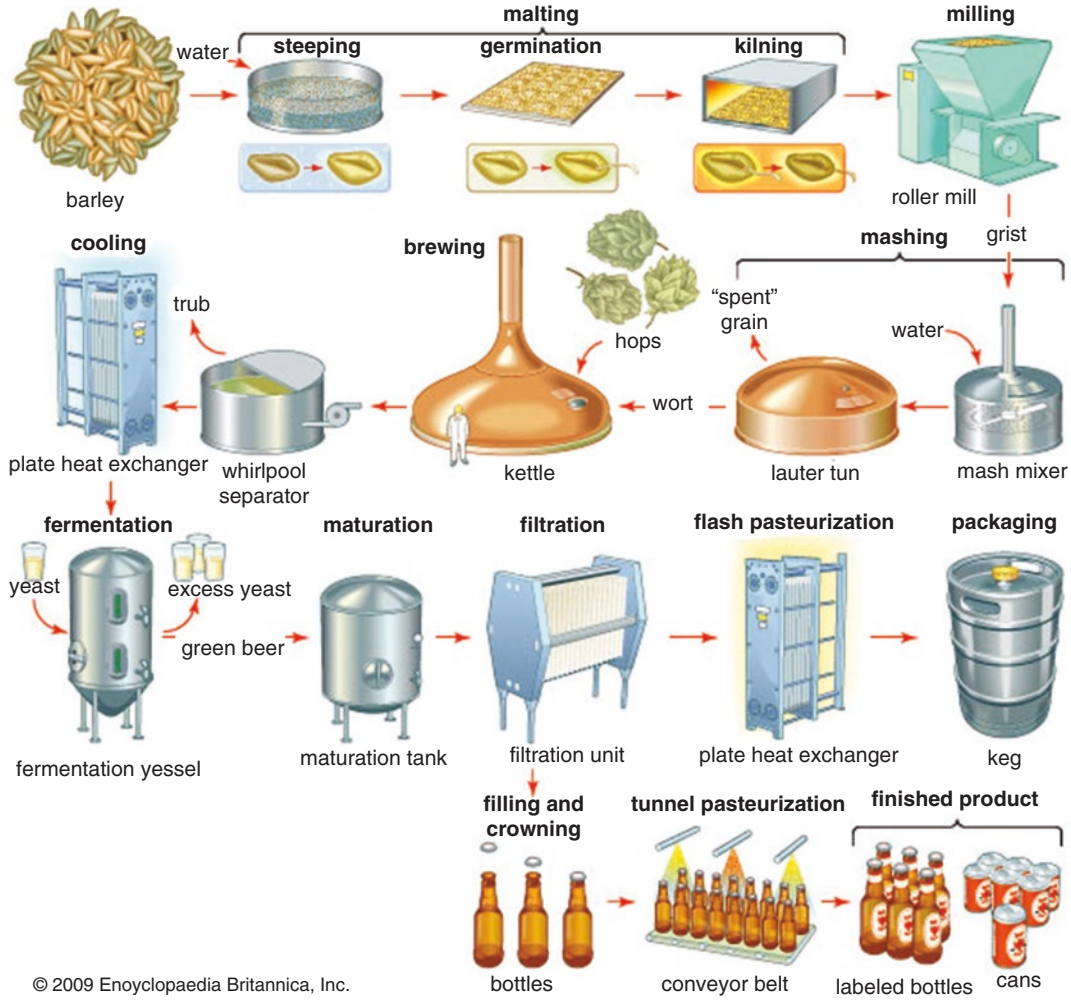


Fig. 13.3 Production of beer

tun, a wide vessel with a false bottom, or a mash filter, a plate-and-frame filter designed the separation. Lautering has two stages: first wort run-off, during which the extract is separated in an undiluted state from the spent grains, and sparging, in which extract which remains with the grains is rinsed off with hot water. A mash rest temperature of 104 °F or 40 °C activates beta-glucanase, which breaks down gummy beta-glucans in the mash, reducing the viscosity of the mash. A mash rest from 120 to 130 °F (49 °C to 55 °C) activates various proteinases, which hydrolyze proteins reducing the chance of haze in the beer. This rest is generally used only with undermodified (i.e. undermalted) malts which are popular in Germany and the Czech Republic, or non-malted grains such as corn and rice, which are widely used as adjuncts in North American beers. Finally, a mash rest temperature of 149–160 °F (65–71 °C) is used to convert the starches in the malt to sugar. Finally the mash temperature may be raised to 165–170 °F (about 75 °C) (known as a mashout) to deactivate enzymes.

After the mashing, the mash is pumped to a lauter tun where the resulting liquid is strained from the grains in a process known as lautering. The lauter tun generally contains a slotted "false bottom" or other form of manifold which acts as a strainer allowing for the separation of the liquid from the grain. A lauter tun is the traditional vessel used for separation of the extracted wort. While the basic principle of its operation has

remained the same since its first use, technological advances have led to better designed lauter tuns capable of quicker and more complete extraction of the sugars from the grain.

The false bottom in a lauter tun has thin slits to hold back the solids and allow liquids to pass through. The solids, not the false bottom, form a filtration medium and hold back small solids, allowing the otherwise cloudy mash to run out of the lauter tun as a clear liquid. The false bottom of a lauter tun is today made of wedge wire, which can provide a free-flow surface in the bottom of the tun.

Sparge water is sprayed on top of the spent grains in the tun to help extract remaining fermentable sugars. http://www.sterkensbrew.be/sbm/beer

A mash filter is a plate-and-frame filter. The empty frames contain the mash, including the spent grains, and have a capacity of around one hectoliter. The plates contain a support structure for the filter cloth The plates, frames, and filter cloths are arranged in a carrier frame like so: frame, cloth, plate, cloth, with plates at each end of the structure. Newer mash filters have bladders that can press the liquid out of the grains between spargings. The grain does not act like a filtration medium in a mash filter.

At this point the liquid is known as wort. The wort is moved into a large tank known as a "cooking tun" or kettle where it is boiled with hops and sometimes other ingredients such as herbs or sugars. The boiling process serves to terminate enzymatic processes, precipitate proteins, isomerize hop resins, concentrate and sterilize the wort. Hops add flavor, aroma and bitterness to the beer.

At the end of the boil, the hopped wort settles to clarify using hop filters. SBM does not use the whirlpool system for hop separation. http://www.sterkensbrew.be/sbm/beer_

Boiling the wort, ensures its sterility, and thus prevents the growth of undesired microorganisms. During the boil hops are added, which contribute bitterness, flavor, and aroma compounds to the beer. The heat of the boil, causes proteins in the wort to coagulate and the pH of the wort to drop slightly.

After the hops are removed by filtration the wort is cooled to fermentation temperatures before yeast is added. The wort is pumped into the heat exchanger, and goes through every other gap between the plates. The cooling medium, usually water, goes through the other gaps. The ridges in the plates ensure turbulent flow. A good heat exchanger can drop 95 °C wort to 20 °C while warming the cooling medium from about 10 °C to 80 °C. After cooling the yeast is added for fermentation.

Fermentation, as a step in the brewing process, starts as soon as yeast is added to the cooled wort. The yeast fermentation converts the sugars in the wort to alcohol and carbon dioxide. Fermentation tanks come in all sorts of forms, from enormous tanks which can look like storage silos, to five gallon glass carboys in a homebrewer's closet.

When the sugars in the fermenting beer have been almost completely digested, the fermentation slows down and the yeast starts to settle to the bottom of the tank. The beer is cooled to almost freezing, which encourages settling of the yeast, and causes proteins to coagulate and settle out with the yeast. Phenolic compounds which can cause unpleasant flavors become insoluble in the cold beer and their removal results in a smoother flavor for the beer.

Some beers undergo a fermentation in the bottle, giving natural carbonation. This may be a second or third fermentation. They are bottled with a viable yeast population in suspension. If there is no residual fermentable sugar left, sugar may be added. The resulting fermentation generates CO_2 which is trapped in the bottle, remaining in solution and providing natural carbonation. http://www.sterkensbrew.be/sbm/beer_

Raw Materials

Barley is the most central ingredient for beer brewing. Different barley strains impart unique characteristic taste and body in different beers. Malted barley is barley that has been allowed to germinate (sprout) to a degree and is then dried.

This is accomplished industrially by increasing the water content of the seed to 40–45% by soaking it for a period close to 40 h. The seed is then drained and held at a constant temperature (60 °F) for close to 5 days until it starts to sprout. The barley is slowly dried in a kiln at temperatures gradually rising to 122 °F for lighter malts and 220 °F for darker malts. This kiln drying takes about 30 h. Finally, the rootlets from the partially germinated seeds are removed.

The germination process converts starch into simpler sugars used by the plant in its initial growing stage. The conversion of starch to sugar is accomplished by amylase enzymes that the seed produces during germination process. The germination and drying stages capture fermentable sugars, soluble starch, and the diastase enzymes for beer brewing. Malted barley is the primary source of the fermentable sugar consumed by the yeast. However many beers add other carbohydrate sources called adjuncts, such as corn, wheat or rice.

Hops

Hops may be the most important ingredient in determining the flavor beer. The current revival craft beer many of which a strong hops flavor as resulted in more studies of hop chemistry.

Hops are the cone-like flowers of the female hop vine (Humulus Lupulus). At the base of hop flower, there is a soft resin called Lupulin Oil which contains the compounds that deliver the bitter flavor and "hoppy" aroma. Hops are grown in Germany, southern England, southern Australia, Tasmania, Oregon and Washington State. The 50 plus varieties of hops deliver unique bitterness in flavor or aroma.

Buttery and Ling (1966) identified over 100 hop oil components. Among the 110 components in beer, Tressl et al. (1978) was able to identify 47 that were derived from hops. Figure 13.4 illustrates the hop oil constituents as defined by Sharpe and Laws 1981, where 50–80% of the hop oil is comprised of hydrocarbons.

Steinhaus and Schieberle (2000) found 23 potent aroma compounds in the hop variety Spalter Select. The compounds were subsequently categorized by flavor dilution factors. The most potent aroma constituents were *trans*-4,5-epoxy-(E)-2-decenal, (which confers a metallic note), linalool (flowery) and myrcene (geranium like). Kishimoto et al. (2006) discovered 4-mercapto-4-methylpentan-2-one (4MMP) added a fruity aroma in American, Australian and New Zealand hops cultivars.

Steinhaus and Schieberle (2000) utilized aroma extract dilution analysis (AEDA) to assess the contributions of various compounds to hoppy

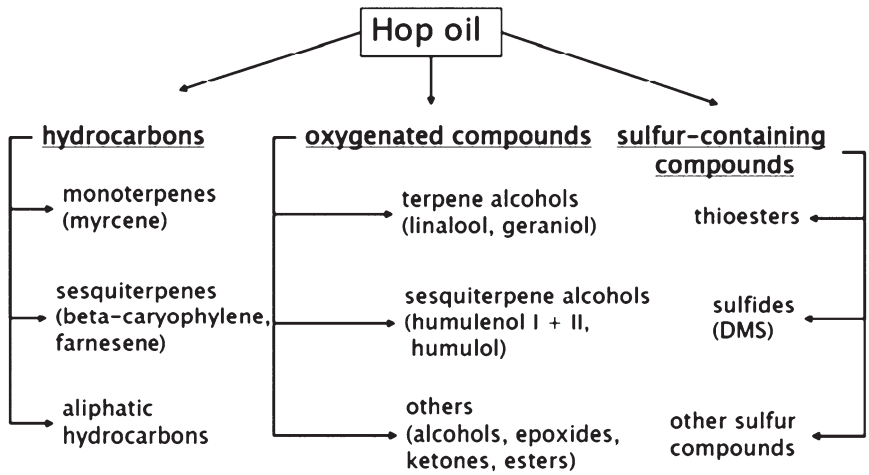


Fig. 13.4 Groups of hop oil constituents (Sharpe and Laws 1981)

aroma in beer. They observed that only a few of the volatiles in hops contributed to the aroma of the beer. Linalool was confirmed in various works to significantly contribute to the hoppy aroma in beer (Sakuma et al. 1991). Takoi et al. (2010) found that linalool acts synergistically with geraniol and citronellol. They also discovered that the presence of β-citronellol (lime aroma) depends on geraniol metabolism by the yeast. Kishimoto et al. (2006) identified 19 hop derived components in hopped beers using GC olfactometry. Within these, 4-mercapto-4-methylpentan-2-one (4MMP, blackcurrant-like) and 3-mercaptohexan-1-ol (muscat-, blackcurrant-like) proved to contribute to the hoppy aroma. Nielsen 43 was also able to identify important contributors to the aroma from hops compounds (Table 13.1).

Schönberger and Kostelecky (2011) summarized the contribution of hops to the aroma of beer:

- Green and grassy flavors can be attributed to aldehydes, e.g., hexanal
- Citrus flavours can be attributed to esters, nerol and linalool
- Floral and fruity flavors can be attributed to linalool, geraniol, β-ionone, citronellol, to -mercapto-4-methylpentan-2-one and 3-mercaptohexan-1-ol and other ketones, epoxides and Esters (Marriott 2001)
- Herbal flavors can be attributed to oxidized sesquiterpenes (Kowaka et al. 1983).

Hanke et al. (2010) studied the effect on threshold values of binary mixtures of hop aroma compounds.

They reported that a mixture of caryophyllene and nerol had a flavor threshold of 170 µg/L whereas the individual compounds had thresholds of 210 mg/L and 1200 µg/L, respectively. Addition of a combination of linalool and farnesene resulted in a combined threshold of 500 µ/L, however, farnesene alone had threshold of about 2000 µg/L.

The contribution of hops to the final aroma of beer is most noticeable when late-hopping or dry-hopping is applied. Late-hoping is the addition at the end of boil. This approach reduces the exposure of the bee with hops to heat which causes unnecessary evaporation of many valuable aroma compounds.

Dry-hopping is mainly applied during the lagering step. Addition at this stage is based on cold extraction of hop material into an alcoholic solution.

The hop oils are water soluble and are highly volatile. The hops are added to the wort at the end of boiling to minimize the loss of volatile components. The choice of hops alters the composition of the hop oils which account for the bitter flavor and hop taste of beer.

Hop oil contains (0.5–3% volatile components, and 3–6% non-volatiles) which are present in the hop polyphenolic fraction. In addition to the aroma and flavor the polyphenolic fraction also contributes to the mouthfeel of the beer.

Table 13.1 Identified aroma compounds in strong hopped beer by Kishimoto et al. (2009), Nielsen (2006), and Lermusieau and Collin (2003)

Sensory character compound	Compound	Threshold in beer
Black currant	4-Mercapto-4-methylpentan-2-one	10–50 ppb
Black currant	3-Mercaptohexan-1-ol	55 ppb
Resinous	Myrcene	30–1000 ppm
Floral, citrus	Linalool	8–80 ppm
Floral, rose like	Geraniol Ethyl 2-methyl-butanoate Ethyl 4-methyl-pentanoate	4–40 ppm 1 ppm
Fruity, herbal	*cis*-Rose oxide	5–50 ppb
Hoppy	Pineapple (e,z)1,3,5-Undecatriene	
Cheesy	2-Methylbutyric acid	
Grape tobacco, black tea	Beta damascenone/phenyl ethyl alcohol	
Cedarwood	Caryophylla-3,8-dien-(13)-dien-5-beta-ol	
Cheesy/onion/ garlic	Various sulfur compounds	

The complex mixtures of hop components in beer are present in very low but they are very important to the flavor, aroma and mouth feel of the final product. In addition to the complex nature of the hops going into the beer many volatile materials are lost during processing and other components are oxidized. The complex composition of the hop oils becomes even more complex during wort boiling. Many brewers conserve part of the aroma compounds in hops by adding the hops near the end of the wort boiling step. Hops also can account for up to one third of the total polyphenols in beer. The low-molecular-weight proanthocyanidins determine the colloidal stability of beer. Boiling results in major changes in the complex polyphenol composition of wort (Forster et al. 1995).

The barley and hops contribute complex mixtures of polyphenolics which are further altered by oxidation during processing. Hop polyphenols are found as monomers, dimers, trimers. In beer they are also found bound to protein. The polyphenols combine slowly with proteins to form chill haze when the beer is cooled after brewing, but the haze re-dissolves when the beer is warmed up. Upon aging the polyphenols polymerize and grow larger, becoming insoluble at room temperature resulting in an irreversible haze (De Keukeleire 2000).

One of the primary contributions of hops in beer is the bitter flavor. Bitter profiles in beer and the bitter components have been carefully studied and are well understood because there are only a few precursors are present in hops (Verzele and De Keukeleire 1991; Benitez et al. 1997). The most important hop compounds are the hop acids, which are defined as alpha-acids or humulones and beta-acids or lupulones (Fig. 13.5). The two groups of compounds each comprise three constituents which differ in the side chains. The three different side chains are derived from the branched chain amino acids, leucine, valine and isoleucine. These hop acids can constitute up to 25% of the dry weight of the hop cones. The proportions of the individual compounds very greatly among hop varieties variety and, and are also influenced by growing conditions. The hop acids occur as pale yellowish solids in the pure state, are weak acids, exhibit very poor solubility in water and have almost no bitter taste.

The most abundant component in alpha-acids is humulone. Depending on the hop variety the relative amounts of humulone range from 20 to 50%, adhumulone is a consistent 15% of the mixture in most hops. Cohumulone is often associated with a poor hop quality (Pollach et al. 1996).

The beta-acids are very sensitive to oxidation and most products result in off flavors. In order to avoid the problem many brewers select hop varieties with low concentrations of beta-acids.

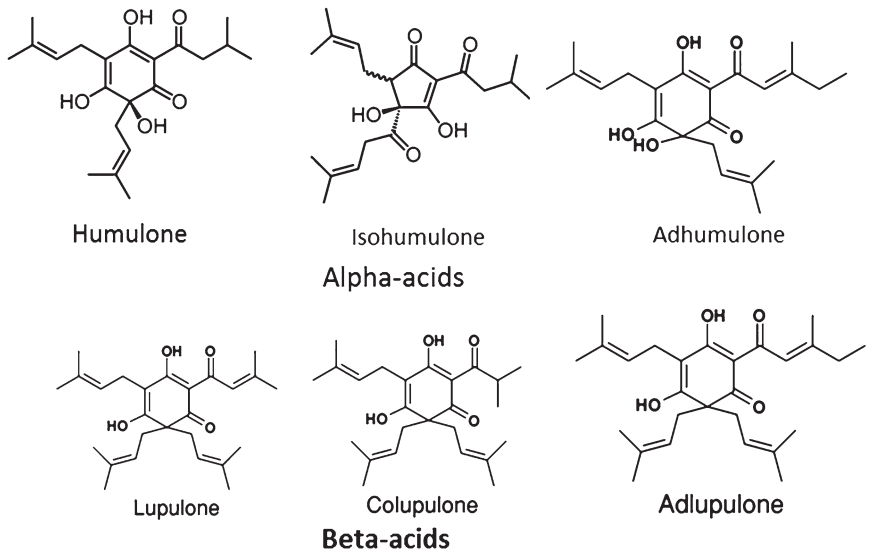


Fig. 13.5 Alpha- and beta acids form hops

Humulone is the most abundant alpha-acid in most hops. The relative amounts of humulone and cohumulone vary widely in different varieties of hops. Adhumulone constitutes invariably approximately 15% of the mixture.

During the boiling of wort, the humalones form the hops undergo major chemical changes. The most significant change is the thermal isomerization of the alpha-acids or humulones to the iso-alpha-acids or isohumcontraction (Fig. 13.6). Each humulone is converted to two epimeric forms (*cis*-isohumulones and *trans*-isohumulones). The difference is based on the spatial arrangement of the tertiary alcohol function at and the prenyl side chain at. The *trans* and *cis* designations refer to the fact that indicate that these groups point in opposite faces and to the same face of the five-membered ring, respectively. Six major iso-alpha-acids (*cis*-isohumulone and *trans*-isohumulone, *cis*-isocohumulone and *trans*-isocohumulone, *cis*-isoadhumulone and *trans*-isoadhumulone) are present in beer as a result of the thermal isomerization of the three major alpha-acids, humulone, cohumulone and adhumulone, respectively. The ratio of the isohumulones depends on the reaction conditions. In wort, the *cis*-/*trans*- ratio is approximately 68:32. The *cis*-isomers are more stable with a half-life of 5 years than the *trans*-isomers which have a half-life of about 1 year. During storage of beer, the changes in the *cis*:*trans* ratio results in changes in the in taste of the beer. Thus, during processing it is desirable to maximize the levels of *cis*-isohumulones. The iso-alpha-acids constitute the are the most significant fraction of hops in beer and account for the bitter taste of beer. The concentrations vary widely, from 15 ppm in typical American lager beers to nearly 100 ppm in bitter English ales. The perceived taste of beer is also influenced complexes formed with the humalones and the sugars in the beer.

To standardize discussions of beer flavors an International Beer Flavor Terminology was developed by the American Society of Brewing Chemists in collaboration with the European Brewery Convention and the Master Brewers Association of the Americas. These organizations felt that by providing brewers, researchers, judges, and marketing people with a simple and easily understandable terminology system, communication among beer industry professionals would improve.

In Fig. 13.7 the descriptive terms are arranged in three tiers. The center circle allows you to classify the terms as they relate to odor and taste. You start with the odor and determine which term best describes the first smell you experience. Then move on to the next level which contains common descriptive terms that allow you to expand on your initial reaction. The third tier makes it possible for you to pinpoint the flavor more specifically. If you have decided that the odor is initially fruity, then you might also be able to specify whether it is more apple-like, banana-like, or both. You can then move on to secondary odors or work on the tastes.

There a wide range of beers and each brewery and beer has unique character. Table 13.2 summarizes the general characteristics of some American beer types. Beers range from very light in color and body to very heavy stouts and brown ales. There is also a wide range of bitterness contributed by the hops.

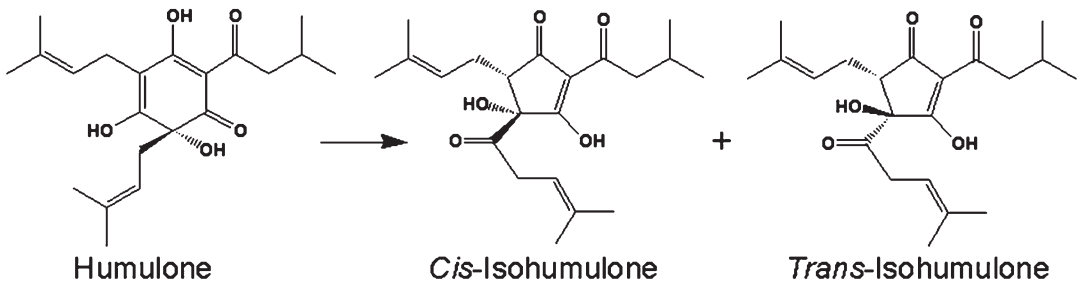


Fig. 13.6 Humalone structures from hops

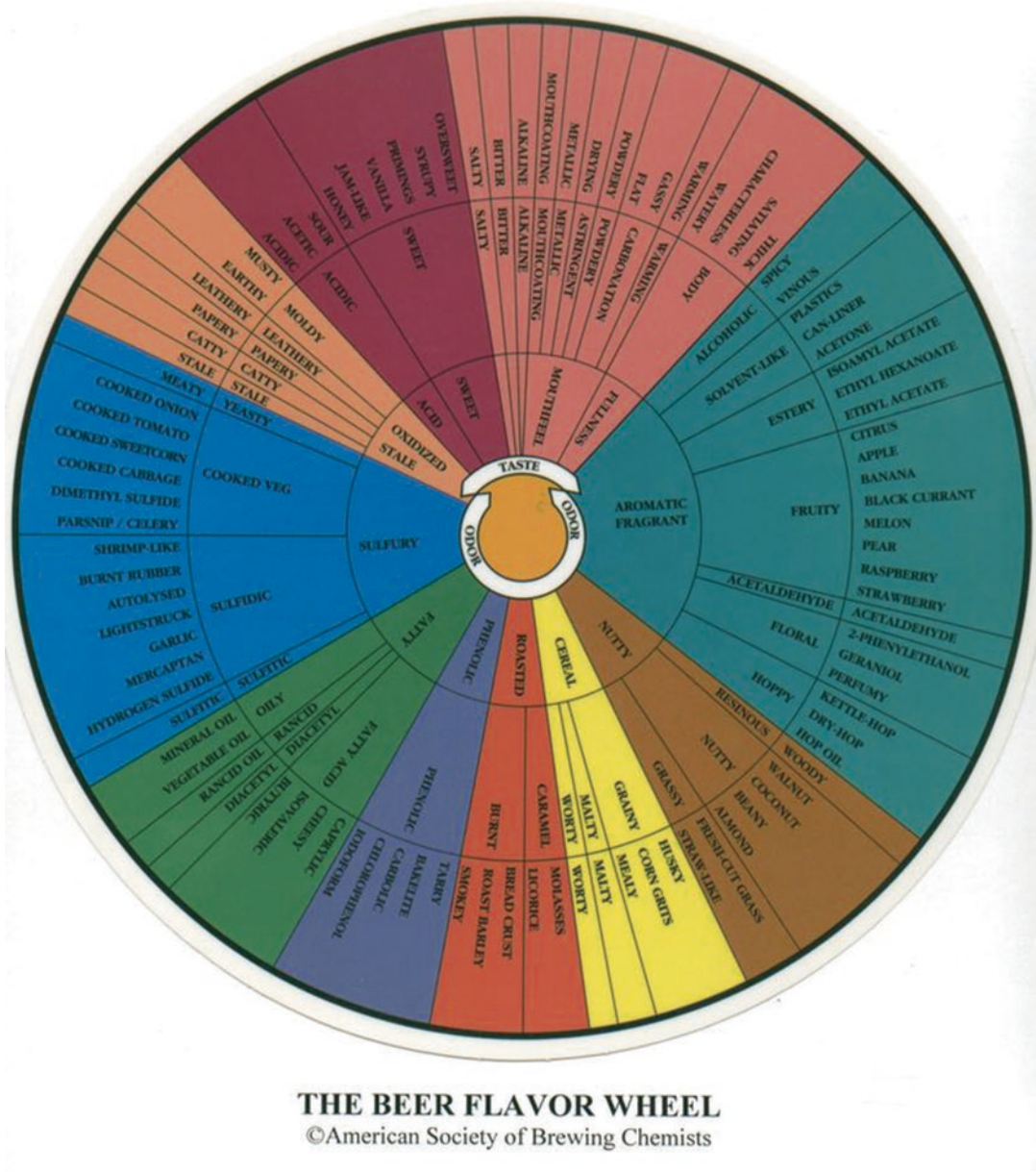


Fig. 13.7 The ASBC Beer Flavor Wheel design combines the original flavor wheel developed by M. C. Meilgaard, C. E. Dalgliesh, and J. F. Clapperton in 1979 with the updated, detailed terminology provided by experts

Yeast

Yeast is responsible for the converting of sugar to alcohol and carbon dioxide in the fermentation stage. It also contributes to the fermentation of the beer. There are thousands of varieties and strains of yeast. Carefully cultivated strains of yeast are used in the brewing of beer. Selection of the most desirable yeast for the specific beer is critical. The two types of yeast used for beer brewing are top-fermenting yeast (*Saccharomyces cerevisiae*) and bottom-fermenting yeast (*Saccharomyces uvarum*). The top-fermenting

Table 13.2 Characteristics of some American type beers

American beer type	Description	Alcohol by weight	Bitterness SRM	Color
Lager	Light fruity-ester aroma, low hop aroma and low malt sweetness Hop bitterness is not perceived to very low Corn, rice, or other grain or sugar adjuncts often used Body is light	3.20–4.00	4 10	1.5–4
Light lager	Pale to medium-amber. "light" refers to relatively low body and reduced calories Hop aroma is absent or low. Malt sweetness is very low but evident Hop flavor is absent or very low. Hop bitterness is very low to low. Corn, rice, or other grain or sugar adjuncts or all-malt formulations are also made. High in carbonation	2.8–3.5	4–10	1.5–4.0
Pilsner	Medium-low to medium malt aroma is present. Hop aroma is medium to high. Up to 25% corn and/or rice in the grist should be used. Medium-low to medium malt flavor. Hop flavor is medium to high. Hop bitterness is medium to high Body is light-medium to medium	3.8–5.0	7–20	2–8
Malt liquor	Fruity-ester and complex alcohol aromas at low levels. Hop aroma is not perceived. Some residual sweetness is perceived. Hop bitterness is very low. High alcoholic strength. Some malt liquors are just slightly stronger than American lagers, while others approach bock strength. Fruity-ester and complex alcohol at low levels	5.0–6.0	18–30	6–14
Dark lager	Dark Lagers are light brown to very dark. Low malt aroma contains contributions from caramel and roasted malts Hop aroma is very low to low. Low malt flavor contains discreet contributions from caramel and roasted malts. Non-malt adjuncts are often used. Hop flavor is very low to low. Hop bitterness is very low to low	3.2–4.4	14–24	14–25
Golden or Blonde Ales	Golden or Blonde Ales are straw to light amber. Hop aroma is low to medium-low. Light malt sweetness is present. Hop flavor is low to medium-low. Hop bitterness is low to medium. Fruity esters may be perceived	3.2–4.0	30–45	3–7
Pale Ale	Pale Ales are deep golden to copper or light brown. Low caramel malt aroma. Moderate fruity-ester aroma Hop aroma is medium to medium-high, exhibiting floral, fruity, sulfur/diesel-like, citrus-like, piney resinous characters. Hop flavor is medium to medium-high Hop bitterness is medium to medium-high. Fruity-ester flavor is moderate to strong	3.5–4.3	30–50	6–14
India Pale Ales	IPAs are gold to copper or red/light brown Fruity-ester aroma is moderate to very high. Hop aroma is high Exhibits floral, fruity, sulfur/diesel-like, citrus-like, piney, resinous characters. Medium maltiness is present. Hop flavor is high	5.0–6.0	50–70	6–15
Brown Ales	Brown Ales are deep copper to very dark brown. Fruity-ester aromas should be subdued. Roasted malt caramel-like and chocolate-like aromas are medium. Hop aroma is low to medium. Roasted malt caramel-like and chocolate-like flavors should be medium. Hop flavor is low to medium. Hop bitterness is medium to high	5.0–6.0	50–70	25–45
Stout	Stouts are black. Head retention is excellent. Fruity-ester aroma is low. Coffee-like roasted barley and roasted malt aromas are prominent. Hop aroma is medium to high. Low to medium malt sweetness with low to medium caramel, chocolate, and/or roasted coffee flavor. Roasted barley and roasted malt contribution to astringency is low. Hop bitterness is medium to high. Fruity-ester flavor is low	4.5–7.0	35–60	40+

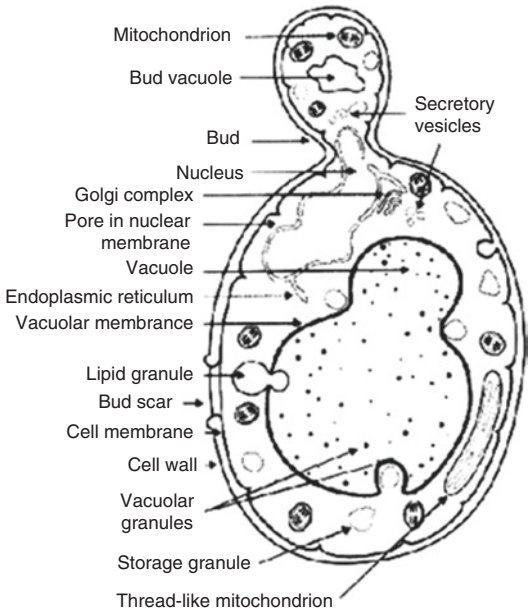


Fig. 13.8 Main components of a typical yeast cell

yeast is similar to the yeast for baking bread. It is applied for making ales and stouts. The bottom-fermenting yeast is used in the production of lagers and steam beer.

Most top fermenting yeast have tendency to flocculate on the surface of the beer during the first few days of fermentation. Some of this yeast settles to the bottom of the fermenter, however the largest portion of the yeast remain dispersed in the fermentation. Top Fermenting yeast, used in ale production, grows best in the temperature range of 55–75 °F.

Bottom fermenting yeast, used in lager production performs better in the temperature range 55–32 °F. The fermentation is much slower using lager yeast, this time is often referred to as *Lagering* (Fig. 13.8).

Wine

Archeological evidence has traced winemaking to the Neolithic period (8500–4000 BC) in the Trans-Caucasus and ancient Near East (Michel et al. 1993). Tartaric acid is an excellent marker for grape wine because few other fruits produce tartaric acid in significant quantities. Measuring residues in pottery provides chemical evidence of ancient wine making (McGovern et al. 1997, 2004; Jackson 2008). Wine production first began in what is now Iran and spread throughout the Fertile Crescent. The technology was further developed by the ancient Mesopotamian, Egyptian, Greek, and Roman civilizations. Once the basic technology was established there was limited progress in wine processing. With the advent of modern chemistry by Dalton and the subsequent investigations of Pasteur brought wine chemistry forward as a modern discipline. The establishment of the role of microorganisms in fermentation in the late 1800s led to an improved understanding of the chemistry pioneered by Pasteur. The focus shifted to analysis of the major components that effect wine quality, including ethanol, sugars, and organic acids. Methods of analysis for ethanol and the other components, including some aroma compounds were described by Mulder in 1857. This was one of the earliest treatises to focus on wine chemistry as opposed to wine production (Ebeler and Thorngate 2009).

Wine is produced in many places around the world. Table 13.3 contains the 2014 wine production form some of the largest wine producing countries and the per capita consumption of some of the largest uses. It is interesting to note that the highest per capita wine consumption is the Vatican City State (54.26 L/year), Andorra (46.26 L/year) and Croatia (44.2 L/year). There

Table 13.3 Leading wine producing countries and wine consumption

Country	Production in liters	Per capita consumption (L/year)
France	4,670,100	42.66
Italy	4,473,900	33.30
Spain	3,820,400	21.26
United States	3,021,400	10.25
Argentina	1,519,700	24.46
Australia	1,200,000	24.53
South Africa	1,131,600	7.38
China	1,117,800	1.18
Chile	1,050,000	17.46

http://www.wineinstitute.org/files/World_Per_Capita_Wine_Consumption_Revised_Nov_2015.pdf

are hundreds of commercial varieties of wine grapes. In Tables 13.4 and 13.5 the flavor/aroma characteristics and the regions of production for major white and red wines are summarized.

There are many wine varieties cultivated but the *Vitis vinifera, L.ssp.* is the most prevalent for wine production. There are over 800 cultivars of *Vitis vinifera* in production world. There is a wide variation in cluster size, size, shape, sugar content and color of the grapes. Grapes are either table grapes for fresh consumption or wine grapes which can be used for Red, rose or white wine production.

Wine Grape Production

In order to maintain identity of grape varieties new grape vines are produced from cuttings of existing varietal vines which can be started directly in soil or they can be grafted onto existing older vines. If grapes were planted from seed, they would no longer be genetically identical to their parent, and would represent a different species. The only way to make more of a desired grape variety (Riesling) is to clonally propagate it, and grafting is a common approach.

During the second half of the nineteenth century phylloxera, a devastating louse was brought into Europe from American vines. Rootstocks were developed from North American species, including *V. riparia*, *V. rupestris*, and *V. berlandieri* which result in more robust vines. Most common are *Vitis riparia* based rootstocks.

Grapevines are trained to grow along a trellis supports it which enables the grower to determine the amount and positioning of both leaves and fruit. Newly planted grapevines require 4–5 years before reaching full productivity. Grape vines can continue producing viable crops for 50–100 years.

Table 13.4 Regions of production and tastes of some white wines

Variety	Regions of production	Flavor descriptors
Chardonnay	Chardonnay is the principle white wine of Burgundy (Bourgogne, France), where it originated. Areas of production include: California, Oregon, Washington, France, Australia, Italy, South Africa, Chili, Argentina	Chardonnay wines are often full-bodied (and more velvety) than other white wines. Notes include raspberry, vanilla, tropical fruit, butterscotch peaches, tea tomato, tobacco with rich citrus (lemon, grapefruit) flavors. Frequently aged in oak which results in buttery notes
Sauvignon blanc	Sauvignon blanc is grown in the Bordeaux region where it is blended with Semillon. The Loire valley and New Zealand produce some excellent sauvignon blanc varietals	Piercing aroma, fruity, grassy, herbal, green fruit, musky
Moscato/Muscat	Grows in most vine-friendly climates, including Italy, the Rhône Valley (where it is called muscat blanc à petits grains) and Austria (where it is called Muskateller) Also produced in California, Australia, and South America	Orange blossom rose petal, peach, apricot
Pinot grigio Pino Gris	Italy, France Pinot grigio is also grown in the western coastal regions of the USA. Loire Valley and Pinot gris in the rest of France. In Germany and Austria Pinot grigio is known as the Ruländer or Grauer Burgunder	Italy it is light bodied, with pear, citrus and mineral flavors French Alsace are richer rounder wines with spicy, honey, peach and pear flavor Oregon Alsace Pinot Gris providing aromatic, fruity flavors
Gewürztraminer	Gewürztraminer is from Alsace, Germany, the US West Coast, and New York. Also New Zealand	Typical taste is fruity flavors with floral aromas of rose petals, peaches, lychees, and allspice
Riesling	Riesling is the classic German grape of the Rhine and Mosel. Alsace and the Eastern US is also excellent but typically drier. Does well in New Zealand and Australia	Riesling wines are much lighter than Chardonnay wines. Green apple, citrus and peach flavors

Table 13.5 Regions of production and tastes of some red wines

Variety	Regions of production	Taste descriptors
Petite Sirah	Syrah excels in California, Australia, Argentina and Chile Burgandy, Oregon and New Zealand	Very dark, big tannins, plum, blackberry fruit flavors
Merlot	A major variety in Bordeaux blends, merlot is now grown in Italy, Romania, California, Washington State, Chile, Australia	Typical scents include black cherry, plums and jammy flavors. When aged on oak secondary flavors of vanilla, leather tobacco and earthy
Cabernet Sauvignon	Bordeayx left, Margaux, Pauillac, St.-Julien and Graves It is part of the great red Médoc wines of France, and among the finest reds in Australia, California and Chile	In Bordeaux the wine it is full bodied with black current high acidity and tannins In warmer climates exhibit black cherry, blueberry, menthol and medium body
Malbec	Malbec has its origins in the French Bordeaux region. Now the signature grape for Argentina vineyards near Mendoza	Malbec's characteristics vary greatly depending on where it is grown and how it is transformed. Ripe tannins and black fruit flavors Malbec is often blended with other varieties such as Cabernet Franc, Cabernet Sauvignon, Merlot
Pinot noir	Produces very high quality red wines of Burgundy, and good wines from Austria, California, Oregon, and New Zealand	Sour cherry and strawberry spicy with medium to low acidity and light tannins
Zinfandel	Primarily found in California, Zinfandel originates from Italy (where it is called primitivo)	Black fruit and spice flavors
Barbera	A classic red of Italian origin. Widespread in California	Juicy black cherry and plum fruit, a silky texture and excellent acidity

Sugar, pH and acid levels are the major factors for determining when grapes are ready for harvesting, however, tannins in the grapes also need to reach a certain level of maturity. Determining the optimum time of harvest is a critical viticultural decision. It is difficult to assess grape maturity in the vineyard and therefore predict the ultimate wine quality. In vineyards now, ripeness evaluation includes more than an analysis of Brix (sugar content), titratable acidity and pH. Winemakers use flavor/aroma assessment in addition to the routine standards.

In viticulture the onset of ripening is called veraison. Veraison represents the transition from berry growth to berry ripening, and many changes occur at veraison. Prior to veraison the grapes are hard and green with low sugar levels and very high acid levels (primarily malic acids). Veraison lasts a week or less. During this period the grapes go through several changes which impact their sugar, acid, tannin and mineral composition. The concentration of phenolic compounds in the skin, primarily anthocyanins in red wine grapes which replace the chlorophyll resulting in the color changes in the berries. Malic acid is consumed as the primary metabolic substrate. Tartaric acid stays unchanged as a result, tartaric acid, becomes the predominant acid in the grape berries Liang et al. (2011). Concomitant with the decrease in acidity hexose sugars accumulate in the berries. Sucrose produced by photosynthesis is transferred from the leaves to the berries where it is hydrolyzed to glucose and fructose. Physiologically, the sugar concentration increases to levels above 18% occasionally reaching 25%. Most grapes are harvested at between 18 and 25% sugar content. The greater the concentration of sugars in the grape juice, the greater the potential for alcohol production. Grapes for sweet wines, such as dessert wines are allowed to ripen longer, producing more sugar than will be fermented leaving a sweet wine.

Also: titratable acidity also decreases because (i) the berry expands, approximately doubling in size, and (ii) there is exchange of H^+ for K^+ and other cations by the berry Veraison occurs from 30 to 70 days depending on the climate.

The decrease in free acids, combined with the buildup of potassium, results in a rise in the pH level of the grape juice. The general relationship between the concentrations of the cations, potassium and sodium, and acidity is expressed as a function of the titratable acidity. The balance among potassium and sodium contents and the tartrate to malate ratio determine the pH and titratable acidity of the grape juice (Boulton 1980).

The color of the grape berries change with the development of phenolic compounds including anthocyanins in the skins. *Vitis vivifera* grapes contain non-volatile aroma precursors including unsaturated lipids, carotenoids, S-cysteine conjugates, glucoconjugates and S-methylmethionine, These non-volatile components are transformed into volatile compounds providing the characteristic aromas of wine varieties during the progression of the grapes from crushing through aging and storage. Flavor precursors are primarily glycosides and S-amino acid conjugates. Additionally, the concentration of tannins in the grape increases in several areas of the grape including the skin, seeds and stem.

Most strains of winemaking yeast have difficulties surviving in an alcohol solution above 15% alcohol This leaves a certain amount of residual sugar which influences the sweetness of the wine.

The presence of alcohol (particularly ethanol) in the wine contributes much more than just intoxication. It has an immense impact of the weight and mouthfeel of the wine as well as the balance of sweetness, tannins and acids. In wine tasting, ethanol diminishes perception of acids and tannins, and increases perception of body.

As sugar levels in the grape rise, acid levels fall. Wines need some degree of acidity in order to have a balanced flavor. The major acids in wine are tartaric and malic acid with citric and succinic acids playing a minor role. Titratable acidity is *not* a measure of tartaric acid. Rather, wine acidity is expressed in units of tartaric acid equivalents. A wine can have 0 g/L tartaric acid, but still have 5 g/L "as tartaric acid".

Wine Production

After the harvest, the grapes are taken into a winery and prepared for the primary fermentation. Figure 13.9 outlines the divergent processes for the production of red or white table wines. Red wine is produced from the must (pulp) of red or black grapes and fermentation occurs together with the grape skins, which give the wine its color. White wine is produced by fermenting juice from crushed grapes where the skins are removed.

Crushing is the process where the berries are squeezed breaking the skins releasing the juice from the berries. The presence of stems in the mix facilitates pressing by allowing juice to flow past flattened skins. More common would be to add only the crushed fruit, sometimes enzyme treated. In red wine production stems of the grapes are usually removed before fermentation because the stems have relatively high tannin content; in addition to tannin they can also give the wine a vegetal aroma (due to extraction of 2-methoxy-3-isobutylpyrazine which has an aroma reminiscent of green bell peppers). This is one of many undesirable compounds; the stems take up room in the fermenter that could otherwise hold grapes.

Crushed grapes are often treated with sulfites or sulfur dioxide which is often added as SO_2 gas, to protect constituents in the juice that are sensitive to oxidation. The treatment prevents enzymatic browning caused by the polyphenoloxidase enzyme. The treatment also suppresses the growth of undesirable microbes including acetic acid producing bacteria, wild yeasts and molds.

Wine fermentation can occur as a result of the natural yeasts found on the surface of the grapes or after inoculation of pasteurized wine must. Wild yeasts include *Saccharomyces apiculatus and exiguous.* Commercial wine yeasts used for inoculation are derived from *Saccharomyces cerevisiae* var. *ellipodes* or *pastorianus.* The pure wine yeast strains use for inoculation are chosen for their desirable fermenting characteristics. These yeast strains can produce high alcohol wines containing up to 15.5% alcohol. Typically,

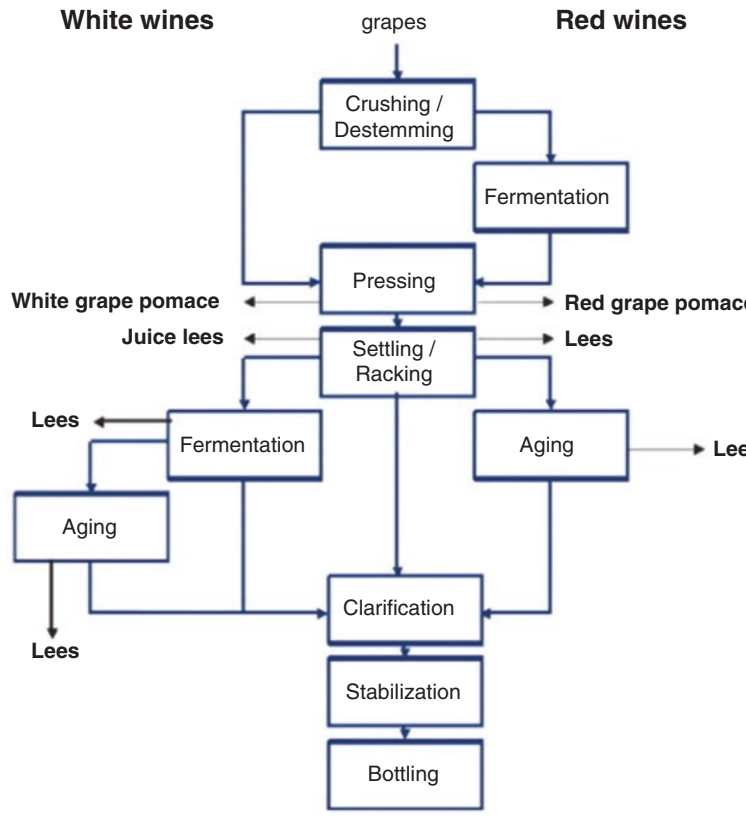


Fig. 13.9 Flow diagram for the production of red and white wine from crushing to bottling

these strains which are used for red wine fermentation are resistant to alcohol and tannins. Some strains of yeast are resistant to sulfites thus they are more useful in sulfite treated wines.

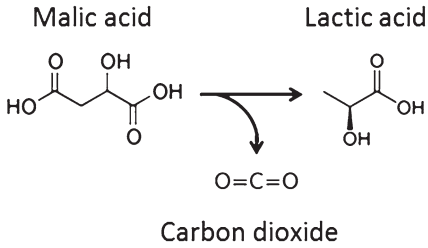
After inoculation of the wine must with yeast, white wines are typically fermented up to 21 days at below 20 °C and red wines are fermented at 20–24 °C. At the end of the primary fermentation (5–7 days) most of the sugar has been converted to alcohol. During this period tannins, protein, tartrate and cell debris settle to the bottom of the fermentation vessel. Yeast is normally already present on the grapes. The primary, or alcoholic fermentation can be done with the natural yeast, however results can be unpredictable depending on the exact types of yeast that are present. For this reason specific cultured yeast is often added to the must.

Red wines derive most of their color from grape skins, therefore contact between the juice and skins are essential for color extraction. Red wines are produced by destemming and crushing the grapes into a tank and leaving the skins in contact with the juice throughout the fermentation (maceration). White wines can be produced from red grapes by pressing of uncrushed fruit minimizing the contact between grape juice and skins. One example is the production of blanc de noirs a sparkling wine, manufactured from Pinot noir, a red vinifera grape. Almost all white wines are produced with crushing and destemming They are pressed directly as picked to avoid extraction of tannins from the skins or grape seeds. Some winemakers crush white grapes allowing a short period of skin contact, usually for 3–24 h.

After alcoholic fermentation, some wines can undergo a secondary fermentation known as malolactic fermentation. This process is particularly desirable for high-acid wine because it reduces the acidity of the wine by the conversion of the dicarboxylic L-malic acid to the

monocarboxylic L-lactic acid and carbon dioxide. This process is conducted by the action of lactic acid bacteria, including Oenococcus oeni, Leuconostoc spp., and Pediococcus spp. (Liu 2002).

During or after the alcoholic fermentation, a secondary, or malolactic fermentation can also take place where specific strains of bacteria (lactobacter) convert malic acid into the milder lactic acid. The fermentation is carried out by the lactic acid bacteria, *Oenococcus oen*i, and various species of *Lactobacillus* or *Pediococcus*. The primary function malolactic fermentation is to convert L-malic acid, to L-lactic acid. This conversion is accompanied by the production of carbon dioxide.



The sourness decreases because the titratable acidity decreases because lactic is monoprotic and malic is diprotic Lactic acid tastes less sour than malic acid.

Although acid reduction is the most obvious result of the growth of lactic acid bacteria in wine, their action can also significantly modify the wine's aroma, flavor and mouthfeel. *Oenococcus oeni* typically produces substances that have pleasant and O. oeni does not produce off-aromas like many other spoilage lactic acid bacteria in wine. The metabolism of sugars by certain lactic acid strains can result in formation of acetic acid and other undesirable compounds: intentional malolactic fermentation can stabilize a wine by preventing growth of spoilage lactic acid bacteria. Lactic fermentation also produces diacetyl is the most important of these substances, it provides the buttery characteristic in the wine.

Pressing is the act of applying pressure to grapes or pomace in order to separate juice or wine from grapes and grape skins. Pressing is not always a necessary act in winemaking; if grapes are crushed there is a considerable amount of juice immediately liberated (called free-run juice) that can be used for vilification. Typically, this free-run juice is of a higher quality than the press juice, however most wineries use presses to increase yield of juice by 15–30%. Increasing the pressure increases the amount of tannin extracted from the skins, rendering the pressed juice excessively tannic or harsh.

In winemaking cold stabilization is used to facilitate the precipitation of potassium tartrate crystals from the wine. These tartrate crystals look like grains of clear sand. The cold stabilizing process is done immediately after fermentation, the temperature of the wine is dropped to near freezing temperatures and is held for 1–2 weeks. Large wineries more commonly use ion exchange, electrodialysis, and/or addition of substances that inhibit precipitation.

Bentonite addition is often used to remove haze-causing proteins from white and rose wines.

Sulfur dioxide is added to wine to prevent oxidation and to undesired fermentation or spoilage. It is normally added either as liquid sulfur dioxide or as sodium or potassium metabisulfite. In the production of white wine sulfur dioxide can be added prior to or immediately after alcoholic fermentation. When it is added after alcoholic fermentation it will have the effect of preventing or stopping malolactic fermentation, bacterial spoilage and help protect against the damaging effects of oxygen.

Wine contains both volatile and non-volatile compounds that contribute to the flavor and aroma of wine. During the fermentation and first months of aging, chemical reactions occur resulting in changes in the aroma. As a wine ages and matures, changes and developments in aroma will continue to take place but at a slower rate.

Wine grapes contain many non-volatile precursors for aroma and flavor including unsaturated lipids, carotenoids, S-cysteine, conjugates, glycoconjugates and S-methylmethionine. These procursors are non-volatile and odorless; however, they are susceptible to chemical and/or enzymatic reactions that occur during processing and aging. These reactions are often driven by the genetic makeup of the particular wine

variety as well as processing and storage conditions (Baumes 2009).

Wine aroma is a compilation of many factors including compounds derived from multiple biochemical sources and the processes of wine making. The contribution of the grape derived aroma compounds include monoterpenes, norisoprenoids, aliphatics, phenylpropanoids, methoxypyrazines and volatile sulfur compounds (Ebler and Thorngate 2009, González-Barreiro et al. 2013). Secondary metabolites from microbial metabolism of sugar, fatty acids, cinnamic acid, organic nitrogen compounds (proteins, nucleic acids and pyrimidines) all can contribute to aroma compounds found in wine (Chatonnet et al. 1992; Herraiz and Ough 1993; Bartowsky and Pretorius 2009; Robinson et al. 2014). Terpenes are altered by acid conditions (Skouromounis and Sefton 2002; Versini et al. 2002) and by enzymatic catalysis modify both nonaroma and aroma active products(Rapp 1998, Ugliano 2009). Oxidative processes in wine take place during winery production of wine, storage and packaging (Karbowiak et al. 2009; Ghidossi et al. 2012).

Tasting wine is essentially the act of smelling these vaporized aroma compounds and sensing the taste factors on the tongue. Wines differ widely in both volatile aroma compounds as well as non-volatile compounds that impact taste. Polaskova et al. (2008) have summarized some of the major interactions associated with grape and wine flavor. Table 13.6 summarizes the taste attributes and chemical compounds responsible for sensor responses in wine. It is also important to remember that the color comes from anthocyanins in the red wine.

As mentioned different grapes and resulting wine result in the wide range of aromas we experience in wine varieties. In Table 13.7 some of the primary odorants and their characteristic aroma are described.

Wine aromas typically contain hundreds of volatile compounds. The use of GCMS combined with aroma ports on the gas chromatograph has allowed identification of the compounds that contribute most to the aroma of the wine. Table 13.7 identifies some of the most important providing the base of wine flavor.

Table 13.6 Sensory modalities and selected chemical components contributing to grape and wine flavor (from Polaskova 2008)

Sensory modality	Attribute	Chemical compounds in wine
Taste	Sweet Sour Salty Bitter	Glucose, fructose, glycerol, ethanol Tartaric acid Sodium chloride, potassium chloride Catechin
Aroma	Floral, Lily of the valley Banana like	Linalool Isoamyl acetate
Tactile	Viscosity Astringency Pungency/heat	Polysaccharides, mannoproteins Tannins Ethanol
Color	Red	Anthocyanins

Wine aroma is highly variable among wines and many classes of compounds contribute. Some of the significant contributors are monoterpenes, norisoprenoids, aliphatics, higher alcohols,esters,phenylpropanoids,methoxypyrazines, and volatile sulfur (Francis and Newton 2005; Ebeler and Thorngate 2009).

In white wine, monoterpenes are contributors to the aroma of Muscat, Gewürztraminer and Riesling wines (Ribéreau-Gayon et al. 1975; Rapp 1998; Mateo and Jimeńez 2000). Linalool and α-terpineol in contribute a floral note in the white wines. (Williams et al. 1981; Günata et al. 1985; De La Presa-Owens and Noble 1997; Lee and Noble 2003, 2006; Campo et al. 2005; Robinson et al. 2014).

Norisoprenoids are present in all wine grapes and are at higher levels in highly aromatic grape cultivars (Winterhalter et al. 1990a; Schneider et al. 2001). The norisoprenoids are important contributors to the aroma of many wine varieties including Semillon, Sauvignon blanc, Chardonnay, Merlot, Syrah, and Cabernet Sauvignon (Razungles et al. 1993; Sefton et al. 1993, 1994, 1996; Sefton 1998). The norisoprenoids are derived from carotenoids found in in grapes and wines. β-Carotene and lutein constitute 85% of the carotenoids, with minor contents of neochrome, neoxanthin, violaxanthin, luteoxanthin, flavoxanthin, lutein-5,6-epoxide

Table 13.7 Impact odorants contributing to varietal aromas of selected wines (from Polaskova 2008)

Variety	Odorant	Odor quality	Sensory[a]	References
Muscat	Linalool, Geraniol, nerol	Floral	170	Stevens et al. (1966), Wenzel and deVries (1968)
Riesling	1,1,6-Trimethyl-1,2-dihydronaphthalene	Kerosene, bottle age	20	Ohloff (1978), Simpson (1978)
Cabernet Sauvignon, Sauvignon blanc Cabernet franc, Merlot Carmenere	3-Isobutyl-2-methoxypyrazines	Bell pepper	2	Lacey et al. (1991), Allen et al. (1991), Roujou et al. (2000), Belancic and Agosin (2007)
Gerwurtzraminer	*Cis*-Rose oxide	Geranium oil, carrot leaves	200	Bauer et al. (1997), Guth (1995, 1996, 1997a, b)
Vitis labrusca, *Vitis rotundfolia*	o-Aminoacetophenone	Foxy, sweet	0.2 (in air)	Acree et al. (1990), Baek et al. (1997)
Sauvignon blanc Scheurebe	4-Methyl-4-mercaptopentan-2-one	Black current	0.6	Guth (1997a, b, 1998)
Grenache rose, Sauvignon blanc, Semillon	3-Mercapto-hexanol	Grapefruit, citrus peel, Passion fruit	50	Ferreira et al. (2002), Tominaga et al. (2006)
Shiraz	Rotundone	Black pepper	16	Siebert et al. (2008), Wood et al. (2008)

[a]Threshold in water ng/L

and zeaxanthin, and *cis* isomers of lutein and β-carotene the next most abundant (Mendes-Pinto 2009). Carotenoids accumulate prior to veraison in the grape exocarp (skin) (Razungles et al. 1988; Robinson et al. 2014).

Volatile phenylpropanoids are derived from hydrolyzed of glycoside in juices and wines, in Chardonnay wine phenylpropanoids make up 10 to 20% of the total hydrolyzed volatile fraction in Chardonnay juice (Sefton et al. 1993). Their flavor attributes include the dried fig, tobacco, and chocolate aromas in Cabernet Sauvignon and Merlot musts (Francis et al. 1998). Methyl anthranilate, which is responsible for the distinctive "foxy" aroma and flavor of the Washington Concord grape (Vitis labrusca) (Wang and De Luca 2005) and may also contribute to the aroma of Pinot noir (Moio and Etievant 1995; Robinson et al. 2014).

The peppery, pea or asparagus aromas in wines come from the 3-alkyl-2-methoxypyrazines, including 3-isobutyl- 2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP), and sec-butyl-2-methoxypyrazine (SBMP) (Sala et al. 2004). These compounds have very strong aromas and are at very low concentrations in wine (ng/L), however they are important to the overall aroma of the wines. It is important to recognize that low levels of methoxypyrazines contribute to varietal character, however, high levels are generally considered undesirable. Methoxypyrazines contribute to the characteristic aroma of both grape juice and wines including Sauvignon blanc (Allen et al. 1991; Lacey et al. 1991), Cabernet Sauvignon (Allen et al. 1991, 1994), Cabernet franc (Roujou de Boubée et al. 2000), Merlot (Sala et al. 2004; Belancic and Agosin 2007; Robinson et al. 2014).

The basis for wine flavor crosses over many varieties of grape and wine. Ferreira (2010) identified the aroma compounds that for base background for many wines. They are summarized in Table 13.8.

The conjugated thiol precursors are highest in the skin (Roland et al. 2011). The glutathione can be conjugated to the C6-aldehyde, (E)-2-hexenal, which is presumably reduced enzymatically to the alcohol by alcohol dehydrogenase (Capone and Jeffery 2011). It has also been discovered that grapes can contain 3-S-cysteinylglycinehexan-1-ol, an intermediate in the catabolism of the glutathione conjugate to the cysteine conjugate (Capone et al. 2011).

Table 13.8 Aroma compounds forming the base of wine flavor (Ferreira 2010)

Miscellaneous	Fusel alcohols	Organic acids	Isoacids	Organic acid ethyl esters	Fusel alcohol acetates	Ethyl esters of isoacids
Ethanol Diacetyl Acetaldehyde	Isobutanol Isoamyl alcohol Hexanol β-Phenylethanol Methionol	Acetic acid Butyric acid Hexanoic acid Octanoic acid Decanoic acid	Isobutyric acid 2-Methyl butyric acid Isovaleric acid	Ethyl acetate Ethyl butyrate Ethyl hexanoate Ethyl octanoate Ethyl decanoate	Isobutyl acetate Isoamyl acetate β-Phenylethyl acetate	Ethyl isobutyrate Ethyl 2-methyl butyrate Ethyl isovalerate

Thiols/Mercaptan-sulfur compound can contribute an aroma of garlic and onion that is considered a wine fault. They have also been found to contribute to some of the varietal aromas associated with Cabernet Sauvignon, Gewürztraminer, Merlot, Muscat, Petit Manseng, Pinot blanc, Pinot griso, Riesling, Scheurebe, Semillon and Sylvaner. Volatile thiols can result in pleasant herbaceous, fruity, mineral, smoky, and toasty aromas in wine (Dubourdieu and Tominaga 2009). The predominant volatile sulfur compounds in wines are H_2S, methanethiol, dimethylmercaptans (dimethylsulfide, dimethyldisulfide, dimethyltrisulfide), methylthioesters (S-methyl thioacetate, S-methyl thiopropanoate, and S-methyl thiobutanoate), and liberated glutathione and cysteine polyfunctional thiols (4-mercapto-4-methylpentan-2-one, 4MMP; 3-mercaptohexan-1-ol, 3MH; and 3-mercaptohexyl acetate, 3MHA) (Swiegers and Pretorius 2007; Dubourdieu and Tominaga 2009; Roland et al. 2010). Many other sulfur-containing compounds have been identified in wines (Mestres et al. 2000, 2002; Bailly et al. 2006; Sarrazin et al. 2007; Dubourdieu and Tominaga 2009).

Some of the aromas perceived in wine are from esters created by the reaction of acids and alcohol in the wine. Esters can develop during fermentation, with the influence of yeast, or during aging. Yeast strain used during fermentation and temperature are the primary factors controlling ester formation. Wines eventually approach an equilibrium where with alcohols, acids, and esters. During processing and aging the bouquet of the wine is constantly changing due to the concentration, formulation and splitting of different esters. This is partly the reason why a wine will have one set of aromas at one time and other aromas later in its life (Bardi et al. 1999).

During alcoholic fermentation yeast can use amino acids in several ways, particularly for protein synthesis, or for other metabolic processes. Because grapes are low in Nitrogen compounds, available Nitrogen often limits growth of yeast. The nitrogen composition of the grape must affects not only the kinetics of alcoholic fermentation, but also the production of aromatic compounds, ethanol, and glycerol (Hernandez-Orte et al. 2005). Yeast strains differ greatly in their ability to use nitrogen and amino acids (Carrau et al. 2008).

Many flavor and aroma compounds are formed from amino acids are alcohols, associated esters and volatile acids Amino acids can be catabolized into higher alcohols by the pathway referred to as the Ehrlich reaction. In Fig. 13.10 the Ehrlich reactions and products marked with an asterisk. The Ehrlich pathway compounds are generally of less importance than the compounds produced by the anabolic pathway. In addition to the three nonpolar branched-chain amino acids (valine, leucine, and isoleucine), other amino acids can also be broken down to other metabolites via this reaction (Table 13.8) (Ardö 2006). The most important odor-related products (higher alcohols and volatile fatty acids) shown in Table 13.8 are produced from valine, leucine, and isoleucine. The first step is a transamination reaction in which the amino group from the amino acid is transferred to α-ketoglutarate to form an α-keto acid and glutamate (Fig. 13.10) (Conway and Hutson 2000; Davoodi et al. 1998; Taylor and Jenkins 1966).

Many fermentation volatiles that are important to wine aroma are metabolic products from yeast metabolism of hexose sugars. Straight-chain fatty acids and their corresponding ethyl esters were derived almost exclusively from hexoses. Most fusel alcohols and their acetate esters are derived from hexose metabolism in the yeast indicating the importance of anabolic pathways in their formation Fig. 13.11 illustrates the ratios of volatile compounds arising from the metabolism of hexoses (Nisbet et al. 2014).

From this it can be seen that the chemical reactions that influencing wine flavor and aroma are complex and varied. Wine flavor and aroma continues to be an intense area of research. Professional wine tasters will often mentally cycle through a list of potential aromas (and may use visual aids like the aroma wheel (Fig. 13.12) developed by Ann C. Noble of University of California, Davis) until one choice stands out and can be identified in the wine (http://winearomawheel.com/).

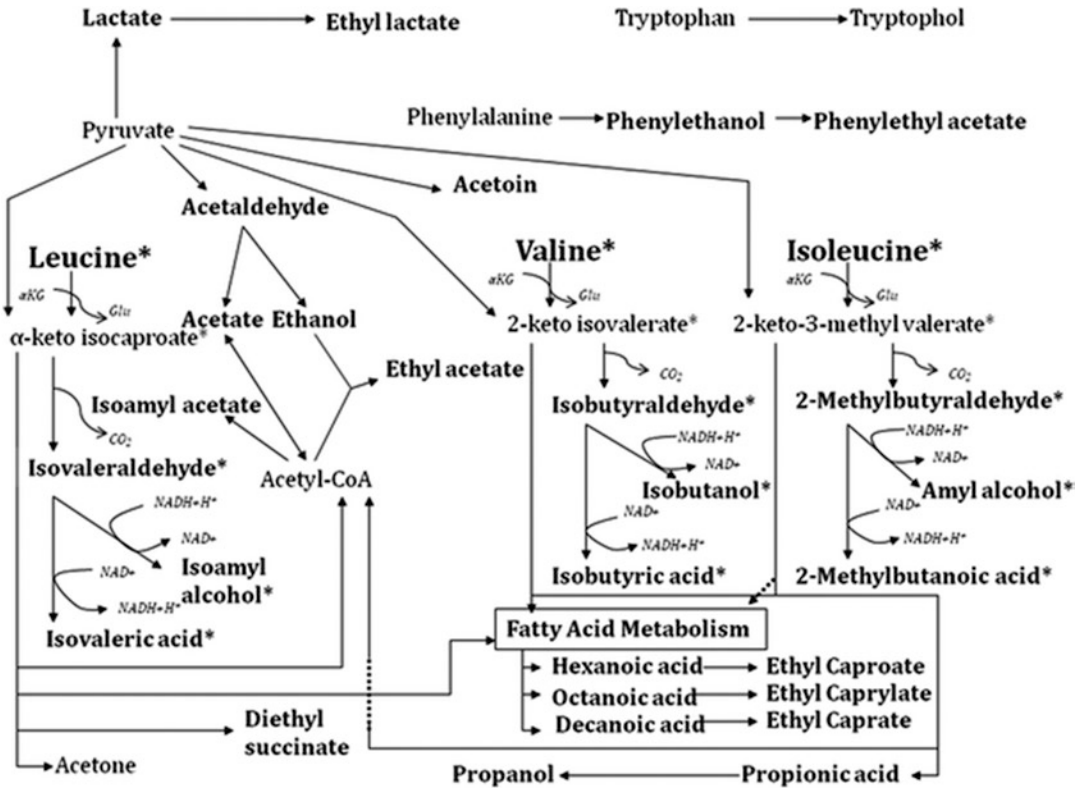


Fig. 13.10 A simplified metabolic map of yeast aroma compound production, indicating known metabolic linkages. *Bold type* indicates aroma compounds important to this study. Compounds marked with an *asterisk* constitute a diagrammatical representation of the Ehrlich pathway, responsible for the production of higher alcohols and volatile acids. Cofactors and transition metabolites are shown in *italics*. αKG is α-keto glutamate and Glu is glutamate (Polášková et al. 2008)

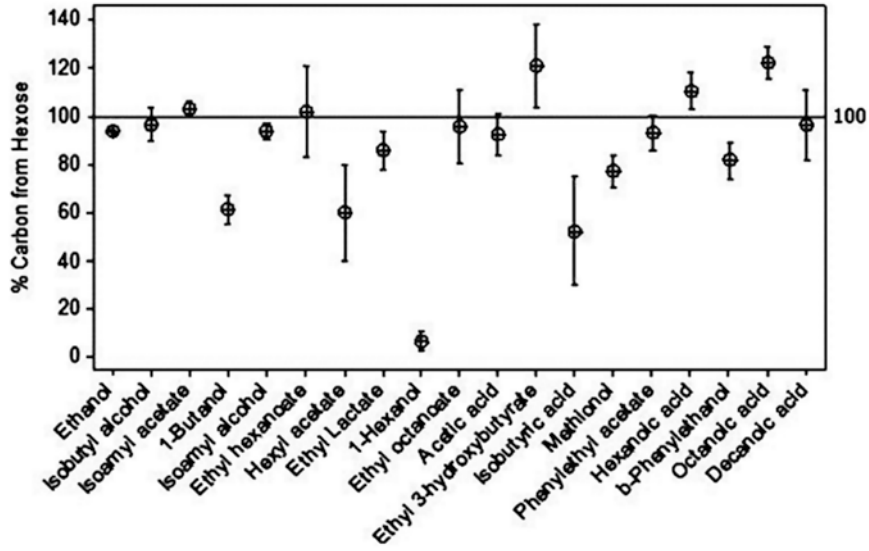


Fig. 13.11 Percentage of carbon found in volatiles that originated from hexoses during yeast metabolism. The mean is indicated by the *circles*, and the error bars represent the 95% confidence interval

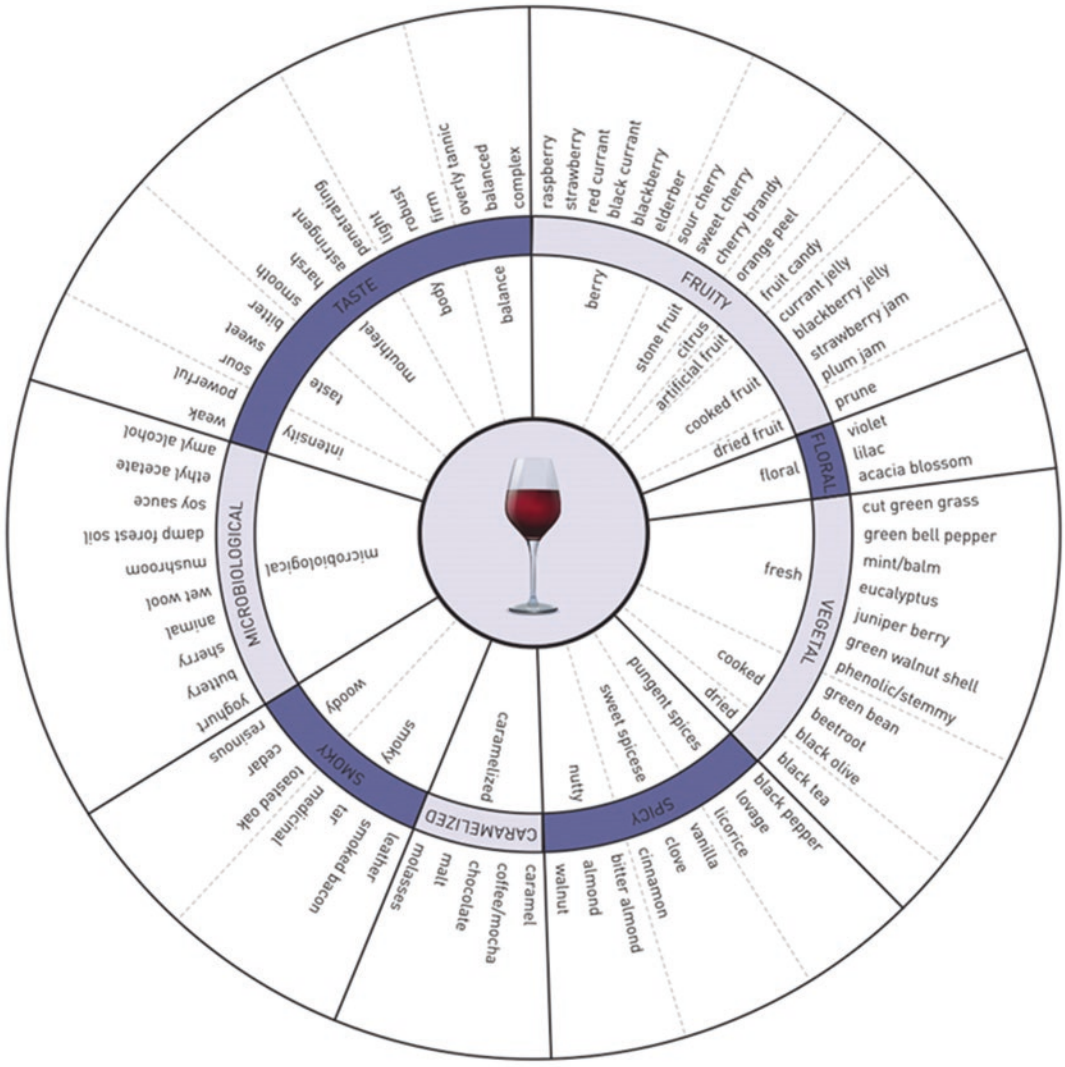


Fig. 13.12 Wine aroma wheel

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Genetically Modified Crops

14

W. Jeffrey Hurst and John W. Finley

When man evolved from a hunter gatherer they began to grow crops for food. They found that selection of crops improved the quality and yield of foods for food production. The selection of seeds led to the evolution of new crops that are more productive and nutritious crops.

The domestication of plants by humans to produce plants with more desirable traits than wild plants drove early man to become dependent on identification and propagation of plants with desirable attributes. Seed selection for desired traits began between 9000–11,000 years ago. Initially early farmers simply selected food plants with particular desirable characteristics, and employed these as progenitors for subsequent generations, resulting in an accumulation of valuable traits over time.

1856 and 1863 Gregor Mendel investigated of plant hybridization led to his laws of inheritance. This work became well known in the 1900s and formed the basis of the science of genetics. The laws he developed stimulated research by many plant scientists dedicated to improving crop production through plant breeding. He conducted hybridization experiments with garden peas (*Pisum sativum*) which led to two generalizations which later became known as *Mendel's Principles of Heredity* or *Mendelian inheritance*. Mendel discovered that, when he crossed purebred white flower and purple flower pea plants (the parental or P generation), the result was not a blend. Rather than being a mix of the two, the offspring (known as the F_1 generation) was purple-flowered. When Mendel self-fertilized the F_1 generation pea plants, he obtained a purple flower to white flower ratio in the F_2 generation of 3 to 1. The results of this cross are tabulated in the Punnett square in Fig. 14.1. Mendel hypothesized that allele pairs segregate, from each other during the production of gametes. Because allele pairs separate during gamete production, a sperm or egg carries only one allele for each inherited trait. When sperm and egg unite at fertilization, each contributes its allele, restoring the paired condition in the offspring. This is called the Law of Segregation. The presence of an allele does not mean that the trait will be expressed in the individual that possesses it. If the two alleles of an inherited pair differ (the heterozygous condition), one determines the organism's appearance and is called the dominant allele; the other has no noticeable effect on the organism's appearance and is called the recessive allele. Thus, in the example above dominant purple flower allele will hide the phenotypic effects of the recessive white flower allele. This is known as the Law of Dominance. These laws formed the basis of understanding inheritance and allowed more effective selection in breeding of plants.

Gregor Mendel's experiments with plant hybridization led to his establishing laws of inheritance. Once this work became well known, it formed the basis of the new science of genetics, which stimulated research by many plant

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_14

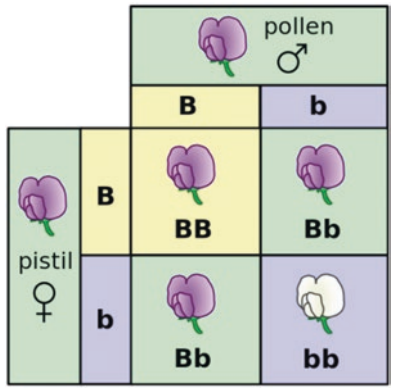

Fig. 14.1 Representation of the Punnett square for inherence of flower color in pea plants the https://commons.wikimedia.org/w/index.php?curid=2063426

scientists dedicated to improving crop production through plant breeding.

However, successful commercial plant breeding concerns began to be founded from the late nineteenth century. Gartons Agricultural Plant Breeders in England was established in the 1890s by John Garton, who was one of the first to cross-pollinate agricultural plants and commercialize the newly created varieties. He began experimenting with the artificial cross pollination first of cereal plants, then herbage species and root crops and developed far reaching techniques in plant breeding (Graften and Ridley 2006; Ford 1960).

In the late nineteenth and early twentieth centuries the better understanding of genetics provided plant breeders, with the tools to make modifications in plants with increasing precision. They changed the expression of traits in plants by crossing specific parent plants to produce new varieties with desired traits. They also developed more rapid methods to generate and detect genetic changes. The improved technology led to targeted and more efficient breeding of improved varieties (Mba2013). DNA mutation rare in nature (Ossowski et al. 2010), but scientists applied mutagenic chemicals or radiation to induce mutations in DNA at increased rates (Roychowdhury and Tah 2013) thus increasing the genetic variation in the species. Both natural and induced mutations are random so breeders must evaluate the progeny sorting for desired changes as well as undesired changes. In 1973, Cohen et al. described recombinant-DNA (rDNA) techniques that allowed scientists to cut gene sequences from the DNA of one organism and splice them into the DNA of another organism (Cohen et al. 1973), the path was paved for a new approach to increase genetic diversity for use in breeding organisms, including crops hence genetic engineering.

Many present-day crops currently under cultivation are traceable to plant domestication in ancient times. Nearly all of the domesticated plants used today for food and agriculture were domesticated in the various centers of origin around the world. In these centers there is still a great diversity of closely related wild plants. For example wheat in its early form came from the Fertile Crescent and corn as we know it came from Central America.

One major technique of plant breeding is selection, the process of selectively propagating plants with desirable characteristics and eliminating or "culling" those with less desirable characteristics.

Deliberate interbreeding of closely or distantly related individuals produces new crop varieties or lines with desirable properties. Plants are crossbred to introduce traits/genes from one variety or line into a new genetic background. One example is when a mildew-resistant pea is crossed with a high-yielding but susceptible pea the new cross bred peas with mildew resistance without losing the high-yield characteristics. Progeny from the first cross would then be crossed with the high-yielding parent to ensure that the progeny were most like the high-yielding parent in a step referred to as backcrossing. Plants may also be crossed with themselves to produce inbred varieties for breeding.

Classical breeding relies largely on homologous recombination between chromosomes to generate genetic diversity. Conventional plant breeders also make use *in vitro* techniques such as protoplast fusion, embryo rescue or mutagenesis to generate diversity and produce hybrid plants that would not exist in nature.

Breeders have attempted to incorporate the following traits into crops using conventional techniques:

1. Improved quality, such as increased nutrition, improved flavor, or greater beauty
2. Increased yield of the crop
3. Increased tolerance of environmental pressures (salinity, extreme temperature, drought)
4. Resistance to viruses, fungi and bacteria
5. Increased tolerance to insect pests
6. Increased tolerance of herbicides
7. Longer storage period for the harvested crop

The development of modern day corn serves as an example of the evolution of corn which began about 10,000 years ago. Starting with Teosinte we see the directed evolution of corn through selective plant breeding. Figure TSC1 illustrates the evolution of corn from teosinte to modern hybrid corn. The farmers selected corn seeds from crops that were easier to grind, tasted better or had larger kernels. The evolution from teosinte to modern corn illustrates man's direction of genetic selection to yield enhanced genotypes of a crop. It is important to realize that this is an early example of a genetically enhanced organism which resulted from selection and cross breeding. Figure 14.2 illustrates the evolution both the tassel (male) and ear (female) portion of corn from the parent teosinte to modern hybrid corn.

These breeding techniques resulted in large yield increase in the United States in the early twentieth century. After World War II, the Green Revolution increased crop production in the developing world in the 1960s. These breakthrough were based on three essential crops; maize, wheat and rice. The development of hybrid maize was followed by high-yielding and input-responsive "semi-dwarf wheat" (for which the CIMMYT breeder N.E. Borlaug received the Nobel prize for peace in 1970), and third came high-yielding "short statured rice" cultivars.

Dwarfing of wheat delivered a critical agronomic quality of dwarf plants with thick stems making them less prone to collapse under the

Fig. 14.2 Evolution of maze from teosinte to modern corn

weight of the extra grain — a trait called lodging. Borlaug worked wheat that had tall, thin stalks. The taller of wheat grasses better compete for sunlight, but tend to collapse under the weight of the extra grain resulting from rapid growth spurts from Nitrogen fertilizer. In 1953, he acquired a Japanese dwarf variety of wheat called Norin 10 developed by Orville Vogel, that had been crossed with a high-yielding American cultivar called Brevor Reitz (1970). Norin 10/Brevor is semi-dwarf (one-half to two-thirds the height of standard varieties) and produces more stalks and thus more heads of grain per plant. Borlaug crossbred the semi-dwarf Norin 10/Brevor cultivar with his disease-resistant cultivars to produce wheat cultivars that were adapted to tropical and sub-tropical climates (Hedden 2003).

In efforts to develop new varieties of grains seeds were submitted to mutation breeding. Mutation breeding is the process of exposing seeds to chemicals or radiation in order to generate mutants with searching for desirable traits to be bred with other cultivars. The mutated seeds were grown to screen for positive characteristics From 1930 to 2014 more than 3200 mutagenic plant varietals have been released (Schouten and Jacobsen (2007; FAO/IAEA 2014). The crops released are either direct mutants (70%) or their progeny (30%) (Maluszynsk et al. 2000), ot which food and agricultural crops account for 75% of released mutagenic species (Ahloowali 2004). The FAO/IAEA reported in 2014 over 1000 mutant varietals of major staple crops were being grown worldwide.

It is important to realize that mutation breeding produces completely random mutations which ultimately result in new or modified proteins in the new plant. The process is very tedious and it can take many years to identify a new and useful plant.

The accumulation of this previous work illustrates that plant scientists have been "engineering" plants for at least 10,000 years. In the early 1990s the next step in the evolution of the technology was to move genes from one species to another by direct gene insertion. The process is significantly faster and more precise than mutation breeding of crossing and back crossing. It also opened the possibility of plants with many new and useful attributes.

These directed changes in plants which includes direct transfer of a desired DNA from a microorganism or another plant into a trans-genic species that has the desired characteristics. Figure 14.3 illustrates the principal of gene insertion in plants.

Intensive research in molecular genetics has led to the development of recombinant DNA technology. Advancement in biotechnological techniques has opened many possibilities for breeding crops. Mendelian genetics allowed plant breeders to perform genetic transformations in a few crops, molecular genetics has provided the key to both the manipulation of the internal genetic structure, and the "crafting" of new cultivars with targeted attributes such as virus resistance, insect resistance and herbicide tolerance.

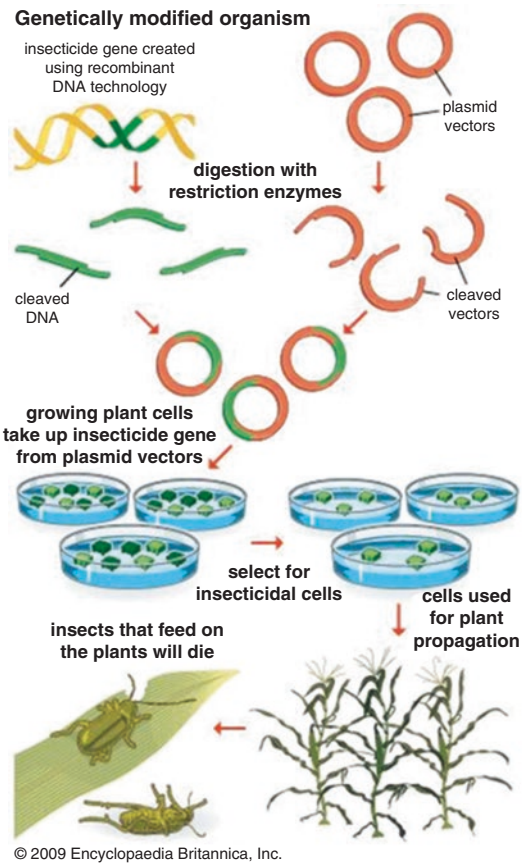

Fig. 14.3 The principal of gene insertion in DNA

Recombinant DNA technology is the joining together of DNA molecules from two different species which are inserted into a host organism to produce new genetic combinations. The recombinant organisms have value and promise in medicine, agriculture, and industry. Recombinant DNA technology allows the isolation of a segment of DNA or a gene for a specific protein. With that fragment the nucleotide sequence can be determined, the transcripts, mutate the sequence in highly specific ways if needed, and reinsert the modified sequence into a living organism. Both agriculture and medicine have benefited significantly from this technology. It should be pointed out that it is one more step in the timeline of plant science and improving our crops. Currently crops or foods modified by modern genic technology are referred to as Genetically Modified Organisms or GMOs. Simplistically GMO's have their genetic composition altered hence they can code for a new property. The gene needs a mechanism to turn it on. This on switch is called the promoter segment. One of the more widely used promoters is named 35S. When a new GMO has been developed with a new trait, the resulting gene construct is called an event with events being developed regularly. These events undergo various regulatory and safety reviews before being approved for use. One area that unfortunately is growing is the development of unapproved events.

Genetically modified (GM) foods were first approved for human consumption in the United States in 1994, and by 2014–2015 about 90% of the corn, cotton, and soybeans planted in the United States were GM. By the end of 2010, GM crops covered more than ten million square kilometres (3.86 million square miles) of land in 29 countries worldwide—one-tenth of the world's farmland. The majority of GM crops were grown in the Americas. In the agricultural arena, the technique was applied to soybeans in 1988, paving the way for one of the most successful crops, glyphosate tolerant soy. While of substantial importance to commercial agriculture, very few consumers were aware of this development. Likely, the introduction of the "Flavr Savr" tomato in 1994 was the first GMO crop many had seen. In the 1980s there was anecdotal information that the enzyme polygalacturonidase was a key since it dissolved cell wall pectin. A group from Celgene proposed to limit this enzyme by developing an antisense gene. The researchers hoped that this would retard ripening allowing it to remain firm longer. In 1987, Calgene identified and cloned the tomato fruit pg gene and in 1992 presented a petition to the FDA and in 1994 approved the addition of a kanamycin resistance gene construct needed to create the PG-antisense tomato. Work continued and in late 1994 the Flavr-Savr tomatoes was introduced. Demand was high and remained high but production costs were also high and the product was not profitable. While it may have been a technological success it was a commercial failure and did nothing for the cause of biotechnology so generally, the application of biotechnology and transgenic foods has become the purview of commercial agriculture. Currently there is a substantial amount of food grown using DNA recombinant technology with approx. 85% of the corn grown in the US being GMO and almost 90% of the soybeans. This is not all the GMO crops but encompass a substantial percentage. The GMO crops have various traits. Examples of two of the more common crops with their associated traits follow. Roundup Ready Soybeans contain a proteins that interferes most with the EPSPS pathway. Round Up known as glyphosate is a general purpose pesticide used not only in agriculture but in homes to eliminate weeds. While good to eliminate weeds, it also eliminates healthy crops such as flowers, crops and ornamentals. In the case of Roundup Ready Soy, the GMO trait allows the farmer the ability to use Round Up to eliminate weeds while not killing the soy. Furthermore, a farmer can be more productive eliminating tedious weeding. The second example is BT corn having been encoded with a gene that eliminates the corn borer allowing for more corn per acre. Based on data from the end of 2012 there were 170 million hectares in production that includes 312 events in 29 species with 3497 approvals in 59 countries. Table 14.1 provides a partial listing of some of the key proteins expressed by some GM crops. Figure 14.4 gives a simplistic view of the steps in genetic modification.

Table 14.1 Genetically engineered traits deregulated and approved for field release in the United States as of 2015

Crop	Scientific Name	Trait	Year approved	Developer
Alfalfa	*Medicago sativa*	Glyphosate HR[a,b]	2005, 2010	Monsanto/Forage Genetics
		Reduced Lignin	2015	Monsanto/Forage Genetics
Apple	*Malus domestica*	Non Browning	2015	Okanagan Specialty Fruits
Canola	*Brassica napus/ Brassica rapa*	Oil Profile Altered[c]	1994	Calgene
		Glufosinate HR	1995	Bayer
		Phytase	1998	BASF
		Glyphosate HR	1999	Monsanto
Maize, field	*Zea mays*	Glufosinate HR	1995	AgrEvo
		Bt IR	1995	Monsanto
		Glyphosate HR	1996	Monsanto
		Increased Lysine	2006	Monsanto
		Alpha-Amylase	2011	Syngenta
		Drought Tolerance	2011	Monsanto
		Male Sterility/Color	2011	DuPont
		ACCase[d] HR	2014	Dow
		2,4-D HR	2014	Dow
		Increased Ear Biomass	2015	Monsanto
Maize, sweet	*Zea mays*	Bt IR[e]	1998	Novartis
		Glyphosate HR	2011	Monsanto
Papaya	*Carica papaya*	Ring Spot Virus VR[f]	1996	Cornell University, University of Hawaii
				USDA Agricultural Research Service
Plum	*Prunus domestica*	Plum Pox VR[c]	2007	USDA Agricultural Research Service
Potato	*Solanum tuberosum*	*Bt* IR	1995	Monsanto
		Potato Virus Y VR[c]	1999	Monsanto
		Potato Leafroll VR[c]	2000	Monsanto
		Low Acrylamide	2015	Simplot Plant Sciences
		Nonbrowning	2015	Simplot Plant Sciences
		Resistance to Late	2015	Simplot Plant Sciences
		Blight Pathogen		
Rice	*Oryza sativa*	Gulfosinate HR	1999	AgrEvo
Squash	*Cucurbita pepo*	Zucchini Yellow VR	1994	Upjohn
		Watermelon Mosaic	1994	Upjohn
		VR	1996	
		Cucumber Mosaic VR		
Soybean	*Glycine max*	Glyphosate HR	1994	Monsanto
		Glufosinate HR	1996	Bayer
		Sulfonylurea HR	2007	DuPont
		Modified Oil	2009	DuPont
		High Oleic Oil	2010	DuPont
		Isoxaflutole HR[c]	2013	Syngenta
		Mesotrione HR[c]	2014	Syngenta
		Imidazolinone HR	2014	BASF
		2,4-D HR	2015	Dow
		Dicamba HR	2015	Monsanto
Sugar Beet	*Beta vulgaris*	Glyphosate HR[g]	2005	Monsanto
		Glufosinate HR	1998	AgrEvo
Tomato	*Solanum lycopersicum*	Fruit Ripening Altered[c]	1992	Calgene

[a]*HR* herbicide resistance
[b]Returned to regulated status in 2007; returned to deregulated status in 2011
[c]Not in production in 2015
[d]Acetyl CoA Carboxylase inhibitor herbicide
[e]*IR* insect resistance (different Bacillus thuringienis Cry genes inserted to encode proteins that kill specific species)
[f]*VR* virus resistance
[g]Returned to regulated status in 2010 because of litigation; Returned to deregulated status in 2011
DATA SOURCES: http://www.cera-gmc.org/GMCropDatabase; http://www.isaaa.org/gmapprovaldatabase/; http://www.aphis. usda.gov/biotechnology/petitions_table_pending.shtml.
Adapted from NAS, 2016

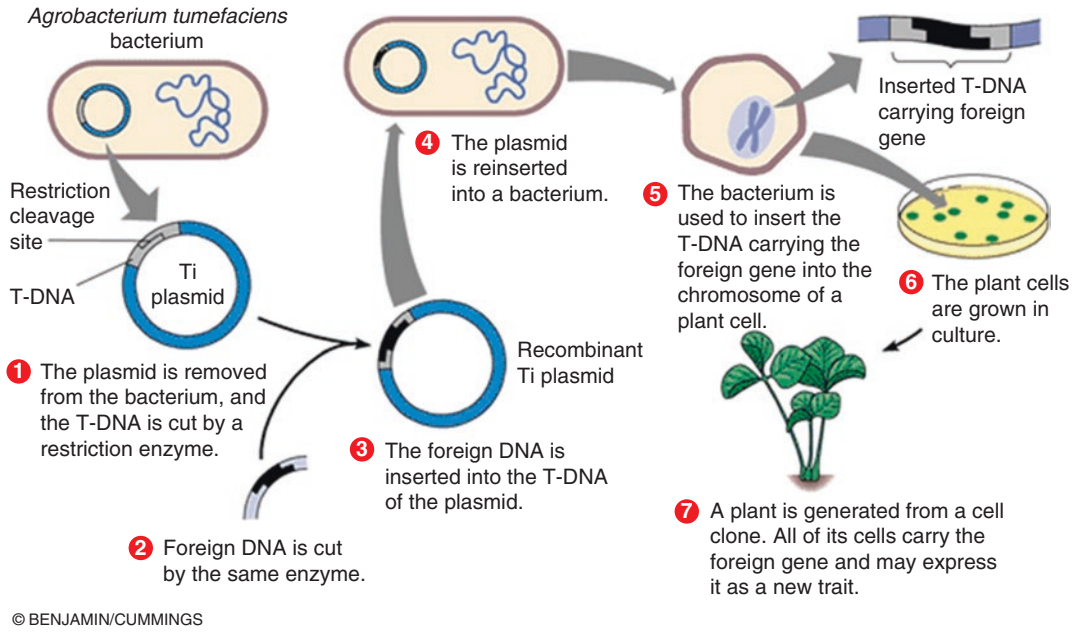

Fig. 14.4 Gene insertion in plants via Agrobacterium tranfection

One of the earlier techniques used to insert genes into plants was the use of the Agrobacterium as a vector to insert the new DNA into a plant. *Agrobacterium tumefaciens* causes a condition known as crown-gall disease in plants. Crown-gall is characterized by a tumor-like growth or gall on the infected plant. The tumors are initiated by the transfer of a DNA segment from the bacterial tumor-inducing plasmid. The plasmid T-DNA is integrated semi-randomly into the genome of the host cell where the tumor morphology genes on the T-DNA are expressed, causing the formation of a gall (Francis and Spiker 2004). The ability of *Agrobacterium* to transfer genes to plants and fungi is used in biotechnology, in particular, genetic engineering for plant improvement. A modified Ti or Ri plasmid can be used. The plasmid is 'disarmed' by deletion of the tumor inducing genes; the only essential parts of the T-DNA are its two small (25 base pair) border repeats, at least one of which is needed for plant transformation. Marc Van Montagu and Jozef Schell at the University of Ghent (Belgium) discovered the gene transfer mechanism between *Agrobacterium* and plants, which resulted in the development of methods to alter *Agrobacterium* into an efficient delivery system for gene engineering in plants (Schell and Van Montagu 1977; Joos et al. 1983). This work then laid the groundwork for the insertion of specific genes into a plant using the Agrobacterium. One can also argue that the gene transfer has been going on for a very long time and we have learned to use it effectively for specific crop improvements.

The genes to be introduced into the plant are cloned into a plant transformation vector that contains the T-DNA region of the bacterial plasmid, together with a selectable marker. Frequently an antibiotic marker gene was incorporated into the plasmid in conjunction with the other desired genes to enable selection for plants that have been successfully transformed. Plants are grown on media containing antibiotic following transformation, and those that do not have the T-DNA integrated into their genome will die. Transformation with *Agrobacterium* can be accomplished by incubating either protoplasts or leaf discs with the *Agrobacterium* to cause the plasmid insertion. From the callus that results, whole plants regenerated using plant tissue culture. The transformation with Agrobacterium is illustrated in Fig. 14.4.

Agrobacterium does not infect all plant species0 but other techniques have been applied for plant transformation one of which is the gene gun. A gene gun is biolistic particle delivery system, originally designed for plant transformation by injecting cells with genetic material. The plasmid DNA is coated on elemental particle of a heavy metal. The gene gun is able to transform almost any type of cell, including plants, and is not limited to genetic material of the nucleus: it can also transform organelles, including plastids.

Gene insertions intended to transform prokaryotic genomes generally have the gene or genes of interest, at least one promoter and terminator sequence, and a reporter gene; which is a gene used to ease detection or removal of those cells which didn't integrate the construct into their DNA.[5] These genes may each have their own promoter and terminator, or be grouped to produce multiple gene products from one transcript, in which case binding sites for translational machinery should be placed between each to ensure maximum translational efficiency. In any case the entire construct is flanked by regions called border sequences which are similar in sequence to locations within the genome, this allows the construct to target itself to a specific point in the existing genome (Slater et al. 2008). The target of a gene gun is often a callus of undifferentiated plant cells growing on gel medium in a Petri dish. After the gold particles have impacted the dish, the gel and callus are largely disrupted. However, some cells are not killed in the impact, and have incorporated enveloped a DNA coated gold particle, which eventually migrates to and integrates into a plant chromosome. Figure 14.5 illustrates the principal of the gene gum. The propellant can be compressed gas or 22 caliber blanks.

The modified cells from the callus are treated with a series of plant hormones, such as auxins and gibberellins, and each may divide and differentiate into the differentiated tissue cells of the plant. This capability of total re-generation is called totipotency. The new plant that originated from a successfully shot cell will express new genetic (heritable) traits as illustrated in Fig. 14.6. In the Figure the use of the gene gun is used to incorporate insect resistance into a tomato plant.

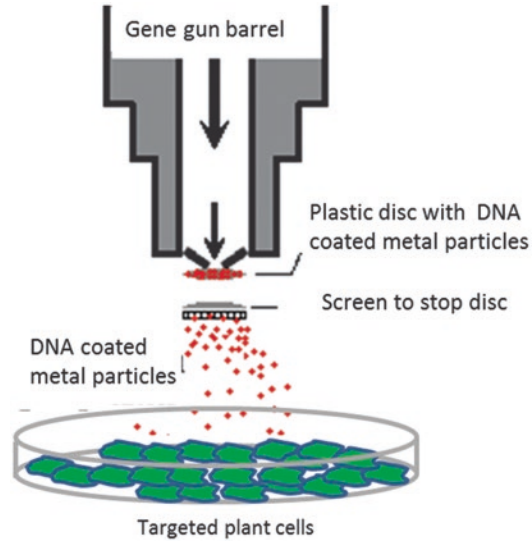

Fig. 14.5 Illustrates the principal of the Gene Gun shooting DNA coated particles into plant callus cells

The term genetic modification and so-called genetically modified organisms (GMOs) is frequently misused. All types (organic, conventional) of agriculture modify the genes of plants so that they will have desirable traits. Traditional forms of breeding change the plant's genetics indirectly by selecting plants with specific traits, while genetic engineering changes the traits by making changes directly to the DNA. In traditional breeding, crosses are made in a relatively uncontrolled manner. In conventional plant breeding, the breeder selects the parents to cross, the results are unpredictable because the DNA from the parents recombines randomly. In contrast, genetic engineering allows highly precise transfer of genes, quick and efficient tracking of genes in new varieties. This ultimately results in increased efficiency in developing new crop varieties with new and desirable traits (Popping 2010).

Applications of Genetically Modified Crops

The introduction of the "Flavr Savr" tomato in 1994 was the first GMO crop to be introduced into the market. In the 1980s there was anecdotal information that the enzyme polygalacturonidase

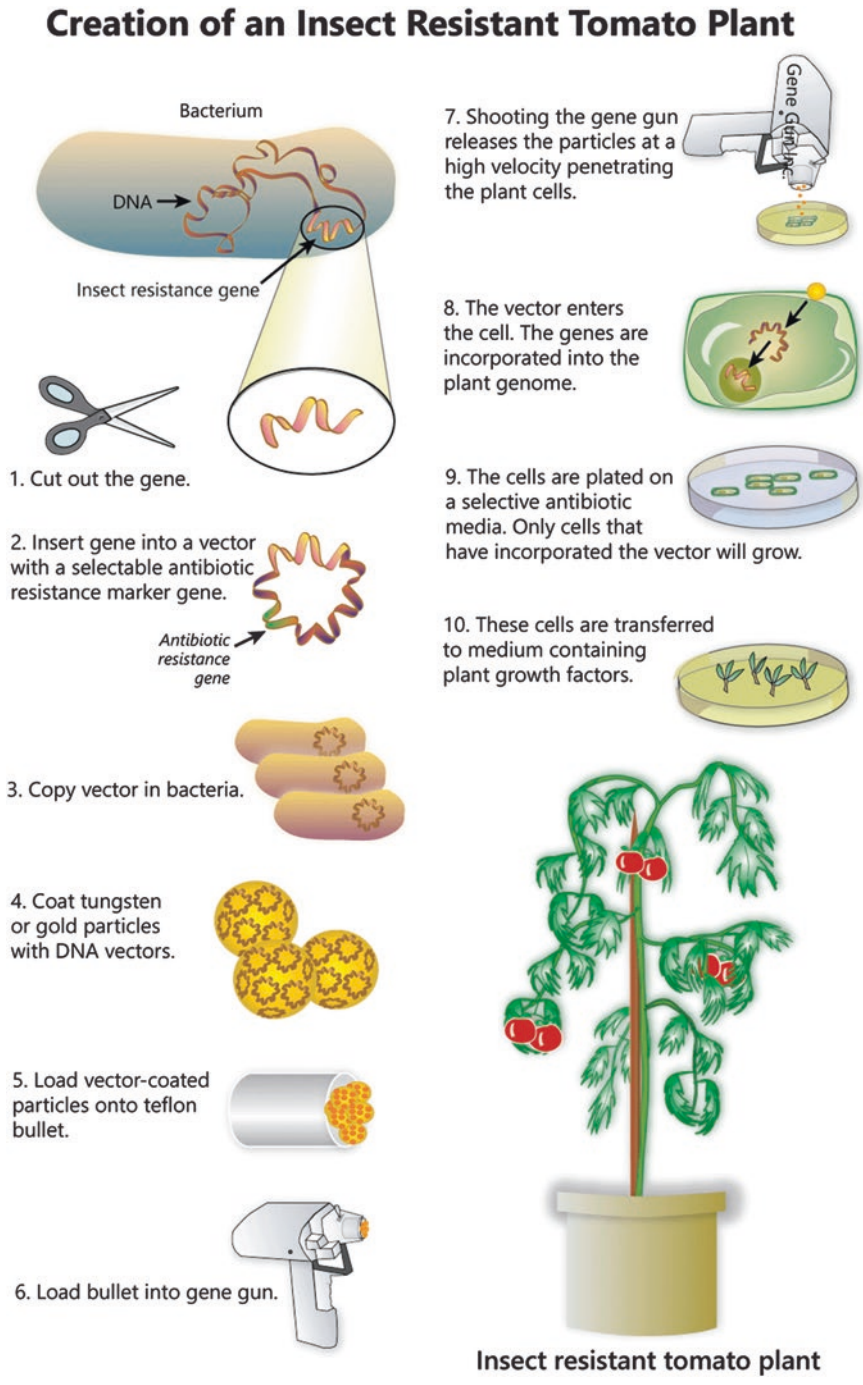

Fig. 14.6 Application of the Gene Gun to introduce insect resistance in a tomato plant

(PG) was key to softening of tomato fruit because it dissolved cell wall pectin. Calgene proposed to limit this enzyme by developing an antisense gene. The goal was to retard ripening allowing the tomatoes to remain firm longer. In 1987, Calegene identified and cloned the tomato fruit PG gene and in 1992 presented a petition to the FDA. In 1994 FDA approved the addition of a

kanamycin resistance gene construct needed to create the PG-antisense tomato. Work continued and in late 1994 the Flavr-Savr tomatoes was introduced. While it may have been a technological success it was a commercial failure and did nothing for the cause of biotechnology so generally, the application of biotechnology and transgenic foods has become a major point of discussion in agriculture. Currently there is a substantial amount of food grown using DNA recombinant technology with approx. 85% of the corn grown in the US being GMO and almost 90% of the soybeans. This is not all the GMO crops but encompass a substantial percentage. The GMO crops have various traits. Examples of two of the more common crops with their associated traits follow. Roundup Ready Soybeans contain a protein that interferes most with the EPSPS pathway. Round Up known as glyphosate is a general purpose pesticide used not only in agriculture but in homes to eliminate weeds. While good to eliminate weeds, it also eliminates healthy crops such as flowers, crops and ornamentals. In the case of Roundup Ready Soy, the GMO trait allows the farmer the ability to use Round Up to eliminate weeds while not killing the soy. Furthermore, a farmer can be more productive eliminating tedious weeding. The second example is BT corn having been encoded with a gene that eliminates the corn borer allowing for more corn per acre. Based on data from the end of 2012 there were 170 million hectares in production that includes 312 events in 29 species with 3497 approvals in 59 countries.

About 12 of global cropland was used to produce genetically modified crops in 2015 (FAO 2015; James 2015). Commercially available crops in production in 2015 included nine food crops, three non-food crops, and two types of flowers. Maize and soybean were the most widely grown genetically modified crops. Production of genetically modified genetically modified maize has increased substantially since its first commercial release in 1996 to include 53.7 million hectares by 2015. Genetically modified soybean also increased rapidly from their introduction in 1996 to over 92 million hectares in 2015 (James 2015).

The seven other food crops of which GE varieties were grown in 2015 were apple (*Malus domestica*), canola (*Brassica napus*), sugar beet (*Beta vulgaris*), papaya (*Carica papaya*), potato, squash (*Cucurbita pepo*), and eggplant (*Solanum melongena*) (James 2015). The contribution of GE varieties to the production of those crops was small, except for canola; GE varieties of canola constituted 24% of the 36 million hectares planted in 2015 (James 2015) rd of all land planted to maize worldwide that year (James 2006, 2015).

The most economically important crop modifications to date are herbicide resistance, Insect resistance and virus resistance.

Herbicide resistance introduces the ability of a crop to resist the application of certain herbicides that are used for weed control. Herbicide resistant traits have been developed for nine different herbicides and introduced into eight herbicide resistant traits for soybeans, six for cotton, three for canola, three for maize, two for sugar beet, and one for alfalfa. Some crop varieties that had stacked traits for resistance to two herbicides (for example, glyphosate and 2,4-D or glyphosate and dicamba). Since it was first introduced in for soybeans in 1996, glyphosate resistance has been introduced in alfalfa, canola, cotton, maize, and sugar beet by 2015.

Insect-resistant (IR) trait incorporates insecticidal properties produced internally by a plant itself. An example of insect resistance is the introduction of transfer of a gene coding for a crystalline (Cry) protein from the soil bacterium *Bacillus thermogenesis*. The Cry is toxic to the target insect when the insect feeds on the plant. The Cry proteins can control many insect pests—primarily moths, beetles, and flies (Höfte and Whiteley 1989). In 2015 insect resistant varieties of cotton, eggplant, maize, poplar, and soybean were in commercial production (NAS 2016).

Virus resistance prevents a plant from being susceptible to specific viral diseases. The virus resistance In crops target the coat-protein gene of the targeted virus. The transgene prevents the virus from replicating successfully in the host plant. Commercially grown virus resistant varieties of papaya were first introduced in the state of

Hawaii in 1998. Virus resistant squash ws also commercialized in the United States in the late 1990s NAS, 2016).

Testing

The ability to determine whether a crop has been genetically modified is important since consumers and regulators require that information. There are two basic types of testing that is performed on selected commodities; protein and DNA. In the development of the gene sequence for a crop, the new gene is sandwiched between two segments; a promoter and a terminator. There are a number of promoter and terminator segments which come from a novel source hence are readily identified. Two of the most common promoter segments are 34S and 35S which come from Cauliflower Mosaic Virus (CaMV) and the Figwort Mosaic Virus (FMV). A relatively common terminator marker is NOS from Nopaline Synthase. When testing for GM content there are two approaches. In the first approach, one can test for the expressed protein using an ELISA or an Immunochromatography method. ELISA tests for a large number of compounds have been in use for decades. While these are useful, the number of possible proteins to test for is limited and proteins are expressed at very low levels. An example of an immunochromatography based method, also called "dipstick" is seen in Fig. 14.7.

A second approach is to test for the fragments such as 34S, 35S and NOS using either PCR or RT-PCR with several commercial test protocols with kits available to test for the specific insert. Obviously before any of these techniques, samples need to be extracted and prepared for analysis using one of several techniques available. In qualitative PCR, the specifity of DNA polymerase is used to allow for amplification of target sequences. In standard PCD, two pairs of primers are used with one being a sense sequence and the other being antisense. These sequences are amplified numbers of times approaching a million. After the amplification, these segments can be separated by agarose gel electrophoresis but other techniques such as HPLC have been used. The approach that is an alternative to the qualitative PCR is Quantitative Real time PCR in which the separation of fragments is performed automatically. Should an organization not choose to perform testing, there are several contract labs that can perform this assay. (Ahmed 2002).

While there is interest in the various technology involved in GMO testing, in recent years a new phenomenon has come into being which is GMO verification services with the most visible

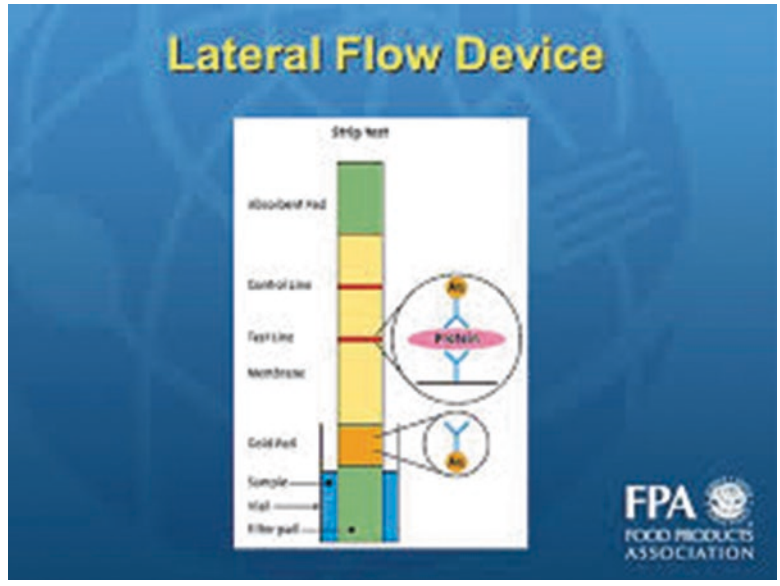

Fig. 14.7 Sample lateral flow device

Fig. 14.8 Non-GMO project verified symbol

Fig. 14.9 USDA PVP symbol

being the non-GMO Verification Project. The project has numerous requirements with the final result being that a manufacturer then can add the symbol to their product (Fig. 14.8).

Additionally, in late 2015, the USDA announced a Process Verified Program (PVP) which allows a supplier manufacturer to place another type of symbol on their project (Fig. 14.9).

Regulation

At the time this chapter is being written, the regulatory landscape is unclear since countries and now individual states in the US are developing action levels that could trigger a requirement for labeling a product as containing GMO. In 1997 the EC developed the novel food regulation 1997/258/EC. For GMO containing foods it required evidence that foods were safe for human consumption and required labeling if foods were not substantially equivalent. It was interesting that this did not trigger labeling. Regulation 1998/1139/EC which regulated Round Up Ready soy and BT137 maize and 1997/1813/EC required labeling of biotech corn and soy based on the presence of transgenic protein or DNA. These regulations created additional confusion as no thresholds were established and 2000/49/EC and 2000/50/EC introduced a 1% labeling threshold for adventitious material which is defined as material that is contained in the food even after all attempts to exclude it. Over the next 3 years there was an evolution of the regulations with 2003/1829/EC/and 2003/1830/EC which added additional biotech crops past Round Up Ready Soy and BT maize and added feed in addition to food. The current level that triggers labeling is 0.9% GMO content but there is still confusion on this topic as there can be different interpretations as what this means. Obviously unapproved events are not allowed. In contrast to Europe, Japan has focused on the GMO content of final food product rather than ingredients with a 5% level (European Union 2011).

The US does not have universal labeling requirements but two bills have been introduced in the US congress that would have standardized labeling with one of them recently defeated. In a parallel fashion a number of states have passed laws requiring GMO labeling with Vermont being the farthest along with food manufacturers preparing for its implementation in 2017 (Figs. 14.10 and 14.11).

Future Challenges

As this topic area evolves, there are going to be challenges on a number of fronts. With the total number of approved events in the hundreds, a category of unapproved events continues to grow. A paper published in the International Journal of Food Contamination on the GMO Contamination Register between the years of 1997 and 2013 indicated that it had recorded 396 incidents across 63 countries (Price and Cotter 2014). An in-depth analysis revealed that rice had the highest number accounting for almost 1/3 of the incidents even though there are is no commercial

Fig. 14.10 Pro-GMO Labeling Ad

Fig. 14.11 Example of a GMO label

growing of gm rice anywhere in the world. The majority of the rice incidents occurred with LLRICE in the US and BT63 Rice from China with the conclusion that the detection of these unapproved events being dependent on both routine and targeted monitoring. As a corollary to these developments, a review of test kits whether it be for expressed protein or genetic markers is limited indicating the need for additional emphasis in the area. A final concern is the need to feed an ever increasing world population making the need for more efficient production of food. In a recent article in **Nature** titled "India needs Home Grown GM Food to Stop Starvation", the author of this commentary stresses the need for India to develop "homegrown" GM IP focusing on commodities that are critical to the country.

The area of transgenic crops is going to continue to evolve with not only development in food for human consumption but also the continuing developments in Pharm animals where modifications are made providing for the development of selected pharmaceuticals. Finally this chapter presents a snapshot of this topic area in early 2016 with changes occurring on a regular basis.

GMO Dictionary

0.9%: The level used in EU countries to determine labeling thresholds.

35S: Promoter DNA fragment from CaMV used as marker to indicate GM content. Also used as a marker when testing samples.

34S: Promoter DNA fragment from FMV used as marker to indicate GM content. Also used as a marker when testing samples.

17,025: A number indicating a lab has met certain quality requirements. Many customers require that 17,025 accredited labs perform the analysis of their samples

Adventitious Contamination The presence of GMOs in traditional crops is difficult to avoid. Minute traces in food products are tolerated if their presence is accidental or the result of technically unavoidable contamination during growing, harvesting, transport or processing.

Base Building blocks of the nucleic acids DNA and RNA**.** Four bases are present in DNA: adenine (A), cytosine (C), guanine (G) and thymine (T). In RNA, thymine is replaced by uracil (U). These four bases encode the genetic information; thus, the four letters A, C, G and T are sometimes called "the alphabet of life".

Bt A protein that is toxic to chewing insects and is produced by the soil bacterium ***Bacillus thuringiensis*** and has long been used as a biological pesticide.

Chromosome: The self-replicating genetic structure of cells, containing genes, which determines inheritance of traits. Chemically, each chromosome is composed of proteins and a long molecule of DNA.

Dipstick; Defined as immunochromatography and used on commodities in the field to determine GM content. Not suitable for processed foods. Uses same technique as home pregnancy test kits.

Cross-pollination: Fertilization of a plant with pollen from another plant. Pollen may be transferred by wind, insects, other organisms, or humans.

DNA (deoxyribonucleic acid): The chemical substance from which genes are made. DNA is a long, double-stranded helical molecule made up of nucleotides which are themselves composed of sugars, phosphates, and derivatives of the four bases adenine (A), guanine (G), cytosine (C), and thymine (T). The sequence order of the four bases in the DNA strands determines the genetic information contained.

EU Labeling: see 0.9%.

Event: A set of trait in a plant giving it unique properties such as herbicide resistance with the provider of the traits having IP.

GMO Analysis: A series of steps including extraction, isolation, analysis and interpretation of data.

Genetic engineering: Manipulation of an organism's genes by introducing, eliminating or rearranging specific genes using the methods of modern molecular biology, particularly those techniques referred to as recombinant DNA techniques.

Genetically engineered organism (GEO): An organism produced through genetic engineering.

Genetic modification: The production of heritable improvements in plants or animals for specific uses, via either genetic engineering or other more traditional methods. Some countries other than the United States use this term to refer specifically to genetic engineering.

Genetics: The study of the patterns of inheritance of specific traits.

Genome: All the genetic material in all the chromosomes of a particular organism.

Herbicide-tolerant crops: Crops that have been developed to survive application(s) of particular herbicides by the incorporation of certain gene(s) either through genetic engineering or traditional breeding methods. The genes allow the herbicides to be applied to the crop to provide effective weed control without damaging the crop itself.

Identity preservation: The segregation of one crop type from another at every stage from production and processing to distribution. This process is usually performed through audits and site visits and provides independent third-party verification of the segregation.

Insecticide resistance: The development or selection of heritable traits (genes) in an insect population that allow individuals expressing the trait to survive in the presence of levels of an insecticide (biological or chemical control agent) that would otherwise debilitate or kill this species of insect. The presence of such resistant insects makes the insecticide less useful for managing pest populations.

Insect-resistance management: A strategy for delaying the development of pesticide resistance by maintaining a portion of the pest population in a refuge that is free from contact with the insecticide. For Bt crops this allows the insects feeding on the Bt toxin to mate with insects not exposed to the toxin produced in the plants.

Insect-resistant crops: Plants with the ability to withstand, deter or repel insects and thereby prevent them from feeding on the plant. The traits (genes) determining resistance may be selected by plant breeders through cross-pollination with other varieties of this crop or through the introduction of novel genes such as Bt genes through genetic engineering.

Intellectual property rights: The legal protection for inventions, including new technologies or new organisms (such as new plant varieties). The owner of these rights can control their use and earn the rewards for their use. This encourages further innovation and creativity for the benefit of us all. Intellectual property rights protection includes various types of patents, trademarks, and copyrights.

Molecular biology: The study of the structure and function of proteins and nucleic acids in biological systems.

Nucleotide: A subunit of DNA or RNA consisting of a nitrogenous base (adenine, guanine,

thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA), a phosphate molecule, and a sugar molecule (deoxyribose in DNA and ribose in RNA). Many of nucleotides are linked to form a DNA or RNA molecule.

Plant-incorporated protectants (PIPs): Pesticidal substances introduced into plants by genetic engineering that are produced and used by the plant to protect it from pests. The protein toxins of Bt are often used as PIPs in the formation of Bt crops.

Polymerase chain reaction (PCR): A technique used to create a large number of copies of a target DNA sequence of interest. One use of PCR is in the detection of DNA sequences that indicate the presence of a particular genetically engineered organism (Vollenhofer et al. (1999)).

Promoter: A region of DNA that regulates the level of function of other genes. Also see 35S and 34S.

Protein: A molecule composed of one or more chains of amino acids in a specific order. Proteins are required for the structure, function, and regulation of the body's cells, tissues, and organs, and each protein has a unique function.

Recombinant DNA (rDNA): A molecule of DNA formed by joining different DNA segments using recombinant DNA technology.

Recombinant DNA technology: Procedures used to join together DNA segments in a cell-free system (e.g. in a test tube outside living cells or organisms). Under appropriate conditions, a recombinant DNA molecule can be introduced into a cell and copy itself (replicate), either as an independent entity (autonomously) or as an integral part of a cellular chromosome.

Ribonucleic Acid (RNA): A chemical substance made up of nucleotides compound of sugars, phosphates, and derivatives of the four bases adenine (A), guanine (G), cytosine (C), and uracil (U). RNAs function in cells as messengers of information from DNA that are translated into protein or as molecules that have certain structural or catalytic functions in the synthesis of proteins. RNA is also the carrier of genetic information for certain viruses. RNAs may be single or double stranded.

Terminator A segment of DNA indicating the end of a particular gene sequence. See NOS

Non-GMO: An internal term indicating a product or ingredient does not require llabeling of GM content

Non-GE: See Non-GMO

Test Free: Test results indicating the sample was Test negative or had detectable but no quantifiable GM content

Test Negative: Test results indicating no detectable GM content

Quantitative Result: A test result indicating the presence or absence of GM content. A result indication the presence of Gm content would like trigger the need for a quantitative test

Qualitative Result; A test result resulting in how much GM content in a sample

RTPCR; The final step in GMO analysis when isolate DNA are amplified 2,000,000 times and are able to be detected

NOS: Terminator DNA fragment from Nopaline Synthase ad used as marker to indicate GM content. Also used as a marker when testing samples

Real Time PCR: See RTPCR

Round up: A type of herbicide used to eliminate weeds. Chemically known as glyphosate

Roundup Ready: A particular type of plant resistant to Roundup allowing for increased productivity

Ingredients

Flour

There are currently no approved non-GMO wheat events. Additionally, based on analytical data and discussions with laboratories and vendors, it is unlikely that one will be able to finds a test negative source of flour. There are vendors that sell a non-GMO flour that tests positive. What this means is the samples likely have been tested with result indicating a detectable but not quantifiable result. One of the reasons for this phenomena is a concept called adventitious contamination. In the case of flour, this concept mean that in each load of wheat there can be several percent GMO corn or soy resulting in a detectable result for the flour and products made with it. A follow on quantitative determination will not be successful as the sample contains not quantifiable DNA. The final result is

that almost 100% of flour will test positive. This should be taken into account as we move forward.

Sunflower Lecithin: With increasing pressure on IP soy lecithin supplies, manufacturers have looked for alternatives such as sunflower lecithin. There currently is no GMO sunflower.

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Additives and Contaminants

15

W. Jeffrey Hurst, John W. Finley,
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Introduction

For centuries, ingredients man has utilized food additives to improve and preserve foods. For centuries, we have used salt to preserve meats and fish, added herbs and spices to improve the flavor of foods, preserved fruit with sugar, and pickled vegetables like in a vinegar solution. Consumers now have the expectation of flavorful, nutritious, safe, convenient, colorful and affordable. Food additives and processing techniques help =deliver foods with these attributes. There is also a strong consumer desire for clean labels and foods without additives. This presents the food chemist with many conundrums about the use of additives.

There are thousands of ingredients approved for use in foods. The Food and Drug Administration (FDA) maintains a list of over 3000 ingredients in its data base "Everything Added to Food in the United States", many of which we use at home every day (e.g., sugar, baking soda, salt, vanilla, yeast, spices and colors) partially sliced loaf of bread.

All food additives are carefully regulated by federal authorities and various international organizations to ensure that foods are safe to eat and are accurately labeled.

In addition to ingredients that are added directly for benefits foods can contain toxic materials that are produced by the plant, animal, fungus or as a result of microbial contamination,

Food Toxins

In the 1500s Paracelsus expressed the classic toxicology maxim "All things are poison and nothing is without poison; only the dose makes a thing not a poison." This is often condensed to: "The dose makes the poison".

There is also the possibility of harmful or toxic substances entering the food supply unintentionally through direct contamination, through environmental pollution, as a result of processing or through deliberate adulteration for economic benefit. In addition, many foods can contain toxic materials. It should be kept in mind that most chemicals have a safe range of use but at very high doses can be toxic. Most compounds that we would consider in food have a dose response where they are inconsequential at low doses then can become toxic and very toxic. This can be seen with ingredients ranging from pesticides to common ingredients like salt. The concentration effects are very different but the typical dose response curve is similar for most chemicals or food ingredients. The key is to identify the safe level and typically we identify the "no adverse effect level" (NOAEL). This can be seen in Fig. 15.1.

The toxicity of some materials will differ greatly among individuals. There is a distribution what concentration of a material will affect individuals. There are variations in sensitivity to even the most common food ingredients. For example,

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8_15

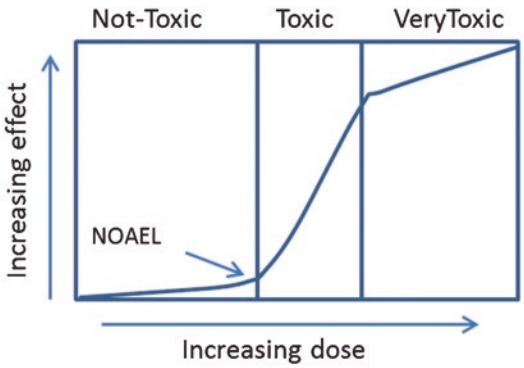


Fig. 15.1 Dose response curve for an ingredient or a drug illustrating the NOAEL

some populations are sensitive to salt while the bulk of the population is unaffected. Figure 15.2 illustrates the distribution in sensitivity to food, drugs or toxins for the same dose of a food ingredient or a drug.aflatoxin.

Many naturally occurring food compounds may be toxic as naturally-occurring constituent, microbial activity handling or processing, the incidence of adverse reactions from food is relatively low. The low incidence of adverse effects is the result solutions by the US Food and Drug Administration (FDA) and other regulatory agencies. The application of specifications, action levels, tolerances, warning labels and prohibitions have been effective in controlling the number of adverse events from food consumption. Regardless of preventative measures to protect consumers from natural food toxins, consumption of small levels of these materials is inevitable. The risk for toxicity due to consumption of food toxins is relatively low, however, there is always the possibility of toxicity due to contamination, overconsumption, allergy or an unpredictable idiosyncratic response.

Sources of food born toxins include certain sea foods, microbial contamination and production of mycotoxins and natural toxins present in plant fungal and sea foods. The increased use of dietary supplements by consumers also present sources of natural toxins. Table 15.1 contains a list of a few natural toxins found in the food supply. These materials cause a range of symptoms from gastrointestinal upset to death. These materials represent both toxins produced by the species and contaminants resulting from microbiological activity such as aflatoxins.

Fungal toxins, also called mycotoxins, are produced by fungi or molds. Most of the interest in fungal toxins is concerned with the so-called storage fungi, molds that grow on relatively dry cereals and oilseeds. These belong to two common genera, Aspergillus and Penicillium. The most common of the fungal toxins are the aflatoxins formed by members of the Aspergillus flavus group. The aflatoxins were discovered as a result of widespread poisoning of turkeys in the early 1960s in England through feeding of toxic peanut meal. The aflatoxins belong to the most powerful toxins known and are highly carcinogenic. A dose of 1 mg given to rats for short or long periods can result in liver cancer, and a diet containing 0.1 ppm of aflatoxin produces liver tumors in 50% of male rats (Spensley 1970). There are at least eight aflatoxins, of which the more important are designated B1, B2, G1, and G2. The names result from the blue and green fluorescence of these compounds when viewed under ultraviolet light. Aflatoxin B1 is a very powerful liver carcinogen; a level of 15 ppb in the diet of rats resulted in tumors in 100% of cases after 68 weeks (Scott 1969). Ducklings are used as test animals because they are especially sensitive to aflatoxins. The aflatoxins, for which the formulas are shown in Fig. 15.3, can occur in many foods but are particularly common in peanuts. Roasting of peanuts reduces the level of aflatoxin; for example, roasting for a half hour at 150° may reduce aflatoxin B1 content by as much as 80% (Scott 1969). However, aflatoxin may still be carried over into peanut butter. In addition, aflatoxins have been found in cottonseed meal, rice, sweet potatoes, beans, nuts, and wheat. Through ingestion of moldy feed by animals, aflatoxins may end up as contaminants in milk and meat. Aflatoxins found in milk may be M1 or M2, where M stands for metabolic; these are also toxic. The development of aflatoxins depends very much on temperature and moisture conditions. With peanuts, contamination occurs mostly during the drying period. Improper drying and storage are responsible for most of the

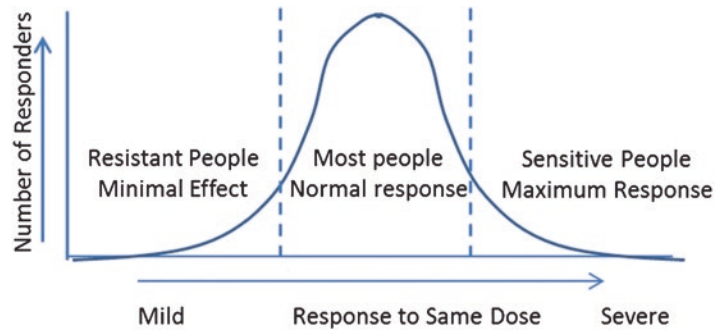


Fig. 15.2 Typical distribution of sensitivities to food ingredients or drugs in a population

Table 15.1 Natural toxins found in foods, their food source and effects when consumed

Toxic material	Food source	Effects
Ciguatoxin from Dinoflagellates (marine algae) in the genus Gambierdiscus	Consumption of subtropical and tropical marine finfish that have accumulated ciguatoxins through their diets	Combination of gastrointestinal, neurological, and, occasionally, cardiovascular disorders
Shellfish poisoning is caused by a group of toxins produced by planktonic algae consumed by shellfish	Paralytic shellfish poisoning(PSP)	PSP—can be deadly
	Diarrhetic shellfish poisoning(DSP)	DSP—nausea, vomiting, diarrhea
	Neurotoxic shellfish poisoning (NSP)	GI effects plus NSP-Neurological loss of short-term memory
	Amnesic shellfish poisoning(ASP)	(ASP) and AZP nausea, vomiting, diarrhea
	Azaspiracid shellfish poisoning(AZP)	
Scombrotoxin is a combination of substances, histamine prominent among them	Scombrotoxin is a combination of substances, histamine prominent among them. Histamine is produced during decomposition of fish	Tingling or burning in or around the mouth or throat, rash or hives, drop in blood pressure, headache, dizziness, itching of the skin, nausea, vomiting, diarrhea
Tetrodotoxin (TTX) and related compounds (e.g. 4,9-anhydroTTX, 4-epiTTX, 11-deoxyTTX, tetrodonic acid)	Pufferfish Multiple species Muscle is edible Toxin is in gonads (mainly ovary), liver, intestines, and skin can contain levels of tetrodotoxin sufficient to produce rapid death	Death is from respiratory-muscle paralysis and usually occurs within 4–6 h, with a known range of about 20 min to 8 h if victims survive the initial 24 h, they are expected to recover full
Mushroom toxins: Amanitin, Gyromitrin, Orellanine, Muscarine, Ibotenic Acid, Muscimol, Psilocybin, Coprine	Consumption of raw or cooked fruiting bodies (mushrooms, toadstools) of a number of species of higher fungi. T	Protoplasmic poisons—life-threatening poisons
		Neurotoxins—profuse sweating, coma, convulsions, hallucinations, excitement, depression, spastic colon
		Gastrointestinal irritants—transient nausea, vomiting, abdominal cramping, and diarrhea
Aflatoxins (AFs) are mycotoxins produced by certain fungi and can cause serious illness in animals and humans	Produced mainly by certain strains of Aspergillus flavus and A. parasiticus	Cancer, impaired protein formation, impaired blood coagulation, toxic hepatitis, AFB1 is the most potent known natural carcinogen and is the most abundant of the AFs
The four major aflatoxins are AFB1, AFB2, AFG1, and AFG2	Occur in a broad range of agricultural commodities, such as corn and nuts	
Pyrrolizidine alkaloids are a large class of naturally occurring alkaloids containing pyrrolizidine rings	Widely distributed in the plant kingdom, particularly in the Boraginaceae, Compositae, and Leguminosae families	pain, particularly in the right upper part of the abdomen; nausea; vomiting; swollen belly; swollen veins on the belly; puffiness from fluid; and fever
Phytohaemagglutinin (kidney bean lectin)	Red kidney bean (Phaseolus vulgaris) poisoning and kinkoti bean poisoning are examples of names for the illness caused by phytohaemagglutinin	Usually begins with extreme nausea and vomiting within 1–3 h of ingestion of the product, with diarrhea developing later

Compiled from: www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/ucm2006773.htm

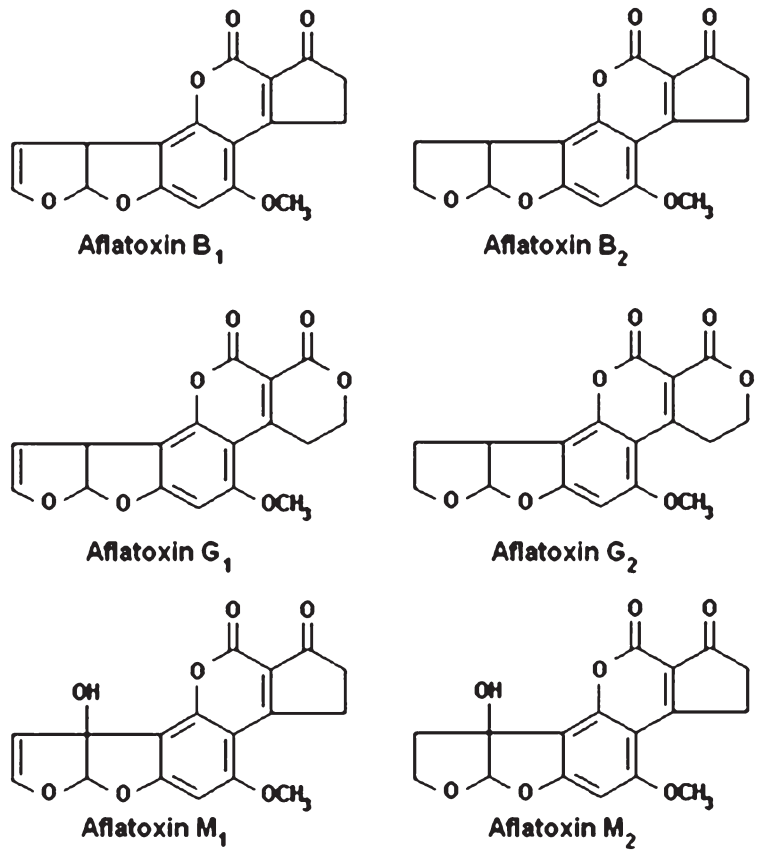


Fig. 15.3 Chemical structure of aflatoxins B_1, B_2, G_1, G_2, M_1 and M_2

contamination. This has been found to apply for rice. Optimum conditions for growth of Aspergillus flavus are 25° to 40 °C with a relative humidity greater than 85%.

Sterigmatocystin, which is a carcinogenic metabolite of Aspergillus ochraceus, has been found to be a natural contaminant of foods, especially com. Molds of the species Fusarium produce several mycotoxins in countries with moderate climates (Andrews et al. 1981). Two of these are zearalenone and deoxynivalenol (Fig. 15.4). Zearalenone, of F-2 toxin, is produced by Fusarium molds that grow on com (Marasas et al. 1979) that is immature or high in moisture at harvest. Deoxynivalenol, also known as vomitoxin, has been found in wheat and barley (Trenholm et al. 1981; Scott et al. 1983). During the wet summer of 1980, wheat grown in Ontario showed sprouting of kernels and pink discoloration. Experiments on milling showed that the vomitoxin was distributed throughout the milled products and was not destroyed by the bread-making process. Patulin is another Aspergillus metabolite and has been indicated as a food contaminant, especially in fruits, as a result of storage rot. It has been found as a constituent of apple juice (Harwig et al. 1973).

Control measures for prevention of aflatoxin production focus on reduction of water activity to a point where the fungus is unable to grow and maintenance of low water activity during storage. A moisture content of 18.0–19.5% in cereal grains is required for growth and toxin production by A. flavus. Aflatoxin-contaminated commodities can be detoxified by treatment with ammonia, calcium hydroxide, or a combination of formaldehyde and calcium hydroxide (Palmgren and Hayes 1987).

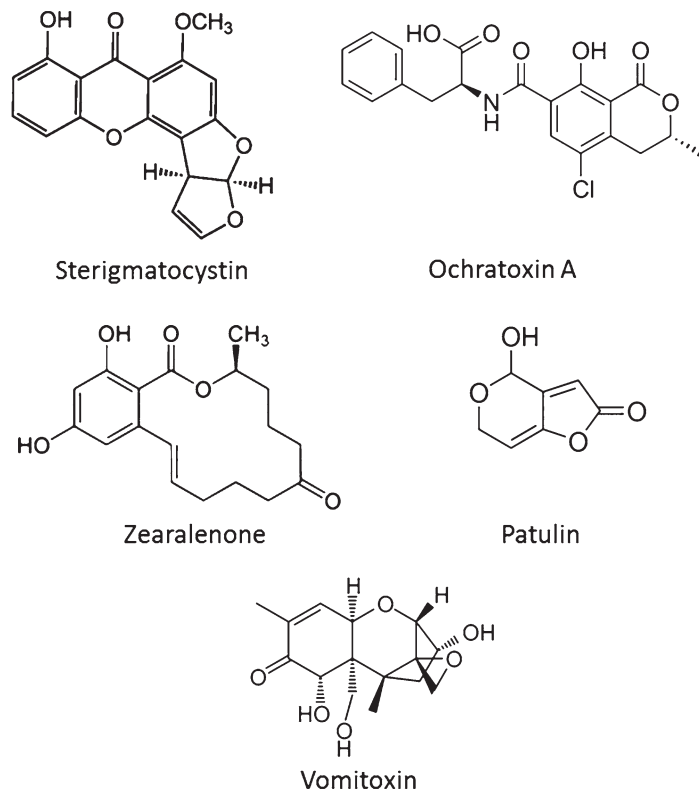


Fig. 15.4 Chemical structures of sterigmatocystin, ochratoxin A, zearalenone, deoxynivalenol (vomitoxin), and patulin

Food Additives

The term food additive means any substance the intended use of which results, or may reasonably be expected to result, directly or indirectly in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use), if such a substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the condition of its intended use; except that such a term does not include pesticides, color additives and substances for which prior sanction or approval was granted.

Food additives can be divided into two major groups, intentional additives and incidental additives. Intentional additives are chemical substances that are added to food for specific purposes. All additives whether intentional, uunintentional or incidental additives are regulated by strict governmental controls. The U.S. law governing additives in foods is the Food Additives Amendment to the Federal Food, Drug and Cosmetic Act of 1958. According to this act, a food additive is defined as follows:

> Any substance that is reasonably expected to become a component of food is a food additive that is subject to premarket approval by FDA, unless the substance is generally recognized as safe (GRAS) among experts qualified by scientific training and experience to evaluate its safety under the conditions of its intended use, or meets one of the other exclusions from the food additive definition in section 201(s) of the Federal Food, Drug, and Cosmetic Act (FFDCA). Any food additive that is intended to have a technical effect in the food is deemed unsafe unless it either conforms to the terms of a regulation prescribing its use or to an exemption for investigational use. Otherwise, in accordance with section 409 of the Act, the substance is deemed an unsafe

food additive. Any food that contains an unsafe food additive is adulterated under section 402(a)(2)(C) of the FFDCA.

Similarly, any substance that is added to food and imparts color to the food is a color additive (see color additive definition in §201(t) of the FFDCA and 21 CFR 70.3(f)). Any color additive in food is deemed unsafe unless its use is either permitted by regulation or exempt by regulation. Unlike the definition for food additive, there is no GRAS exemption for color additives. Any food that contains an unsafe color additive is adulterated under section 402(c) of the FFDCA.

The decision tree below will help in determining the regulatory status of a food ingredient. It is the responsibility of the manufacturer of any food to ensure that all ingredients used are of food-grade purity and comply with specifications and limitations in all applicable authorizations. The overall regulatory status of a food is affected by the regulatory status of each individual food ingredient. To determine compliance, consider each authorization to be composed of three parts:

- The *identity* of the substance,
- *Specifications* including purity and physical properties, and
- *Limitations* on the conditions of use.

To assure a customer that an ingredient that is being shipped to them is not adulterated or misbranded, the ingredient manufacturer may want to provide a letter of guaranty with the shipment (see 21 CFR 7.13 for suggested forms of guaranty).

Food additives perform a variety of useful functions in foods. Food ingredients that are intentionally introduced into foods to aid in processing, to act as preservatives, or to improve the quality of the food are called intentional additives. Food ingredients have been used for many years to preserve, flavor, blend, thicken and color foods, and have played an important role in reducing serious nutritional deficiencies among consumers. These ingredients also help ensure the availability of flavorful.

The FDA defines the utility of food additives as follows:

To Maintain or Improve Safety and Freshness: Preservatives slow product spoilage caused by mold, air, bacteria, fungi or yeast. In addition to maintaining the quality of the food, they help control contamination that can cause foodborne illness, including life-threatening botulism. One group of preservatives—antioxidants—prevents fats and oils and the foods containing them from becoming rancid or developing an off-flavor. They also prevent cut fresh fruits such as apples from turning brown when exposed to air.

To Improve or Maintain Nutritional Value: Vitamins and minerals (and fiber) are added to many foods to make up for those lacking in a person's diet or lost in processing, or to enhance the nutritional quality of a food. Such fortification and enrichment has helped reduce malnutrition in the U.S. and worldwide. All products containing added nutrients must be appropriately labeled.

Improve Taste, Texture and Appearance: Spices, natural and artificial flavors, and sweeteners are added to enhance the taste of food. Food colors maintain or improve appearance. Emulsifiers, stabilizers and thickeners give foods the texture and consistency consumers expect. Leavening agents allow baked goods to rise during baking. Some additives help control the acidity and alkalinity of foods, while other ingredients help maintain the taste and appeal of foods with reduced fat content.

The use of food additives strictly regulated by national and international laws. The National Academy of Sciences (1973) has listed the purposes of food additives as follows:

- to improve or maintain nutritional value
- to enhance quality
- to reduce wastage
- to enhance consumer acceptability
- to improve keeping quality
- to make the food more readily available
- to facilitate preparation of the food

The use of food additives is in effect a food processing method, because both have the same objective—to preserve the food and/or make it more attractive. In many food processing techniques, the use of additives is an integral part of the method, as is smoking, heating, and fermenting. The National Academy of Sciences (1973) has listed the following situations in which additives should *not* be used:

- to disguise faulty or inferior processes
- to conceal damage, spoilage, or other inferiority
- to deceive the consumer

- if use entails substantial reduction in important nutrients
- if the desired effect can be obtained by economical, good manufacturing practices
- in amounts greater than the minimum necessary to achieve the desired effects

There are several ways of classifying intentional food additives. One such method lists the following three main types of additives:

1. complex substances such as proteins or starches that are extracted from other foods (for example, the use of caseinate in sausages and prepared meats)
2. naturally occurring, well-defined chemical compounds such as salt, phosphates, acetic acid, and ascorbic acid
3. substances produced by synthesis, which may or may not occur in nature, such as coal tar dyes, synthetic β-carotene, antioxidants, preservatives, and emulsifiers

Some of the more important groups of intentional food additives and their functions are described in Table 15.2.

Under the Food Additives Amendment, two groups of ingredients were exempted from the regulation process.

GROUP I—Prior-sanctioned substances—are substances that FDA or USDA had determined safe for use in food prior to the 1958 amendment. Examples are sodium nitrite and potassium nitrite used to preserve luncheon meats.

GROUP II—GRAS (generally recognized as safe) ingredients—are those that are generally recognized by experts as safe, based on their extensive history of use in food before 1958 or based on published scientific evidence. Among the several hundred GRAS substances are salt, sugar, spices, vitamins and monosodium glutamate (MSG). Manufacturers may also request that FDA review the industry's determination of GRAS Status (Hall 1975).

Preservatives or antimicrobial agents play an important role in today's supply of safe and stable foods. Increasing demand for convenience foods and reasonably long shelf life of processed foods make the use of chemical food preservatives imperative. Some of the commonly used preservatives—such as sulfites, nitrate, and salt—have been used for centuries in processed meats and wine. The choice of an antimicrobial agent has to be based on a knowledge of the antimicrobial spectrum of the preservative, the chemical and physical properties of both food and preservative, the conditions of storage and handling, and the assurance of a high initial quality of the food to be preserved (Davidson and Juneja 1990).

Benzoic Acid

Benzoic acid occurs naturally in many types of berries, plums, prunes, and some spices. As an additive, it is used as benzoic acid or as benzoate. The latter is used more often because benzoic acid is sparsely soluble in water (0.27% at 18 °C) and sodium benzoate is more soluble (66.0 g/100 mL at 20 °C). The undissociated form of benzoic acid is the most effective antimicrobial agent. With a pKa of 4.2, the optimum pH range is from 2.5 to 4.0. This makes it an effective antimicrobial agent in high-acid foods, fruit drinks, cider, carbonated beverages, and pickles. It is also used in margarines, salad dressings, soy sauce, and jams.

Parabens

Parabens are alkyl esters of p-hydroxybenzoic acid. The alkyl groups may be one of the following: methyl, ethyl, propyl, butyl, or heptyl. Parabens are colorless, tasteless, and odorless (except the methyl paraben). They are nonvolatile and nonhygroscopic. Their solubility in water depends on the nature of the alkyl group; the longer the alkyl chain length, the lower the solubility. They differ from benzoic acid in that they have antimicrobial activity in both acid and alkaline pH regions.

The antimicrobial activity of parabens is proportional to the chain length of the alkyl group. Parabens are more active against molds and

Table 15.2 Common food additives and their functions

Types of ingredients	Applications	Examples of uses	Products in foods
Preservatives	Prevent food spoilage from bacteria, molds, fungi, or yeast (antimicrobials); slow or prevent changes in color, flavor, or texture and delay rancidity (antioxidants); maintain freshness	Fruit sauces and jellies, beverages, baked goods, cured meats, oils and margarines, cereals, dressings, snack foods, fruits and vegetables	Ascorbic acid, citric acid, sodium benzoate, calcium propionate, sodium erythorbate, sodium nitrite, calcium sorbate, potassium sorbate, BHA, BHT, EDTA, tocopherols (Vitamin E)
Sweeteners	Add sweetness with or without the extra calories	Beverages, baked goods, confections, table-top sugar, substitutes, many processed foods	Sucrose (sugar), glucose, fructose, sorbitol, mannitol, corn syrup, high fructose corn syrup, saccharin, aspartame, sucralose, acesulfame potassium (acesulfame-K), neotame
Color additives	Offset color loss due to exposure to light, air, temperature extremes, moisture and storage conditions; correct natural variations in color; enhance colors that occur naturally; provide color to colorless and "fun" foods	Many processed foods, (candies, snack foods margarine, cheese, soft drinks, jams/jellies, gelatins, pudding and pie fillings)	FD&C Blue Nos. 1 and 2, FD&C Green No. 3, FD&C Red Nos. 3 and 40, FD&C Yellow Nos. 5 and 6, Orange B, Citrus Red No. 2, annatto extract, beta-carotene, grape skin extract, cochineal extract or carmine, paprika oleoresin, caramel color, fruit and vegetable juices, saffron (Note: Exempt color additives are not required to be declared by name on labels but may be declared simply as colorings or color added)
Flavors and spices	Add specific flavors (natural and synthetic)	Pudding and pie fillings, gelatin dessert mixes, cake mixes, salad dressings, candies, soft drinks, ice cream, BBQ sauce	Natural flavoring, artificial flavor, and spices
Flavor enhancers	Enhance flavors already present in foods (without providing their own separate flavor)	Many processed foods	Monosodium glutamate (MSG), hydrolyzed soy protein, autolyzed yeast extract, disodium guanylate or inosinate
Fat replacers (and components of formulations used to replace fats)	Provide expected texture and a creamy "mouth-feel" in reduced-fat foods	Baked goods, dressings, frozen desserts, confections, cake and dessert mixes, dairy products	Olestra, cellulose gel, carrageenan, polydextrose, modified food starch, microparticulated egg white protein, guar gum, xanthan gum, whey protein concentrate
Nutrients	Replace vitamins and minerals lost in processing (enrichment), add nutrients that may be lacking in the diet (fortification)	Flour, breads, cereals, rice, macaroni, margarine, salt, milk, fruit beverages, energy bars, instant breakfast drinks	Thiamine hydrochloride, riboflavin (Vitamin B_2), niacin, niacinamide, folate or folic acid, beta carotene, potassium iodide, iron or ferrous sulfate, alpha tocopherols, ascorbic acid, Vitamin D, amino acids (L-tryptophan, L-lysine, L-leucine, L-methionine)

Emulsifiers	Allow smooth mixing of ingredients, prevent separation	Salad dressings, peanut butter, chocolate, margarine, frozen desserts	Soy lecithin, mono- and diglycerides, egg yolks, polysorbates, sorbitan monostearate
	Keep emulsified products stable, reduce stickiness, control crystallization, keep ingredients dispersed, and to help products dissolve more easily		
Stabilizers and thickeners, binders, texturizers	Produce uniform texture, improve "mouth-feel"	Frozen desserts, dairy products, cakes, pudding and gelatin mixes, dressings, jams and jellies, sauces	Gelatin, pectin, guar gum, carrageenan, xanthan gum, whey
pH control agents and acidulants	Control acidity and alkalinity, prevent spoilage	Beverages, frozen desserts, chocolate, low acid canned foods, baking powder	Lactic acid, citric acid, ammonium hydroxide, sodium carbonate
Leavening agents	Promote rising of baked goods	Breads and other baked goods	Baking soda, monocalcium phosphate, calcium carbonate
Anti-caking agents	Keep powdered foods free-flowing, prevent moisture absorption	Salt, baking powder, confectioner's sugar	Calcium silicate, iron ammonium citrate, silicon dioxide
Humectants	Retain moisture	Shredded coconut, marshmallows, soft candies, confections	Glycerin, sorbitol
Yeast nutrients	Promote growth of yeast	Breads and other baked goods	Calcium sulfate, ammonium phosphate
Dough strengtheners and conditioners	Produce more stable dough	Breads and other baked goods	Ammonium sulfate, azodicarbonamide, L-cysteine
Firming agents	Maintain crispness and firmness	Processed fruits and vegetables	Calcium chloride, calcium lactate
Enzyme preparations	Modify proteins, polysaccharides and fats	Cheese, dairy products, meat	Enzymes, lactase, papain, rennet, chymosin
Gases	Serve as propellant, aerate, or create carbonation	Oil cooking spray, whipped cream, carbonated beverages	Carbon dioxide, nitrous oxide

Adapted from: http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm094211.htm#types accessed 8/4/2016

yeasts than against bacteria, and more active against gram-positive than gram negative bacteria. They are used in fruitcakes, pastries, and fruit fillings. Methyl and propyl parabens can be used in soft drinks. Combinations of several parabens are often used in applications such as fish products, flavor extracts, and salad dressings.

Sorbic Acid

Sorbic acid is a straight-chain, *trans-trans* unsaturated fatty acid, 2,4-hexadienoic acid. As an acid, it has low solubility (0.15 g/100 mL) in water at room temperature. The salts, sodium, or potassium are more soluble in water. Sorbates are stable in the dry form; they are unstable in aqueous solutions because they decompose through oxidation. The rate of oxidation is increased at low pH, by increased temperature, and by light exposure.

Sorbic acid and sorbates are effective against yeasts and molds. Sorbates inhibit yeast growth in a variety of foods including wine, fruit juice, dried fruit, cottage cheese, meat, and fish products. Sorbates are most effective in products of low pH including salad dressings, tomato products, carbonated beverages, and a variety of other foods.

The effective level of sorbates in foods is in the range of 0.5–0.30%. Some of the common applications are shown in Table 15.3. Sorbates are generally used in sweetened wines or wines that contain residual sugars to prevent refermentation. At the levels generally used, sorbates do not affect food flavor. However, when used at higher levels, they may be detected by some people as an unpleasant flavor. Sorbate can be degraded by certain microorganisms to produce off-flavors. Molds can metabolize sorbate to produce 1,3 pentadiene, a volatile compound with an odor like kerosene. High levels of microorganisms can result in the degradation of sorbate in wine and result in the off-flavor known as geranium off-odor (Edinger and Splittstoesser 1986). The compounds responsible for the flavor defect are ethyl sorbate, 4-hexenoic acid, 1-ethoxyhexa-2,4-diene, and 2-ethoxyhexa-3,5-diene. The same problem may occur in fermented vegetables treated with sorbate.

Table 15.3 Applications of sorbates as antimicrobial agents

Products	Levels (%)
Dairy products: aged cheeses, processed cheeses, cottage cheese, cheese spreads, cheese dips, sour cream, yogurt	0.05–0.30
Bakery products: cakes, cake mixes, pies, fillings, mixes, icings, fudges, toppings, doughnuts	0.03–0.30
Vegetable products: fermented vegetables, pickles, olives, relishes, fresh salads	0.02–0.20
Fruit products: dried fruit, jams, jellies, juices, fruit salads, syrups, purees, concentrates	0.02–0.25
Beverages: still wines, carbonated and noncarbonated beverages, fruit drinks, low-calorie drinks	0.02–0.10
Food emulsions: mayonnaise, margarine, salad dressings	0.05–0.10
Meat and fish products: smoked and salted fish, dry sausages	0.05–0.30
Miscellaneous: dry sausage casings, semimoist pet foods, confectionery	0.05–0.30

Source: Reprinted with permission from J.N. Sofos and F.F. Busta, Sorbic Acid and Sorbates, in *Antimicrobials in Foods,* RM. Davidson and A.L. Branen, eds., p. 62, 1993, by courtesy of Marcel Dekker, Inc.

Table 15.4 Sources of SO_2 and their content of active SO_2

Chemical	Formula	Content of active SO_2 (%)
Sulfur dioxide	SO_2	100.00
Sodium sulfite, anhydrous	Na_2SO_3	50.82
Sodium sulfite, heptahydrate	$Na_2SO_3 \cdot 7 H_2O$	25.41
Sodium hydrogen sulfite	$NaHSO_3$	61.56
Sodium metabisulfite	$Na_2S_2O_5$	67.39
Potassium metabisulfite	$K_2S_2O_5$	57.63
Calcium sulfite	$CaSO_3$	64.00

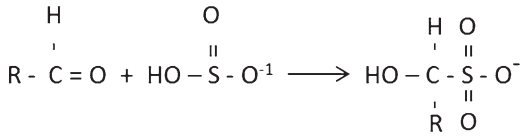
Sulfites

Sulfur dioxide and sulfites have long been used as preservatives, serving both as antimicrobial substance and as antioxidant. Their use as preservatives in wine dates back to Roman times. Sulfur dioxide is a gas that can be used in compressed form in cylinders. It is liquid under pressure of 3.4 atm and can be injected directly in liquids. It can also be used to prepare solutions in ice cold water. It dissolves to form sulfurous acid. Instead of sulfur dioxide solutions, a number of sulfites can be used (Table 15.4) because, when dissolved

in water, they all yield active SO_2. The most widely used of these sulfites is potassium metabisulfite. In practice, a value of 50% of active SO_2 is used. When sulfur dioxide is dissolved in water, the following ions are formed:

$$SO_2\,(gas) \rightarrow SO_2\,(aq)$$
$$SO_2\,(aq)^{+} \rightarrow H_2O \rightarrow H_2SO_3$$
$$H_2SO_3 \rightarrow H^{+} + HSO_3^{-}\left(K_1 = 1.7\times10^{-2}\right)$$
$$HSO_3^{-} \rightarrow H^{+} + SO_3^{2-}\left(K_2 = 5\times10^{-6}\right)$$
$$2HSO_3^{-} \rightarrow S_2O_5^{2-} + H_2O$$

All of these forms of sulfur are known as free sulfur dioxide. The bisulfite ion (HSO_3^-) can react with aldehydes, dextrins, pectic substances, proteins, ketones, and certain sugars to form addition compounds.



The addition compounds are known as bound sulfur dioxide. Sulfur dioxide is used extensively in wine making, and in wine acetaldehyde reacts preferentially with bisulfite. Excess bisulfite reacts with sugars. It is possible to classify bound SO_2 into three forms: aldehyde sulfurous acid, glucose sulfurous acid, and rest sulfurous acid. The latter holds the SO_2 in a less tightly bound form. Sulfites in wines serve a dual purpose: (1) antiseptic or bacteriostatic and (2) antioxidant. These activities are dependent on the form of SO_2 present. The various forms of SO_2 in wine are represented schematically in Fig. 15.5. The free SO_2 includes the water-soluble SO_2 and the undissociated H_2SO_3 and constitutes about 2.8% of the total. The bisulfite form constitutes 96.3% and the sulfite form 0.9% (all at pH 3.3 and 20 °C). The bound SO_2 is mostly (80%) present as acetaldehyde SO_2, 1% as glucose SO_2, and 10–20% as rest SO_2. The various forms of sulfite have different activities. The two free forms are the only ones with antiseptic activity. The antioxidant activity is limited to the SO_3^{2-} ion (Fig. 15.5).

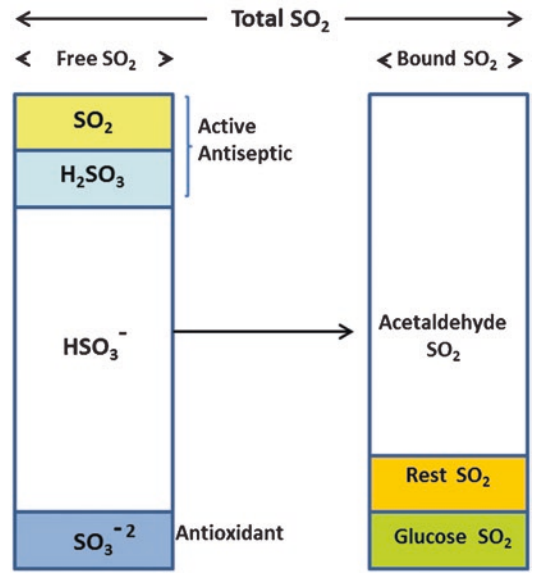


Fig. 15.5 The various forms of SO_2 in wine and their activity

Table 15.5 Effect of pH on the proportion of active antiseptic SO_2 of wine containing 100 mg/L free SO_2

pH	Active SO_2 (mg/L)
2.2	37.0
2.8	8.0
3.0	5.0
3.3	3.0
3.5	1.8
3.7	1.2
4.0	0.8

The antiseptic activity of SO_2 is highly dependent on the pH, as indicated in Table 15.5. The lower the pH, the greater the antiseptic action of SO_2. The effect of pH on the various forms of sulfur dioxide is shown in Fig. 15.6.

Sulfurous acid inhibits molds and bacteria and to a lesser extent yeasts. For this reason, SO2 can be used to control undesirable bacteria and wild yeast in fermentations without affecting the SO_2-tolerant cultured yeasts. According to Chichester and Tanner (1968), the undissociated acid is 1000 times more active than HSO_3—for Escherichia coli, 100–500 times for Saccharomyces cerevisiae, and 100 times for Aspergillus niger.

The amount of SO_2 added to foods is self-limiting because at levels from 200 to 500 ppm

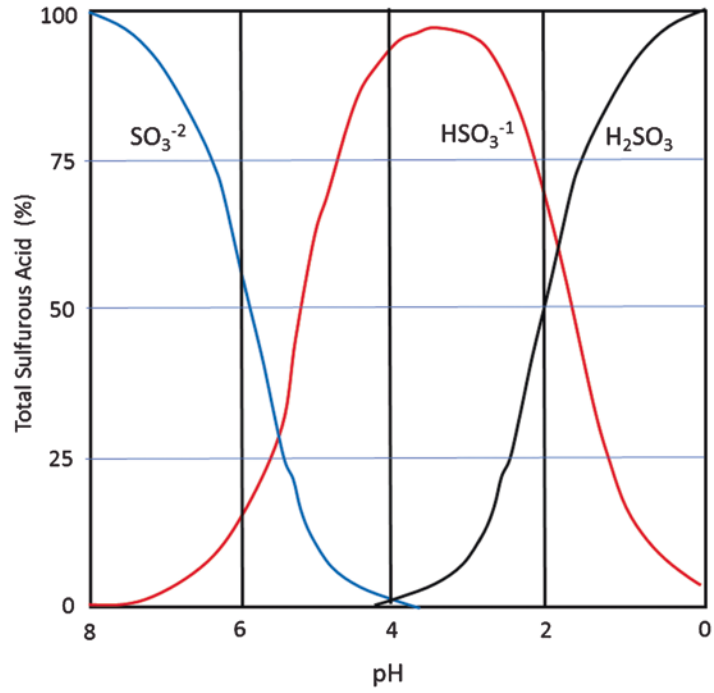


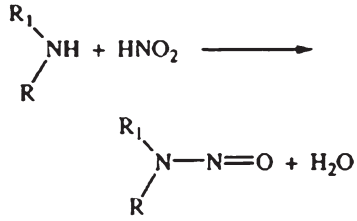
Fig. 15.6 Effect of pH on the ionization of sulfurous acid in water

the product may develop an unpleasant off-flavor. The acceptable daily intake (ADI) is set at 1.5 mg/kg body weight. Because large intakes can result from consumption of wine, there have been many studies on reducing the use of SO_2 in wine making. Although some other compounds (such as sorbic acid and ascorbic acid) may partially replace SO_2, there is no satisfactory replacement for SO_2 in wine making.

The use of SO_2 is not permitted in foods that contain significant quantities of thiamine, because this vitamin is destroyed by SO_2. In the United States, the maximum permitted level of SO_2 in wine is 350 ppm. Modern practices have resulted in much lower levels of SO_2. In some countries SO_2 is used in meat products; such use is not permitted in North America on the grounds that this would result in consumer deception. SO_2 is also widely used in dried fruits, where levels may be up to 2000 ppm. Other applications are in dried vegetables and dried potato products. Because SO_2 is volatile and easily lost to the atmosphere, the residual levels may be much lower than the amounts originally applied.

Nitrates and Nitrites

Curing salts, which produce the characteristic color and flavor of products such as bacon and ham, have been used throughout history. Curing salts have traditionally contained nitrate and nitrite; the discovery that nitrite was the active compound was made in about 1890. Currently, nitrate is not considered to be an essential component in curing mixtures; it is sometimes suggested that nitrate may be transformed into nitrite, thus forming a reservoir for the production of nitrite. Both nitrates and nitrites are thought to have antimicrobial action. Nitrate is used in the production of Gouda cheese to prevent gas formation by butyric acid–forming bacteria. The action of nitrite in meat curing is considered to involve inhibition of toxin formation by *Clostridium botulinum*, an important factor in establishing safety of cured meat products. Major concern about the use of nitrite was generated by the realization that secondary amines in foods may react to form nitrosamines, as follows:



The nitrosamines are powerful carcinogens, and they may be mutagenic and teratogenic as well. It appears that very small amounts of nitrosamines can be formed in certain cured meat products. These levels are in the ppm or the ppb range and, because analytical procedures are difficult, there is as yet no clear picture of the occurrence of nitrosamines. The nitrosamines may be either volatile or nonvolatile, and only the latter are usually included in analysis of foods. Nitrosamines, especially dimethyl-nitrosamine, have been found in a number of cases when cured meats were surveyed at concentrations of a few μg/kg (ppb). Nitrosamines are usually present in foods as the result of processing methods that promote their formation (Havery and Fazio 1985). An example is the spray drying of milk. Suitable modifications of these process conditions can drastically reduce the nitrosamine levels. Considerable further research is necessary to establish why nitrosamines are present only in some samples and what the toxicological importance of nitrosamines is at these levels. There appears to be no suitable replacement for nitrite in the production of cured meats such as ham and bacon. The ADI of nitrite has been set at 60 mg per person per day. It is estimated that the daily intake per person in Canada is about 10 mg.

Cassens (1997) has reported a dramatic decline in the residual nitrite levels in cured meat products in the United States. The current residual nitrite content of cured meat products is about 10 ppm. In 1975 an average residual nitrite content in cured meats was reported as 52.5 ppm. This reduction of nitrite levels by about 80% has been attributed to lower ingoing nitrite, increased use of ascorbates, improved process control, and altered formulations.

The nitrate-nitrite intake from natural sources is much higher than that from processed foods. Fassett (1977) estimated that the nitrate intake from 100 g of processed meat might be 50 mg and from 100 g of high-nitrate spinach, 200 mg. Wagner and Tannenbaum (1985) reported that nitrate in cured meats is insignificant compared to nitrite produced endogenously. Nitrate is produced in the body and recirculated to the oral cavity, where it is reduced to nitrite by bacterial action.

Hydrogen Peroxide

Hydrogen peroxide is a strong oxidizing agent and is also useful as a bleaching agent. It is used for the bleaching of crude soya lecithin. The antimicrobial action of hydrogen peroxide is used for the preservation of cheese milk. Hydrogen peroxide decomposes slowly into water and oxygen; this process is accelerated by increased temperature and the presence of catalysts such as catalase, lacto-peroxidase and heavy metals. Its antimicrobial action increases with temperature. When hydrogen peroxide is used for cheese making, the milk is treated with 0.02% hydrogen peroxide followed by catalase to remove the hydrogen peroxide. Hydrogen peroxide can be used for sterilizing food processing equipment and for sterilizing packaging material used in aseptic food packaging systems.

Sodium Chloride

Sodium chloride has been used for centuries to prevent spoilage of foods. Fish, meats, and vegetables have been preserved with salt. Today, salt is used mainly in combination with other processing methods. The antimicrobial activity of salt is related to its ability to reduce the water activity (a_w,), thereby influencing microbial growth. Salt has the following characteristics: it produces an osmotic effect, it limits oxygen solubility, it changes pH, sodium and chloride ions are toxic, and salt contributes to loss of magnesium ions (Banwart 1979). The use of sodium chloride is self-limiting because of its effect on taste.

Bacteriocins

Nisin is an antibacterial polypeptide produced by some strains of *Lactococcus lactis.* Nisin-like substances are widely produced by lactic acid bacteria. These inhibitory substances are known as bacteriocins. Nisin has been called an antibiotic, but this term is avoided because nisin is not used for therapeutic purposes in humans or animals. Nisin-producing organisms occur naturally in milk. Nisin can be used as a processing aid against gram-positive organisms. Because its effectiveness decreases as the bacterial load increases, it is unlikely to be used to cover up unhygienic practices.

Nisin is a polypeptide with a molecular weight of 3500, which is present as a dimer of molecular weight 7000. It contains some unusual sulfur amino acids, lanthionine and β-methyl lanthionine. It contains no aromatic amino acids and is stable to heat.

The use of nisin as a food preservative has been approved in many countries. It has been used effectively in preservation of processed cheese. It is also used in the heat treatment of nonacid foods and in extending the shelf life of sterilized milk.

A related antibacterial substance is natamycin, identical to pimaricin. Natamycin is effective in controlling the growth of fungi but has no effect on bacteria or viruses. In fermentation industries, natamycin can be used to control mold or yeast growth. It has a low solubility and therefore can be used as a surface treatment on foods. Natamycin is used in the production of many varieties of cheese.

Acids

Acids as food additives serve a dual purpose, as acidulante and as preservatives. Phosphoric acid is used in cola soft drinks to reduce the pH. Acetic acid is used to provide tartness in mayonnaise and salad dressings. A similar function in a variety of other foods is served by organic acids such as citric, tartaric, malic, lactic, succinic, adipic, and fumarie acid. The properties of some of the common food acids are listed in Table 15.6 (Peterson and Johnson 1978). Members of the straight-chain carboxylic acids, propionic and sorbic acids, are used for their antimicrobial properties. Propionic acid is mainly used for its antifungal properties. Propionic acid applied as a 10% solution to the surface of cheese and butter retards the growth of molds. The fungistatic effect is higher at pH 4 than at pH 5. A 5% solution of calcium propionate acidified with lactic acid to pH 5.5 is as effective as a 10% unacidified solution of propionic acid. The sodium salts of propionic acid also have antimicrobial properties.

Antioxidants

Food antioxidants in the broadest sense are all of the substances that have some effect on preventing or retarding oxidative deterioration in foods. They can be classified into a number of groups (Kochhar and Rossell 1990).

Primary antioxidants terminate free radical chains and function as electron donors. They include the phenolic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), alkylgalates, usually propylgallate (PG), and natural and synthetic tocopherols and tocotrienols.

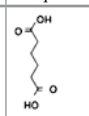
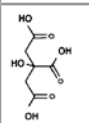
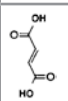
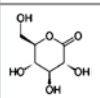
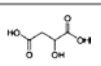
Oxygen scavengers can remove oxygen in a closed system. The most widely used compounds are vitamin C and related substances, ascorbyl palmitate, and erythorbic acid (the D-isomer of ascorbic acid).

Chelating agents or sequestrante remove metallic ions, especially copper and iron, that are powerful prooxidants. Citric acid is widely used for this purpose. Amino acids and ethylene diamine tetraacetic acid (EDTA) are other examples of chelating agents.

Enzymic antioxidants can remove dissolved or head space oxygen, such as glucose oxidase. Superoxide dismutase can be used to remove highly oxidative compounds from food systems.

Natural antioxidants are present in many spices and herbs (Lacroix et al. 1997; Six 1994). Rosemary and sage are the most potent antioxidant spices (Schuler 1990). The active principles in rosemary are carnosic acid and camosol (Fig. 15.7). Antioxidants from spices can be

Table 15.6 Properties of some common food acids

Property	Acetic acid	*Adipic acid*	Citric acid	Fumaric acid	Glucono-Delta lactone	lactic acid	Malic acid	Phos-phoric acid	Tartaric acid
									
Empirical formula	$C_2H_4O_2$	$C_6H_{10}O_4$	$C_6H_8O_7$	$C_4H_4O_4$	$C_6H_{10}O_6$	$C_3H_6O_3$	$C_4H_6O_5$	H_3PO_4	$C_4H_6O_6$
Physical form	Oily Liquid	Crystalline	Crystalline	Crystalline	Crystalline	85% Water Solution	Crystalline	85% Water Solution	Crystalline
Molecular weight	60.05	146.14	192.12	116.07	178.14	90.08	134.09	82.00	150.09
Equivalent weight	60.05	73.07	64.04	58.04	178.14	90.08	67.05	27.33	75.05
Physical form	Oily Liquid	Crystal-line	Crystal-line	Crystal-line	Crystal-line	85% Water	Crystal-line	85% Water Solution	Crystal-line
Molecular weight	60.05	146.14	192.12	116.07	178.14	90.08	134.09	82.00	150.09
Equivalentweight	60.05	73.05	64.04	58.04	178.14	90.08	67.05	27.03	75.05
Sol. in water (g/100 ml)	∞	1.4	181.00	0.63	59.0	∞	144.0	∞	147.0
K1	8×10^{-5}	3.7×10^{-5}	8.2×10^{-4}	1×10^{-3}	2.5×10^{-4} (gluconic acid)	1.37×10^{-4}	4×10^{-4}	7.25×10^{-3}	1.04×10^{-3}
K2		2.4×10^{-6}	1.77×10^{-5}	3×10^{-5}			9×10^{-6}	6.23×10^{-8}	5.55×10^{-3}
K3			3.9×10^{-6}					3×10^{-13}	

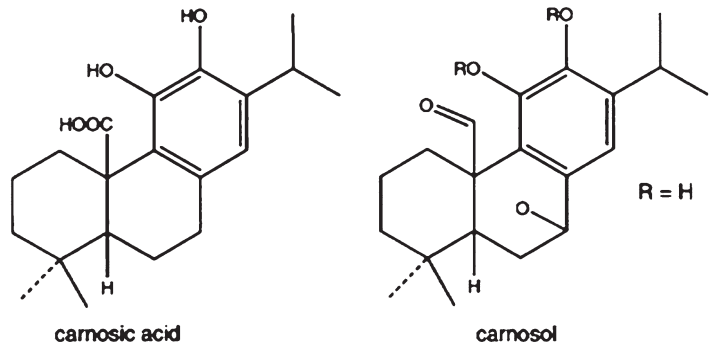


Fig. 15.7 Chemical structure of the active antioxidant principles in rosemary

obtained as extracts or in powdered form by a process described by Bracco et al. (1981).

The level of phenolic antioxidants permitted for use in foods is limited. U.S. regulations allow maximum levels of 0.02% based on the fat content of the food.

Sometimes the antioxidants are incorporated in the packaging materials rather than in the food itself. In this case, a larger number of antioxidants is permitted, provided that no more than 50 ppm of the antioxidants become a component of the food.

Emulsifiers

With the exception of lecithin, all emulsifiers used in foods are synthetic. They are characterized as ionic or nonionic and by their hydrophile/lipophile balance (HLB). All of the synthetic emulsifiers are derivatives of fatty acids.

Lecithin is the commercial name of a mixture of phospholipids obtained as a byproduct of the refining of soybean oil. Phosphatidylcholine is also known as lecithin, but the commercial product of that name contains several phospholipids including phosphatidylcholine. Crude soybean lecithin is dark in color and can be bleached with hydrogen peroxide or benzoyl peroxide. Lecithin can be hydroxylated by treatment with hydrogen peroxide and lactic or acetic acid. Hydroxylated lecithin is more hydrophilic, and this makes for a better oil-in-water emulsifier. The phospholipids contained in lecithin are insoluble in acetone.

Monoglycerides are produced by transesterification of glycerol with triglycerides. The reaction proceeds at high temperature, under vacuum and in the presence of an alkaline catalyst. The reaction mixture, after removal of excess glycerol, is known as commercial monoglyceride, a mixture of about 40% monoglyceride and di- and triglycerides. The di- and triglycerides have no emulsifying properties. Molecular distillation can increase the monoglyceride content to well over 90%. The emulsifying properties, especially HLB, are determined by the chain length and unsaturation of the fatty acid chain.

Hydroxycarboxylic and fatty acid esters are produced by esterifying organic acids to monoglycerides. This increases their hydrophilic properties. Organic acids used are acetic, citric, fumarie, lactic, succinic, or tartaric acid. Succinylated monoglycerides are synthesized from distilled monoglycerides and succinic anhydride. They are used as dough conditioners and crumb softeners (Krog 1981). Acetic acid esters can be produced from mono- and diglycerides by reaction with acetic anhydride or by transesterification. They are used to improve aeration in foods high in fat content and to control fat crystallization. Other esters may be prepared: citric, diacetyl tartaric, and lactic acid. A product containing two molecules of lactic acid per emulsifier molecule, known as stearoyl-2-lactylate, is available as the sodium or calcium salt. It is used in bakery products.

Polyglycerol esters of fatty acids are produced by reacting polymerized glycerol with edible fats. The degree of polymerization of the glycerol

and the nature of the fat provide a wide range of emulsifiers with different HLB values.

Polyethylene or propylene glycol esters of fatty acids are more hydrophilic than monoglycerides. They can be produced in a range of compositions.

Sorbitan fatty acid esters are produced by polymerization of ethylene oxide to sorbitan fatty acid esters. The resulting polyoxyethylene sorbitan esters are nonionic hydrophilic emulsifiers. They are used in bakery products as antistaling agents. They are known as polysorbates with a number as indication of the type of fatty acid used (e.g., lauric, stearic, or oleic acid).

Sucrose fatty acid esters can be produced by esterification of fatty acids with sucrose, usually in a solvent system. The HLB varies, depending on the number of fatty acids esterified to a sucrose molecule. Monoesters have an HLB value greater than 16, triesters less than 1. When the level of esterification increases to over five molecules of fatty acid, the emulsifying property is lost. At high levels of esterification the material can be used as a fat replacer because it is not absorbed or digested and therefore yields no calories.

Bread Improvers

To speed up the aging process of wheat flour, bleaching and maturing agents are used. Benzoyl peroxide is a bleaching agent that is frequently used; other compounds—including the oxides of nitrogen, chlorine dioxide, nitrosyl chloride, and chlorine—are both bleaching and improving (or maturing) agents. Improvers used to ensure that dough will ferment uniformly and vigorously include oxidizing agents such as potassium bromate, potassium iodate, and calcium peroxide. In addition to these agents, there may be small amounts of other inorganic compounds in bread improvers, including ammonium chloride, ammonium sulfate, calcium sulfate, and ammonium and calcium phosphates. Most of these bread improvers can only be used in small quantities, because excessive amounts reduce quality. Several compounds used as bread improvers are actually emulsifiers and are covered under that heading.

Sweeteners

Sweeteners can be divided into two groups, nonnutritive and nutritive sweeteners. The nonnutritive sweeteners include saccharin, cyclamate, aspartame, acesulfame K, and sucralose. Plant extracts from Stevia and Monk Fruit extract where the main sweetener is Mogroside V are plant extracts that have been approved for food uses. The nutritive sweeteners are sucrose; glucose; fructose; invert sugar; and a variety of polyols including sorbitol, mannitol, maltitol, lactitol, xylitol, and hydrogenated glucose syrups are discussed in Chap. 2.

The chemical structure of the most important nonnutritive sweeteners is shown in Fig. 15.8. Saccharin is available as the sodium or calcium salt of orthobenzosulfimide. The cyclamates are the sodium or calcium salts of cyclohexane sulfamic acid or the acid itself. Cyclamate is 30–40 times sweeter than sucrose, and about 300 times sweeter than saccharin. Organoleptic comparison of sweetness indicates that the medium in which the sweetener is tasted may affect the results. There is also a concentration effect. At higher concentrations, the sweetness intensity of the synthetic sweeteners increases at a lower rate than that which occurs with sugars. This has been ascribed to the bitterness and strong aftertaste that appears at these relatively high concentrations.

Cyclamates were first synthesized in 1939 and were approved for use in foods in the United States in 1950. Continued tests on the safety of these compounds resulted in the 1967 finding that cyclamate can be converted by intestinal flora into cyclohexylamine, which is a carcinogen. Apparently, only certain individuals have the ability to convert cyclamate to cyclohexylamine (Collings 1971). In a given population, a portion are nonconverters, some convert only small amounts, and others convert large amounts.

Aspartame is a dipeptide derivative, L-aspartyl-L-phenylalanine methyl ester, which was approved in the United States in 1981 for use as a tabletop sweetener, in dry beverage mixes, and in foods that are not heat processed. This substance is metabolized in the body to phenylalanine, aspartic acid, and methanol. Only people with phenylketonuria cannot break down

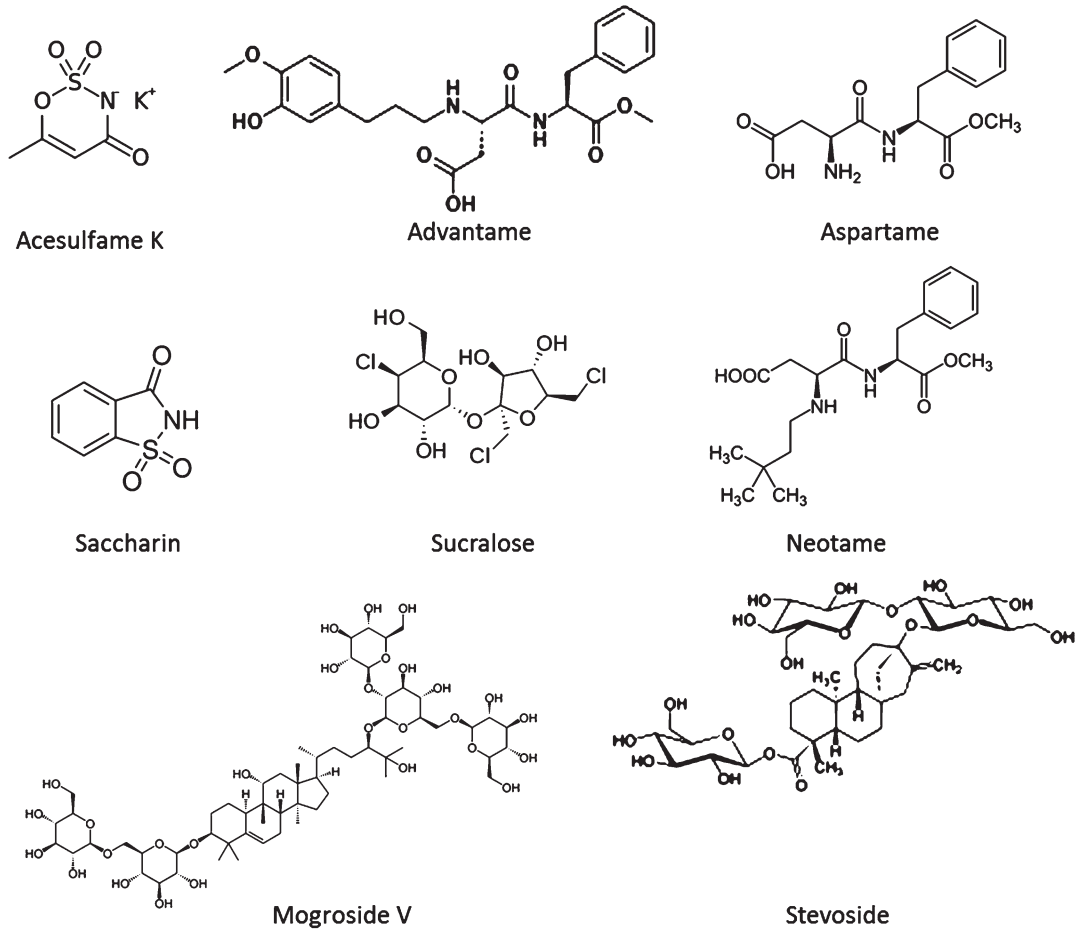


Fig. 15.8 Chemical structure of approved intense sweeteners

phenylalanine. Another compound, diketopiperazine, may also be formed. However, no harmful effects from this compound have been demonstrated. The main limiting factor in the use of aspartame is its lack of heat stability (Homler 1984).

Acesulfame K is the potassium salt of 6-methyl-1,2,3-oxathiozine-4(3H)-one-2, 2-dioxide (Fig. 15.8). It is a crystalline powder that is about 200 times sweeter than sugar. The sweetening power depends to a certain degree on the acidity of the food it is used in. Acesulfame K is reportedly more stable than other sweeteners. The sweet taste is clean and does not linger. Sucralose is a trichloroderivative of the C-4 epimer galactosucrose. It is about 600 times sweeter than sucrose and has a similar taste profile. One of its main advantages is heat stability, so it can be used in baking.

Table 15.7 Summarizes the regulatory status, brand names and applications of currently available intense sweeteners.

Stevioside and mogroside are glycosides of phenolic groups as can be seen in Fig. 15.6. Blending of nonnutritive sweeteners may lead to improved taste, longer shelf life, lower production cost, and reduced consumer exposure to any single sweetener (Verdi and Hood 1993).

Phosphates

These compounds are widely used as food additives, in the form of phosphoric acid as acidulant, and as monophosphates and polyphosphates in a large number of foods and for a variety of purposes.

Table 15.7 Regulatory stauus, brand names, relative sweetness and daily intake for currently approved intense sweeteners

Sweetener	Regulatory status	Examples of brand names containing sweetener	Sweetness intensity compared to sucrose	ADI
Acesulfame potassium (Ace-K)	Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry) 21 CFR 172.800	Sweet One® Sunett®	200×	15
Advantame	Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry) 21 CFR 172.803		20,000×	32.8
Aspartame	Approved as a sweetener and flavor enhancer in foods generally 21 CFR 172.804	Nutrasweet® Equal® Sugar Twin®	200×	50
Neotame	Approved as a sweetener and flavor enhancer in foods generally (except in meat and poultry) 21 CFR 172.829	Newtame®	7000–13,000×	0.3
Saccharin	Approved as a sweetener only in certain special dietary foods and as an additive used for certain technological purposes 21 CFR 180.37	Sweet and Low® Sweet Twin® Sweet'N Low® Necta Sweet®	200–700×	15
Mogroside Siraitia grosvenorii Swingle extracts (SGFE)	SFGE containing 25%, 45% or 55% Mogroside V is the subject of GRAS notices for specific conditions of use GRAS Notice Inventory	Nectresse® Monk Fruit in the Raw® PureLo®	100–250×	NS
Certain high purity steviol glycosides purified from the leaves of *Stevia rebaudiana*(Bertoni) Bertoni	≥95% pure glycosides Subject of GRAS notices for specific conditions of use GRAS Notice Inventory	Truvia® PureVia® Enliten®	200–400×	4
Sucralose	Approved as a sweetener in foods generally 21 CFR 172.831	Splenda®	600×	5

ADI Acceptable daily Intake in milligrams per kilogram body weight per day (mg/kg bw/d)
Adapted from: http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm397725.htm#Luo_Han_Guo_fruit_extracts

Phosphates serve as buffering agents in dairy, meat, and fish products; anticaking agents in salts; firming agents in fruits and vegetables; yeast food in bakery products and alcoholic beverages; and melting salts in cheese processing. Phosphorus oxychloride is used as a starch-modifying agent.

The largest group of phosphates and the most important in the food industry is the orthophosphates (Fig. 15.9). The phosphate group has three replaceable hydrogens, giving three possible sodium orthophosphates—monosodium, disodium, and trisodium phosphate. The phosphates can be divided into orthophosphates, polyphosphates, and metaphosphates, the latter having little

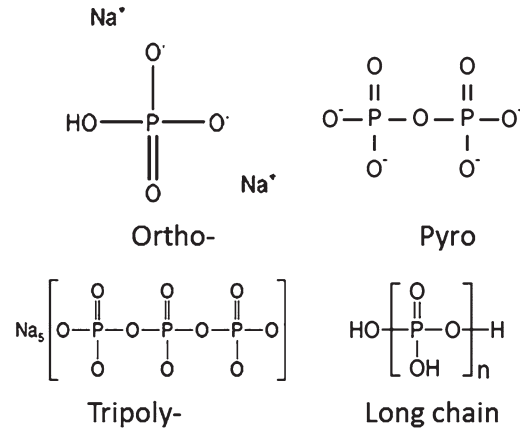


Fig. 15.9 Structure of ortho- and polyphosphate salts

practical importance. Polyphosphates have two or more phosphorus atoms joined by an oxygen bridge in a chain structure. The first members of this series are the pyrophosphates, which have one P-O-P linkage. The condensed phosphate with two linkages is tripolyphosphate. Alkali metal phosphates with chain lengths greater than three are usually mixtures of polyphosphates with varied chain lengths. The best known is sodium hexametaphosphate. The longer chain length salts are glasses. Hexametaphosphate is not a real metaphosphate, since these are ring structures and hexametaphosphate is a straight-chain polyphosphate. Sodium hexametaphosphate has an average chain length of 10–15 phosphate units.

Phosphates are important because they affect the absorption of calcium and other elements. The absorption of inorganic phosphorus depends on the amount of calcium, iron, strontium, and aluminum present in the diet. Chapman and Pugsley (1971) have suggested that a diet containing more phosphorus than calcium is as detrimental as a simple calcium deficiency. The ratio of calcium to phosphorus in bone is 2 to 1. It has been recommended that in early infancy, the ratio should be 1.5 to 1; in older infants, 1.2 to 1; and for adults, 1 to 1. The estimated annual per capita intake in the United States is 1 g Ca and 2.9 g P, thus giving a ratio of 0.35. The danger in raising phosphorus levels is that calcium may become unavailable.

Coloring Agents

In the United States two classes of color additives are recognized: colorants exempt from certification and colorants subject to certification. The former are obtained from vegetable, animal, or mineral sources or are synthetic forms of naturally occurring compounds. The latter group of synthetic dyes and pigments is covered by the Color Additives Amendment of the U.S. Food, Drug and Cosmetic Act. In the United States these color compounds are not known by their common names but as FD&C colors (Food, Drug and Cosmetic colors) with a color and a number (Noonan 1968). As an example, FD&C red dye no. 2 is known as amaranth outside the United States. Over the years the originally permitted fat-soluble dyes have been removed from the list of approved dyes, and only water-soluble colors remain on the approved list (Newsome 1990).

Color additives listed in 21 CFR Parts 74 must be analyzed and batch certified by FDA before they can be used in any food in the United States. This requirement applies to products imported into this country as well as those manufactured domestically. Straight colors required to be certified are listed in 21 CFR Part 74. Most lakes are provisionally listed under 21 CFR 81.1 for use as listed in 21 CFR 82.51 (food, drugs, and cosmetics), All FD&C Red No. 40 lakes are permanently listed under 21 CFR 74.340 (food),. FD&C Table 15.8 summarizes the approved colors which are exempt from batch certification. Colors requiring batch certification are included in Table 15.9.

Lakes are insoluble forms of the dyes and are obtained by combining the color with aluminum or calcium hydroxide. The dyes provide color in solution, and the lakes serve as insoluble pigments.

Food Irradiation

Food irradiation is the treatment of foods by ionizing radiation in the form of beta, gamma, or X-rays. The purpose of food irradiation is to preserve food and to prolong shelf life, as other processing techniques such as heating or drying have done. For regulatory purposes irradiation is considered a process, but in many countries it is considered to be an additive. This inconsistency in the interpretation of food irradiation results in great obstacles to the use of this process and has slowed down its application considerably. Several countries are now in the process of reconsidering their legislation regarding irradiation. Depending on the radiation dose, several applications can be distinguished. The unit of radiation is the Gray (Gy), which is a measure of the energy absorbed by the food. It replaced the older unit rad (1 Gy = 100 rad).

Table 15.8 Color additives approved for use in human food Part 73, Subpart A: Color additives exempt from batch certification

21 CFR section	Straight color	Year approved	Uses and restrictions
§73.30	Annatto extract	1963	Foods generally
§73.40	Dehydrated beets (beet powder)	1967	Foods generally
§73.75	Canthaxanthin(3)	1969	Foods generally, NTE(7) 30 mg/lb of solid or semisolid food or per pint of liquid food; May also be used in broiler chicken feed
§73.85	Caramel	1963	Foods generally
§73.90	β-Apo-8′-carotenal	1963	Foods generally, NTE(7): 15 mg/lb solid, 15 mg/pt liquid
§73.95	β-Carotene	1964	Foods generally
§73.100	Cochineal extract	1969	Foods generally
		2009	Food label must use common or usual name “cochineal extract”; effective January 5, 2011
	Carmine	1967	Foods generally
		2009	Food label must use common or usual name “carmine”; effective January 5, 2011
§73.125	Sodium copper chlorophyllin(3)	2002	Citrus-based dry beverage mixes NTE(7) 0.2% in dry mix; extracted from alfalfa
§73.140	Toasted partially defatted cooked cottonseed flour	1964	Foods generally
§73.160	Ferrous gluconate	1967	Ripe olives
§73.165	Ferrous lactate	1996	Ripe olives
§73.169	Grape color extract(3)	1981	Non-beverage food
§73.170	Grape skin extract (enocianina)	1966	Still & carbonated drinks & ades; beverage bases; alcoholic beverages (restrict. 27 CFR Parts 4 & 5)
§73.200	Synthetic iron oxide(3)	1994	Sausage casings NTE(7) 0.1% (by wt)
		2015	Hard and soft candy, mints and chewing gum
		2015	For allowed human food uses, reduce lead from ≤20 ppm to ≤5 ppm
§73.250	Fruit juice(3)	1966	Foods generally
		1995	Dried color additive
§73.300	Carrot oil	1967	Food generally
§73.340	Paprika	1966	Food Generally
§73.345	Paprika oleoresin	1966	Food generally
§73.350	Mica-based pearlescent pigments	2006	Cereals, confections and frostings, gelatin desserts, hard and soft candies (including lozenges), nutritional supplement tablets and gelatin capsules, and chewing gum
		2013	Distilled spirits containing not less than 18% and not more than 23% alcohol by volume but not including distilled spirits mixtures containing more than 5% wine on a proof gallon basis
		2015	Cordials, liqueurs, flavored alcoholic malt beverages, wine coolers, cocktails, nonalcoholic cocktail mixers and mixes and in egg decorating kits
§73.450	Riboflavin	1967	Foods generally
§73.500	Saffron	1966	Foods generally
§73.530	Spirulina extract	2013	Candy and chewing gum
		2014	Coloring confections (including candy and chewing gum), frostings, ice cream and frozen desserts, dessert coatings and toppings, beverage mixes and powders, yogurts, custards, puddings, cottage cheese, gelatin, breadcrumbs, and ready-to-eat cereals (excluding extruded cereals)
§73.575	Titanium dioxide	1966	Foods generally; Not to exceed 1% by wt
§73.585	Tomato lycopene extract; tomato lycopene concentrate(3)	2006	Foods generally
§73.600	Turmeric	1966	Foods generally
§73.615	Turmeric oleoresin	1966	Foods generally

http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm#table4A

Table 15.9 Color additives approved for use in human food Part 74, Subpart A: Color additives subject to batch certification

21 CFR section	Straight color	Year approved	Uses and restrictions
§74.101	FD&C Blue No. 1	1969	Foods generally
		1993	Added Mn spec
§74.102	FD&C Blue No. 2	1987	Foods generally
§74.203	FD&C Green No. 3	1982	Foods generally
§74.250	Orange B(3)	1966	Casings or surfaces of frankfurters and sausages; NTE(7) 150 ppm (by wt)
§74.302	Citrus Red No. 2	1963	Skins of oranges not intended or used for processing; NTE(7) 2.0 ppm (by wt)
§74.303	FD&C Red No. 3	1969	Foods generally
§74.340	FD&C Red No. 40(3)	1971	Foods generally
§74.705	FD&C Yellow No. 5	1969	Foods generally
§74.706	FD&C Yellow No. 6	1986	Foods genera

http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm#table4A

Radiation sterilization produces foods that are stable at room temperature and requires a dose of 20–70 kGy. At lower doses, longer shelf life may be obtained, especially with perishable foods such as fruits, fish, and shellfish. The destruction of *Salmonella* in poultry is an application for radiation treatment. This requires doses of 1–10 kGy. Radiation disinfestation of spices and cereals may replace chemical fumigants, which have come under increasing scrutiny in recent years. Dose levels of 8–30 kGy would be required. Other possible applications of irradiation processing are inhibition of sprouting in potatoes and onions and delaying of the ripening of tropical fruits.

Nutrition Supplements

There are two fundamental reasons for the addition of nutrients to foods consumed by the public: (1) to correct a recognized deficiency of one or more nutrients in the diets of a significant number of people when the deficit actually or potentially adversely affects health; and (2) to maintain the nutritional quality of the food supply at a level deemed by modern nutrition science to be appropriate to ensure good nutritional health, assuming only that a reasonable variety of foods are consumed (Augustin and Scarbrough 1990).

A variety of compounds are added to foods to improve the nutritional value of a product, to replace nutrients lost during processing, or to prevent deficiency diseases. Most of the additives in this category are vitamins or minerals. Enrichment of flour and related products is now a well-recognized practice. The U.S. Food and Drug Administration (FDA) has established definitions and standards of identity for the enrichment of wheat flour, farina, com meal, com grits, macaroni, pasta products, and rice. These standards define minimum and maximum levels of addition of thiamin, riboflavin, niacin, and iron. In some cases, optional addition of calcium and vitamin D is allowed. Margarine contains added vitamins A and D, and vitamin D is added to fluid and evaporated milk. The addition of the fat-soluble vitamins is strictly controlled, because of the possible toxicity of overdoses of these vitamins. The vitamin D enrichment of foods has been an important measure in the elimination of rickets. Another example of the beneficial effect of enrichment programs is the addition of iodine to table salt. This measure has virtually eliminated goiter.

One of the main potential deficiencies in the diet is calcium. Lack of calcium is associated with osteoporosis and possibly several other diseases. The recommended daily allowance for adolescents/young adults and the elderly has increased from the previous recommendation of 800–1200 mg/day to 1500 mg/day. This level is difficult to achieve, and the use of calcium citrate in fortified foods has been recommended by Labin-Goldscher and Edelstein (1996). Sloan

and Stiedemann (1996) highlighted the relationship between consumer demand for fortified products and complex regulatory issues.

Migration from Packaging Materials

When food packaging materials were mostly glass or metal cans, the transfer of packaging components to the food consisted predominantly of metal (iron, tin, and lead) uptake. With the advent of extensive use of plastics, new problems of transfer of toxicants and flavor and odor substances became apparent. In addition to polymers, plastics may contain a variety of other chemicals, catalysts, antioxidants, plasticizers, colorants, and light absorbers. Depending on the nature of the food, especially its fat content, any or all of these compounds may be extracted to some degree into the food (Bieber et al. 1985).

Awareness of the problem developed in the mid-1970s when it was found that mineral waters sold in polyvinyl chloride (PVC) bottles contained measurable amounts of vinyl chloride monomer. Vinyl chloride is a known carcinogen. The Codex Alimentarius Committee on Food Additives and Contaminants has set a guideline of 1 ppm for vinyl chloride monomer in PVC packaging and 0.01 ppm of the monomer in food (Institute of Food Technologists 1988). Another additive found in some PVC plastics is octyl tin mercaptoacetate or octyl tin maleate. Specific regulations for these chemicals exist in the Canadian Food and Drugs Act.

The use of plastic netting to hold and shape meat during curing resulted in the finding of N-nitrosodiethylamine and N-nitrosodibutylamine in hams up to levels of 19 ppb (parts per billion) (Sen et al. 1987). Later research established that the levels of nitrosamines present were not close to violative levels (Marsden and Pesselman 1993).

Plasticizers, antioxidants, and colorants are all potential contaminants of foods that are contained in plastics made with these chemicals. Control of potential migration of plastic components requires testing the containers with food simulants selected to yield information relevant to the intended type of food to be packaged (DeKruyf et al. 1983; Bieber et al. 1984).

Incidental Additives or Contaminants

Pesticides

Contamination of food with residues of pesticides may result from the application of these chemicals in agricultural, industrial, or household use. Nearly 300 organic pesticides are in use, including insecticides, miticides, nematocides, rodenticides, fungicides, and herbicides. The most likely compounds to appear as food contaminants are insecticides, of which there are two main classes—chlorinated hydrocarbon insecticides and organophosphorous insecticides.

The chlorinated hydrocarbon insecticides can be divided into three classes—oxygenated compounds, benzenoid nonoxygenated compounds, and nonoxygenated nonben-zenoid compounds (Table 15.10) (Mitchell 1966). In addition to the pesticide compounds, there may be residues of their metabolites, which may be equally toxic. Two important properties of the chlorinated hydrocarbons are their stability, which leads to persistence in the environment, and their solubility in fat, which results in their deposition and accumulation in fatty tissues. The structure of some of the chlorinated hydrocarbon insecticides is given in Fig. 15.10. Aldrin is a technical com-

Table 15.10 Classes of chlorinated hydrocarbon insecticides

Class I—Oxygenated compounds	
Chlorobenzilate	Methoxychlor
Dicofol	Neotran
Dieldrin	Ovex
Endosulfan	Sulfenone
Endrin	Tetradifon
Kepone	
Class II—Benzenoid, nonoxygenated compounds	
BHC	Perthane
Chlorobenside	TDE
DDT	Zectran
Lindane	
Class III—Nonoxygenated, nonbenzenoid compounds	
Aldrin	Mirex
Chlordan	Strobane
Heptachlor	Toxaphene

Source: From L.E. Mitchell, Pesticides: Properties and Prognosis, in *Organic Pesticides in the Environment*, R.F. Gould, ed., 1966, American Chemical Society

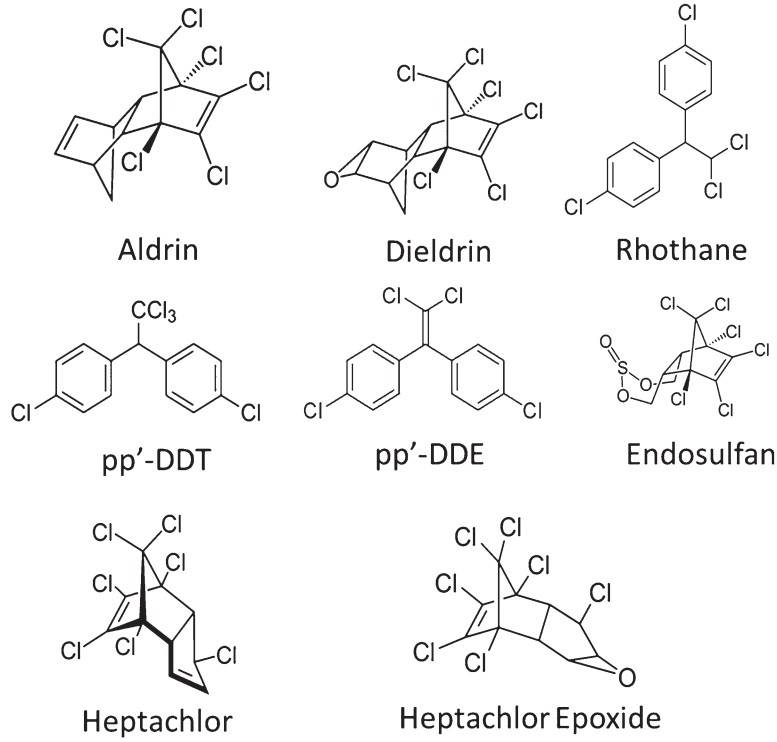


Fig. 15.10 Structure of some chlorinated hydrocarbon pesticides

pound containing about 95% of the compound 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4-*endo*-exo-5,8-dimethanonaphthalene. It has a molecular weight of 365, formula $C_{12}H_8Cl_6$, and contains 58% chlorine. Residues of this compound in animal and plant tissues are converted into dieldrin by epoxidation. The epoxide is the stable form and, thus, it is usual to consider these compounds together.

Dieldrin contains about 85% of the compound 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-exo-l, 4-endo-exo-5, 8-dimethano-naphthalene (HEOD). It has a molecular weight of 381, formula $C_{12}H_8Cl_6O$, and contains 56% chlorine. DDT is a technical compound that contains about 70% of the active ingredient pp′-DDT. In addition, there are other isomers, including op′-DDT, as well as related compounds such as TDE or rhothane. The insecticide pp′-DDT is 1,1,1-trichloro-2,2-di-(4-chlorophenyl) ethane, formula $C_{14}H_9Cl_5$. It has a molecular weight of 334.5 and contains 50% chlorine. Residues of DDT in animal tissue are slowly dehydrochlorinated to pp'-DDE, which may occur at levels of up to 70% of the original DDT. It is usual to combine DDT, DDE, and TDE in one figure as "total DDT equivalent."

Heptachlor contains about 75% of 1,4,5,6,7,10,10-heptachloro-4,7,8,9-tetrahyro-4,7-methyleneindene, formula $C_{10}H_5Cl_7$. It has a molecular weight of 373.5 and contains 67% chlorine. In animal and plant tissues, it epoxidizes to heptachlor epoxide, which is analogous in structure to HEOD (dieldrin).

The organophosphorous insecticides are inhibitors of Cholinesterase and, because of their water solubility and volatility, create less of a problem as food contaminants than the chlorinated hydrocarbons. A large number of organophosphorous insecticides are in use; these can act by themselves or after oxidative conversions in plants and animals (Table 15.11). The water solubility of these compounds varies widely, as is indicated by Table 15.12. The organophosphorous insecticides may be subject to oxidation, hydrolysis, and demethylation (Fig. 15.11). Thiophosphates may be changed to sulfoxides and sulfones in animals and plants.

Table 15.11 Classification of organophosphorous insecticides

Aliphatic derivatives	
Butonate	Mevinphos
Demeton	Mipefox
Dichlorvos	Naled
Dimefox	Phorate
Dimethoate	Phosphamidon
Dithiodemeton	Schradan
Ethion	Sulfotepp
Malathion	Tepp
Methyl demeton	Trichlorofon
Aromatic (Cyclic) derivatives	
Azinphosmethyl	EPN
Carbophenothion	Fenthion
Diazinon	Methyl parathion
Dicapthon	Parathion
Endothion	Ronnel

Source: From L.E. Mitchell, Pesticides: Properties and Prognosis, in *Organic Pesticides in the Environment*, R.F. Gould, ed., 1966, American Chemical Society

Table 15.12 Water solubilities of some organophosphorus insecticides

Insecticide	(ppm)
Carbophenothion	2
Parathion	24
Azinphosmethyl	33
Diazinon	40
Methyl parathion	50
Phorate	85
Malathion	145
Dichlorvos	1000
Dimethoate	7000
Mevinphos	∞

Source: From L.E. Mitchell, Pesticides: Properties and Prognosis, in *Organic Pesticides in the Environment,* R.F. Gould, ed., 1966, American Chemical Society

In animal products, chlorinated hydrocarbon residues are predominantly present in the lipid portion, organophosphates in both lipid and aqueous parts. In plant materials, the residue of chlorinated hydrocarbons are mostly surface bound or absorbed by waxy materials, but some can be translocated to inner parts. Extensive research has demonstrated that processing methods such as washing, blanching, heating, and canning may remove large proportions of pesticide residues (Liska and Stadelman 1969; Farrow et al. 1969). A summary of how different food processing approaches can influence pesticide residue in foods are found in Table 15.13. It is important to recognize that levels of pesticides can be lowered during processing but complete removal is unlikely.

It has been reported (Farrow et al. 1969) that 48% of DDT residues on spinach and 91% on tomatoes are removed by washing. Elkins (1989) reported that washing and blanching reduced carbaryl residues on spinach and broccoli by 97% and 98%, respectively. Washing, blanching, and canning reduced carbaryl pesticides on tomatoes and spinach by 99%. Although this pattern of removal generally holds true, Peterson et al. (1996) have pointed out that there are exceptions. Pesticides may accumulate in one part of an agricultural product. Friar and Reynolds (1991) reported that baking does not result in a decline in thiabendazole residues in potatoes, and Elkins et al. (1972) found that thermal processing does not result in a reduction of methoxychlor residues on apricots. Sometimes processing may cause a chemical to degrade, producing a compound that is more toxic than the original one.

The dietary intake of pesticide chemicals from foods is well below the acceptable daily intake (ADI) levels set by the FAO/WHO (Table 15.14). In recent years, severe restrictions on the use of many chlorinated hydrocarbon pesticides have been instituted in many areas. It can be seen that in most cases the allowable daily intake is at least one order of magnitude than the No Observed Effect Level, constituting a reasonable degree of safety.

Dioxin

The term dioxin is used to represent two related groups of chlorinated organic compounds, polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (Fig. 15.12). A total of eight carbon atoms in each molecule can carry chlorine substitution, which produces 75 possible isomers for PCDD and 135 for PCDF.

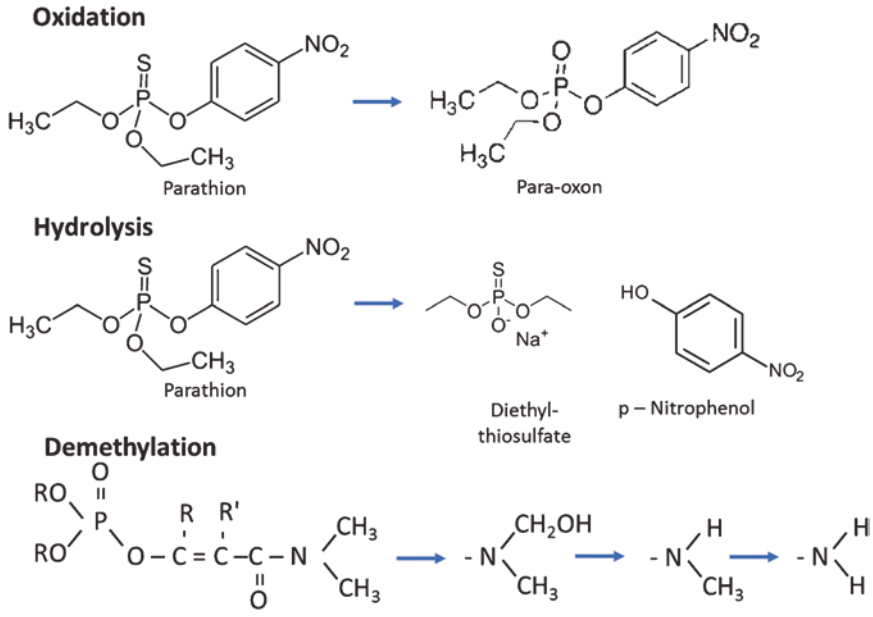


Fig. 15.11 Oxidation, hydrolysis, and demethylation reactions of organophosphorous insecticides. *Adapted from* L.E. Mitchell, Pesticides: Properties and Prognosis, in *Organic Pesticides in the Environment*, R.F. Gould, ed., 1966, American Chemical Society

These compounds are lipophilic, have low volatility, and are extremely stable. They are also very toxic, although the toxicity of each isomer may vary widely. These compounds may exhibit acute toxicity, carcinogenicity, and teratogenicity (birth defects). They are ubiquitous environmental contaminants and are present in human tissues.

The dioxins are produced as contaminants in the synthesis of certain herbicides and other chlorinated compounds, as a result of combustion and incineration, in the chlorine bleaching of wood pulp for paper making, and in some metallurgical processes (Startin 1991). Dioxins first attracted attention as a contaminant of the herbicide 2,4,5-trichloro-phenoxyacetic acid (2,4,5-T). The particular compound identified was 2,3,7,8-TCDD, which was for some time associated with the name *dioxin.* This compound was present in substantial concentration in the defoliant "Agent Orange" used by U.S. forces during the war in Vietnam.

The various isomers, also known as congeners, vary in toxicity with the 2,3,7,8-substituted ones being the most toxic. Humans appear to be less sensitive than other species.

Dioxins can be generated from chlorine bleaching of wood pulp in the paper- and cardboard-making process. This can not only lead to environmental contamination but also to incorporation of the dioxins in the paper used for making coffee filters, tea bags, milk cartons, and so forth. Dioxins can migrate into milk from cartons, even if the cartons have a polyethylene plastic coating. Unbleached coffee filters and cardboard containers have been produced to overcome this problem, and there have also been improvements in the production of wood pulp using alternative bleaching agents. The FDA guideline for dioxin in fish is 25 parts per trillion (Cordle 1981). Dioxin is considered a very potent toxin, but information on harmful effects on humans is controversial.

Polychlorinated Biphenyls (PCBs)

The PCBs are environmental contaminants that are widely distributed and have been found as residues in foods. PCBs are prepared by chlorination of biphenyl, which results in a mixture of

Table 15.13 Influence of various types of food processing on reduction of residual pesticide levels in foods (adapted from Kaushik et al. 2009)

Commodity	Pesticide	Process	Initial ppm	Final ppm	% Loss by Dissipation	Reason	References
Potato	Profenofos	Baking microwave oven	11.48	0.22 0.19		Loss of pesticide due to physico-chemical processes, e.g., evaporation, codistillation, and thermal degradation	Sharma et al. (2005)
Wheat flour	Endosulfan	Bread making			70	Bread makingprocess involves yeast-mediated fermentation and baking which contribute to degradation of pesticides	Sharma et al. (2005)
	Deltamethrin				63		
	Malathion				60		
	Propiaconazole				52		
	Chlorpyriphos				51		
	Hexaconazole				46		
Milk	Leptophos	Cheese making	100	1.84		Heating and salting stages in cheese making caused the greatest degradation of leptophos	Abu-Elamayem et al. (1979)
	Leptophos-oxon			0.76			
	Phenol derivative			32.26			
Rice	Ekalux 25 EC	Parboiling	0.078		49	Inactivation or degradation of the pesticides during parboiling at high temperature	Krishnamurthy and Sreeramulu (1982)
	Dursbsan 25 EC		3 to 0.042		51		
	Lebaycid ECO all (0.05%)		6		68		
Rough rice	Malathion	Parboiling	14	0.013			Cogburn et al. (1990)
Maize and beans	Malathion	12 Months storage open basket	7.73 7.52		64 47	Volatilization and possible settling of pesticide dust formulation to the bottom and on the sides of basket during storage in the open and windy tropical laboratory	Lalah and Wandiga (2002)
Tomatoes	HCB	Wash with acetic acid			42.9	Reduction by dissolution of phosalone in water	Mergnat et al. (1995) residue
	Lindane				46.1	Effectiveness of washing in removing residues depends upon: 1. Location of residue 2. Age of the residue 3. Water solubility of the pesticide 4. Temperature and type of wash 5. Effectiveness of washing may be improved addition of a detergent	Holland et al. (1994)
	p,p-DDT				27.2		
	Dimethoate				90.8		
	Profenofos				82.4		
	Pirimiphos-Methyl				91.4		
Tomatoes	HCB	Wash with tap water			9.62		
	Lindane				15.3		
	p,p-DDT				9.17		
	Dimethoate				18.8		
	Profenofos				22.7		
	Pirimiphos-Methyl				16.2		

Table 15.14 The use, allowable daily intake and NOAEL levels for some pesticides and herbicides

Compound	Use	ADI (mg/kg bw)	NOAEL
Aldrin	Insecticide	0.0001	0.025
Carbamyl	Insecticide	0.01	0.06
Chlordane	Insecticide	0.0005	0.05
Cycloxydim	Herbicide	0.07	7
DDT	Insecticide	0.02	0.25
Diazinon	Herbicide	0.002	0.025
Dieldrin	Insecticide	0.0001	0.025
Diquat	0.002	0.19	
Glyphosate	Herbicide	0.3	31
Heptachlor-heptachlor epoxide	Insecticide	0.0001	0.025
Lindane	Insecticide	0.008	0.75
Malathion	Insecticide	0.02	0.2
Methoxychlor	Insecticide	0.1	10
Paraquat	Insecticide	0.004	1.6
Parathion	Insecticide	0.005	0.05
Pyrethrins	Insecticide	0.04	10
2,4,D	Herbicide	0.3	31
2,4,5-T	Herbicide	0.03	3

Adapted from Lu 1995

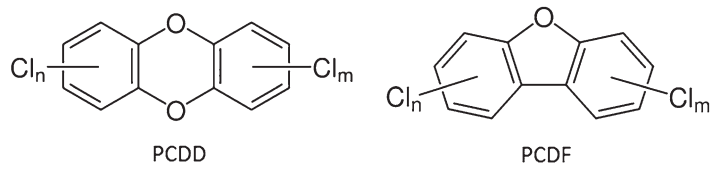


Fig. 15.12 Chemical structure of Polychlorinated Dibenzo-*p*-dioxins (PCDD) and Polychlorinated Dibenzofurans (PCDF)

isomers that have different chlorine contents. In North America, the industrial compounds are known as Aroclor; these are used industrially as dielectric fluids in transformers, as plasticizers, as heat transfer and hydraulic fluids, and so forth. The widespread industrial use of these compounds results in contamination of the environment through leakages and spills and seepage from garbage dumps. The PCBs may show up on chromatograms at the same time as chlorinated hydrocarbon pesticides. The numbering system used in PCBs and the prevalent substitution pattern are presented in Fig. 15.13. Table 15.15 presents information on commercial Aroclor compounds. In the years prior to 1977 production of PCBs in North America amounted to about 50 million pounds per year. PCBs were first discovered in fish and wildlife in Sweden in 1966, and they can now be found in higher concentrations in fish than organochlorine pesticides (Zitco 1971).

PCBs decompose very slowly. It is estimated that between 1929 and 1977, about 550 million kg of PCBs were produced in the United States. Production was stopped voluntarily after a serious poisoning occurred in Japan in 1968. Large amounts are still present in, for example, transformers and could enter the environment for many years. Federal regulations specify the following limits in foods: 1.5 ppm in milk fat, 1.5 ppm in fat portion of manufactured dairy products, 3 ppm in poultry, and 0.3 ppm in eggs. The tolerance level for PCB in fish was reduced from 5 to 2 ppm in 1984. Although there has been a good deal of concern about the possible toxicity

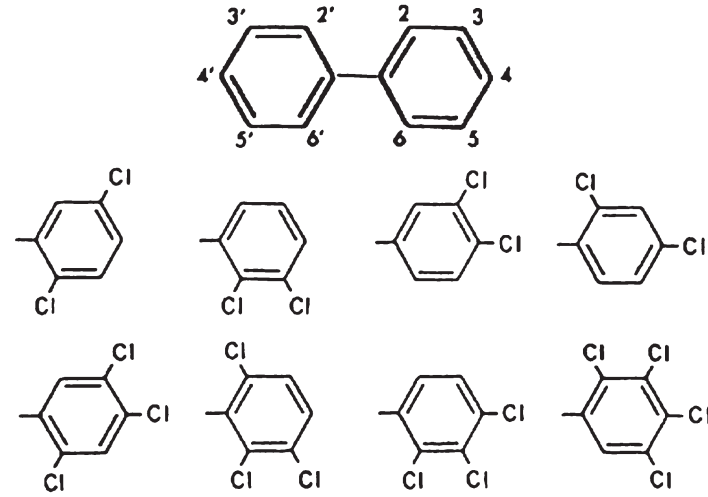


Fig. 15.13 The numbering system used in PCBs and the prevalent substitution patterns of chlorine

Table 15.15 Information on aroclor preparations

Aroclor	% Cl	Average number of Cl per molecule	Average molecular weight
Aroclor 1221	21	1.15	192
Aroclor 1232	32	2.04	221
Aroclor 1242	42	3.10	261
Aroclor 1248	48	3.90	288
Aroclor 1254	54	4.96	327
Aroclor 1260	60	6.30	372
Aroclor 1262	62	6.80	389
Aroclor 1268	68	8.70	453

of PCBs, there is now evidence that PCBs are much less toxic than initially assumed (American Council on Science and Health 1985).

Zabik and Zabik (1996) have reviewed the effect of processing on the removal of PCBs from several foods. In the processing of vegetable oil the PCB present in the crude oil was completely removed; some was removed by the hydrogenation catalyst, but most was lost by deodorization. The PCB was recovered in the deodorizer distillate.

Antibiotics

Growth-retarding or antimicrobial substances may be present in foods naturally, may be produced in a food during processing, or may occur incidentally through the treatment of diseased animals. The latter problem has created the greatest concern. The use of antibiotics in therapy, prophylaxis, and growth promotion of animals may result in residues in foods. These residue levels rarely exceed the range of 1–0.1 ppm (where toxicological interest ceases). However, levels well below those of toxicological interest may be important in food processing, for example, in cheese making, by preventing starter development. The low levels may also be important in causing allergies and development of resistant organisms. Highly sensitized persons may experience allergic reactions from milk that contains extremely low amounts of penicillin. The various antibiotics used in agriculture, including some used in food processing, are listed in Table 15.16 The tetracyclines, CTC and OTC, are broad-spectrum antibiotics and act against both gram-positive and gram-negative bacteria. The action is bacteriostatic and not bactericidal. The tetracyclines have been used to delay spoilage in poultry and fish. Their effectiveness seems to decrease quite rapidly, because the contaminating flora quickly become resistant. Nisin, which is one of the few antibiotics not used in human therapeutics, has been found to be effective as an aid in heat sterilization of foods. It is a polypeptide with a molecular weight of about 7000 and contains 18 amino acid residues. It is active against certain gram-positive organisms only, and all spores are sensitive to it.

Table 15.16 Antibiotics used in animal production

Drug	Desired effect in animals	References
Bambermycin	Improves feed conversion ratio and weight gain in chickens, beef cattle, swine, and turkeys	Reinhardt (2012) and Allen and Stanton (2014)
Lasalocid	Improves feed conversion ratio and weight gain in beef cattle	Reinhardt (2012) and Allen and Stanton (2014)
Monensin	Increase feed conversion and weight gain in beef cattle and sheep	Reinhardt (2012)
	Improves milk production in dairy cows	Allen and Stanton (2014)
Salinomycin	Increase weight gain in chickens	Reinhardt (2012)
Virginiamycin	Improved feed conversion and weight gain in chickens, swine, turkeys, and beef cattle	Reinhardt (2012) and Allen and Stanton (2014)
Bacitracin	Increase weight gain in chickens, turkeys, beef cattle, and swine; promotes egg production in chickens	Reinhardt (2012) and Allen and Stanton (2014)
Carbadox	Increases feed conversion and weight gain in swine	Allen and Stanton (2014)
Laidlomycin	Increase feed conversion and weight gain in beef cattle	Allen and Stanton 2014
Lincomycin	Increase feed conversion ratio and weight gain in chickens and swine	Allen and Stanton (2014)
Neomycin/ oxytetracyclinee	Increase weight gain and feed conversion ratio in chickens, turkeys, swine, and beef cattle	Allen and Stanton (2014)
Penicillin	Increase feed conversion ratio and weight gain in chickens, turkeys, and swine	Allen and Stanton (2014)
Roxarsone	Increase feed conversion ratio and weight gain in chickens and turkeys	Allen and Stanton (2014)
Tylosin	Increase feed conversion ratio and weight gain in chickens and swine	Allen and Stanton (2014)

Trace Metals

A variety of trace metals (such as mercury and lead) may become components of foods through industrial contamination of the environment. Some trace metals (such as tin and lead) may be introduced into foods through pickup from equipment and containers (especially tin cans).

Mercury

Large amounts of mercury are released into the environment by several industries. Major mercury users are the chloralkali industry, where mercury is used in electrolytic cells; traditional gold extraction and the pulp and paper industry.

Mercury (Hg) occurs naturally in the Earth's crust and is present in the environment and atmosphere at low levels. Concerns about Mercury are related to its release through anthropogenic emissions. Mercury is detrimental to humans, animals and plants (Eisler 2004).

Natural sources of mercury in the environment include elemental mercury vapor from volcanoes and forest fires and the release of inorganic mercury from movement of water (Bose-O'Reilly et al. 2010). Environmental sources of Mercury include burning of coal and fossil fuels, mining of mercury, precious metal refinement, electrical and automotive part manufacture, and chemical processing, and release through waste incineration, landfills, and industrial contamination of water systems. Methylmercury (MeHg) contamination of fish is still a significant source of human exposure to mercury compounds in fish-eating populations. As much as 90% of ingested MeHg is absorbed through the intestine and forms thiol (-SH) complexes with proteins and amino acids in the liver. Some MeHg enters the general circulation, and is distributed throughout all tissues including the brain. However, most of the absorbed MeHg is incorporated into bile, secreted into the intestine, and reabsorbed through the enterohepatic circulation (Martinez-Finley and Aschner 2014).

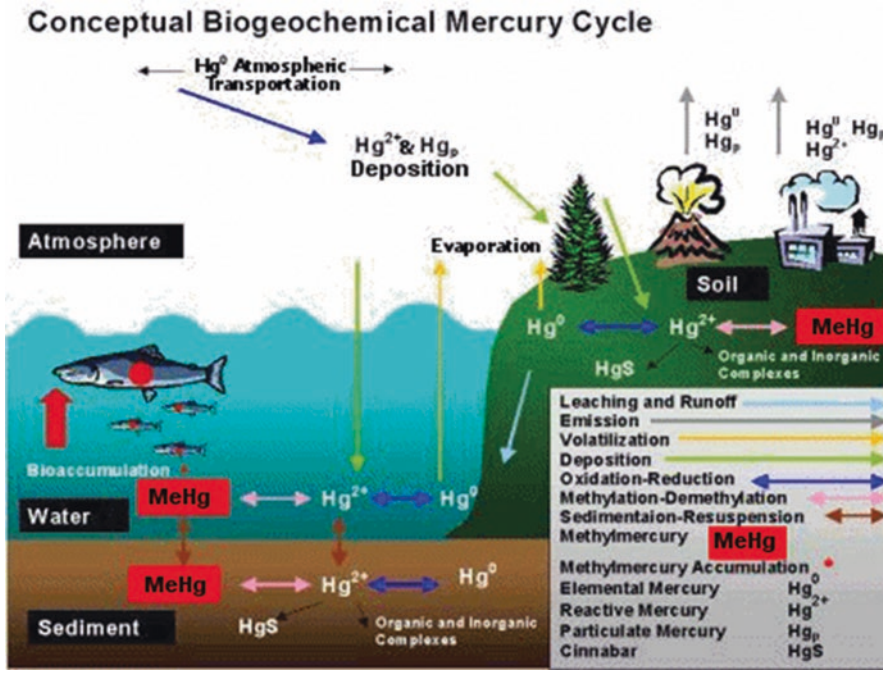


Fig. 15.14 The environmental conversion of inorganic mercury and some mercury-containing compounds to methyl mercury. *Source:* https://www.ec.gc.ca/mercure-mercury/default.asp?lang=En&n=67E16201-1#mercurymethylation

The discoveries of biomethylation and bioaccumulation aroused intense interest in the environmental fate of mercury and its pathways to human exposure. Methyl mercury is found in most fish species and in fish consuming animals including humans. The cyscle of mercury in the environment ae illustrated in Fig. 15.14 where elemental mercury is biomethylated by microorganisms in sediments found in both fresh and ocean water. Historically environmental mercury came from Chloralkali plants which discharged large amounts of inorganic mercury into rivers, lakes, and ocean bays. Other sources included paper pulp factories. These practices have been eliminated, however gold mining operations can result in large quantities of liquid mercury being deposited in river beds (Pfeiffer and Lacerda 1988).

Formation of the more volatile dimethyl mercury is favored at alkaline pH. The less volatile monomethyl form is favored at acid pH. Because much of the mercury pollution ends up in rivers and lakes where it is converted into methyl mercury, contamination of fish with mercury has been a great concern. In many animal tissues, methyl mercury may comprise as much as 99% of the total mercury present. The present interest in mercury and its effect on humans and wildlife originated with the discovery of mercury as the causative agent in the Minamata disease in Japan. Near the town of Minamata, a chemical industry used mercury compounds as catalysts for the conversion of acetylene into acetaldehyde and vinyl chloride. Organic mercury compounds were released into the waters of Minamata Bay and contaminated fish and shellfish. Many cases of mercury poisoning occurred, resulting in the death of close to 50 patients. This event triggered research into mercury contamination in many areas of the world. As a result, there is now much improved control of environmental release of mercury. It should be noted that some natural sources such as volcanic eruptions still contribute to volatile mercury being released to the environment.

Fresh and saltwater seafood remain the largest dietary source of mercury. The higher the species in the food chain the greater the bioaccumulation. The US. FDA has summarized th mercury levels in commercial fish and shell fish over the period of 1990–2012. Table 15.17 summarizes the FDA report.

Table 15.17 Mercury concentration found in fish. http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm115644.htm

Species	Mean (PPM)	Minimum (PPM)	Maximum (PPM)	Num. of samples	Data source
Anchovies	0.016	ND	0.049	15	FDA 2007–2009
Bass (Saltwater, Black, striped, rockfish)	0.167	ND	0.96	101	FDA 1991–2010
Bass chilean	0.354	ND	2.18	74	FDA 1994–2010
Bluefish	0.368	0.089	1.452	94	FDA 1991–2009
Buffalofish	0.137	0.032	0.43	17	FDA 1992–2008
Butterfish	0.058	ND	0.36	89	NMFS report 1978
Carp	0.110	ND	0.271	14	FDA 1992–2007
Catfish	0.024	ND	0.314	59	FDA 1991–2010
Clam	0.009	ND	0.028	15	FDA 1991–2010
Cod	0.111	ND	0.989	115	FDA 1991–2010
Crab	0.065	ND	0.61	93	FDA 1991–2009
Crawfish	0.033	ND	0.051	46	FDA 1991–2007
Croaker atlantic (Atlantic)	0.069	ND	0.193	90	FDA 2002–2011
Croaker white (Pacific)	0.287	0.18	0.41	15	FDA 1997
Flatfish	0.056	ND	0.218	71	FDA 1991–2009
Grouper (all species)	0.448	0.006	1.205	53	FDA 1991–2005
Haddock (Atlantic)	0.055	ND	0.197	50	FDA 1991–2009
Hake	0.079	ND	0.378	49	FDA 1994–2009
Halibut	0.241	ND	1.52	101	FDA 1992–2009
HERRING	0.078	ND	0.56	27	FDA 2005–2012
Jacksmelt	0.081	0.011	0.5	23	FDA 1997–2007
Lobster (Northern/American)	0.107	ND	0.23	9	FDA 2005–2007
Lobster (Species unknown)	0.166	ND	0.451	71	FDA 1991–2008
Lobster (Spiny)	0.093	ND	0.27	13	FDA 1991–2005
Mackerel atlantic (N.Atlantic)	0.05	0.02	0.16	80	NMFS report 1978
Mackerel chub (Pacific)	0.088	0.03	0.19	30	NMFS report 1978
Mackerel king	0.73	0.23	1.67	213	Gulf of Mexico report 2000
Mackerel spanish (Gulf of Mexico)	0.454	0.07	1.56	66	NMFS report 1978
Mackerel spanish (S. Atlantic)	0.182	0.05	0.73	43	NMFS report 1978
Mahi mahi	0.178	ND	0.45	29	FDA 1991–2005
Marlin	0.485	0.1	0.92	16	FDA 1992–1996
Monkfish	0.161	ND	0.289	11	FDA 1994–2007
Mullet	0.050	ND	0.27	20	FDA 1991–2008
Orange roughy	0.571	0.265	1.12	81	FDA 1991–2009
Oyster	0.012	ND	0.25	61	FDA 1991–2009
Perch (Freshwater)	0.150	ND	0.325	19	FDA 1991–2007
Perch ocean	0.121	ND	0.578	31	FDA 1991–2010
Pickerel	0.095	ND	0.31	16	FDA 1991–2007
Pollock	0.031	ND	0.78	95	FDA 1991–2008
Sablefish	0.361	0.09	1.052	26	FDA 2004–2009
Salmon (Canned)	0.014	ND	0.086	19	FDA 1993–2009
Salmon (Fresh/frozen)	0.022	ND	0.19	94	FDA 1991–2009
Sardine	0.013	ND	0.083	90	FDA 2002–2010
Scallop	0.003	ND	0.033	39	FDA 1991–2009

(continued)

Table 15.17 (continued)

Species	Mean (PPM)	Minimum (PPM)	Maximum (PPM)	Num. of samples	Data source
Scorpionfish	0.233	0.098	0.456	6	FDA 2006–2007
Shad	0.038	ND	0.186	15	FDA 2007–2011
Shark	0.979	ND	4.54	356	FDA 1991–2007
Sheepshead	0.090	ND	0.17	8	FDA 1992–2007
Shrimp	0.009	ND	0.05	40	FDA 1991–2009
Skate	0.137	0.04	0.36	56	NMFS REPORT 1978
Snapper	0.166	ND	1.366	67	FDA 1991–2007
Squid	0.024	ND	0.07	36	FDA 2005–2009
Swordfish	0.995	ND	3.22	636	FDA 1990–2010
Tilapia	0.013	ND	0.084	32	FDA 1991–2008
Tilefish (Atlantic)	0.144	0.042	0.533	32	FDA 1994–2004
Tilefish (Gulf of Mexico)	1.123	0.65	3.73	60	NMFS report 1978
Trout (Freshwater)	0.071	ND	0.678	35	FDA 1991–2008
Tuna (Canned, albacore)	0.350	ND	0.853	451	FDA 1991–2009
Tuna (Canned, light)	0.126	ND	0.889	545	FDA 1991–2010
Tuna (Fresh/frozen, albacore)	0.358	ND	0.82	43	FDA 1992–2008
Tuna (Fresh/frozen, All)	0.386	ND	1.816	420	FDA 1991–2010
Tuna (Fresh/frozen, bigeye)	0.689	0.128	1.816	21	FDA 1993–2005
Tuna (Fresh/frozen, skipjack)	0.144	0.022	0.26	3	FDA 1993–2007
Tuna (Fresh/frozen, species unknown)	0.410	ND	1.3	122	FDA 1991–2010
Tuna (Fresh/frozen, yellowfin)	0.354	ND	1.478	231	FDA 1993–2010
Weakfish (Sea trout)	0.235	ND	0.744	46	FDA 1991–2005
Whitefish	0.089	ND	0.317	37	FDA 1991–2008
Whiting	0.051	ND	0.096	13	FDA 1991–2008

Source of data: FDA 1990–2012, "National Marine Fisheries Service Survey of Trace Elements in the Fishery Resource" Report 1978, "The Occurrence of Mercury in the Fishery Resources of the Gulf of Mexico" Report 2000
ND-mercury concentration below detection level (Level of Detection (LOD) = 0.01 ppm)
N/A-data not available

Lead and Tin

The presence of lead in foods may be the result of environmental contamination, pickup of the metal from equipment, or the solder of tin cans. It has been estimated that nearly 90% of the ingested lead is derived from food (Somers and Smith 1971). However, only 5% of this is absorbed. In the early 1970s, the average North American car was reported to emit 2.5 kg of lead per year (Somers and Smith 1971), and Zuber et al. (1970) reported that crops grown near busy highways had a high lead content (in some cases, exceeding 100 ppm of lead in the dry matter). The removal of lead from gasoline has eliminated this source of contamination. Lead can also be picked up by acid foods such as fruit juices that are kept in glazed pottery made with lead-containing glazes. Both lead and tin may be taken up by foods from the tin of cans and from the solder used in their manufacture. The amounts of lead and tin taken up depend on the type of tin plate and solder used and on the composition and properties of the canned foods. In a study on the detinning of cans by spinach, Lambeth et al. (1969) found that detinning was significantly related to the oxalic acid content and pH of the product. Detinning in excess of 60% was observed during 9 months' storage of high-oxalate spinach.

The present levels of lead that humans ingest cause concern because ADI calculations range from 0.1 to 0.8 mg lead per day. The average daily intake is in the vicinity of 0.4 mg lead per day. This means that lead is one of the few toxic food components for which the acceptable daily

intake is approached or exceeded by the general population (Clarkson 1971).

Cadmium

As are lead and mercury, cadmium is a nonessential trace metal with high toxicity. Crustaceans have the ability to accumulate cadmium as well as other trace metals, such as zinc. Cadmium levels in oysters may reach 3–4 ppm, whereas in other foods, levels are only one-tenth or one-hundredth of these (Underwood 1973).

Arsenic

Arsenic is found in the environment from both natural and human sources. Environmental Aarsenic can come from erosion of arsenic-containing rocks, volcanic eruptions, contamination from mining and smelting ores. Environmental arsenic can contaminate food products from absorption through soil and water. Foods that are produced following prolonged contact with water, such as seafood and rice frequently have higher levels of arsenic contamination.

Arsenic is used as a pesticide primarily to preserve wood from rot and decay. Historically, arsenic was also used in rat poisons, ant poisons and weed killers. Agricultural soils can contain high levels of arsenic resulting from its former agricultural uses. Most forms of arsenic tend to stick to soil or sediment particles however, some arsenic can dissolve in water, leaching into lakes, rivers, or ground water.

Exposure to arsenic can come from many different foods and beverages. Consumer awareness of dietary arsenic exposure in a publication by Consumer Reports magazine analyzing arsenic content of commercially available fruit juices (January, 2012) and rice-based products (November, 2012). In July 2013, the FDA proposed an action level of 10 parts per billion (ppb), which is similar to the U.S. Environmental Protection agency (EPA) drinking water standard, for inorganic arsenic in apple juice. The FDA has not yet set an arsenic standard for rice-based products, however, the Joint FAO-WHO Codex Alimentarius Commission in July 2014 established a maximum level of 200 ppb for inorganic arsenic in polished rice. Based on its testing, the FDA on April 1, 2016 proposed an action level, or limit, of 100 parts per billion (ppb) for inorganic arsenic in infant rice cereal. This level, which is based on the FDA's assessment of a large body of scientific information, seeks to reduce infant exposure to inorganic arsenic.

http://www.consumerreports.org/cro/magazine/2012/11/arsenic-in-your-food/index.htm

https://medicalxpress.com/news/2015-01-arsenic-food.html#jCp

https://medicalxpress.com/news/2015-01-arsenic-food.html#jCp

Polycyclic Aromatic Hydrocarbons (PAHs)

These compounds form a large group of materials that are now known to occur in the environment. The structural formulas of the major members of this group are presented in Fig. 15.15. Several of these, especially benzo(a)pyrene (3,4-benzopyrene), have been found to be carcinogenic. Usually, the polycyclic hydrocarbons occur together in foods, especially in smoked foods, because the aromatic hydrocarbons are constituents of wood smoke. Trace quantities of PAHs have been found in a variety of foods, and this may be the result of environmental contamination.

The PAHs may be carcinogenic and mutagenic. The level of carcinogenicity may vary widely between different members of this group. Minor constituents of PAH mixtures may make large contributions to the carcinogenic activity of the mixture. Certain methylchrysenes, particularly the 5-isomer, which is one of the most carcinogenic compounds known, may dominate the carcinogenic activity of a mixture (Bartle 1991).

Rhee and Bratzler (1968) analyzed hydrocarbons in smoke, and the amounts found in smoke and in the vapor phase (smoke filtered to remove particles) are listed in Table 15.18. Small amounts of these smoke constituents may be transferred to

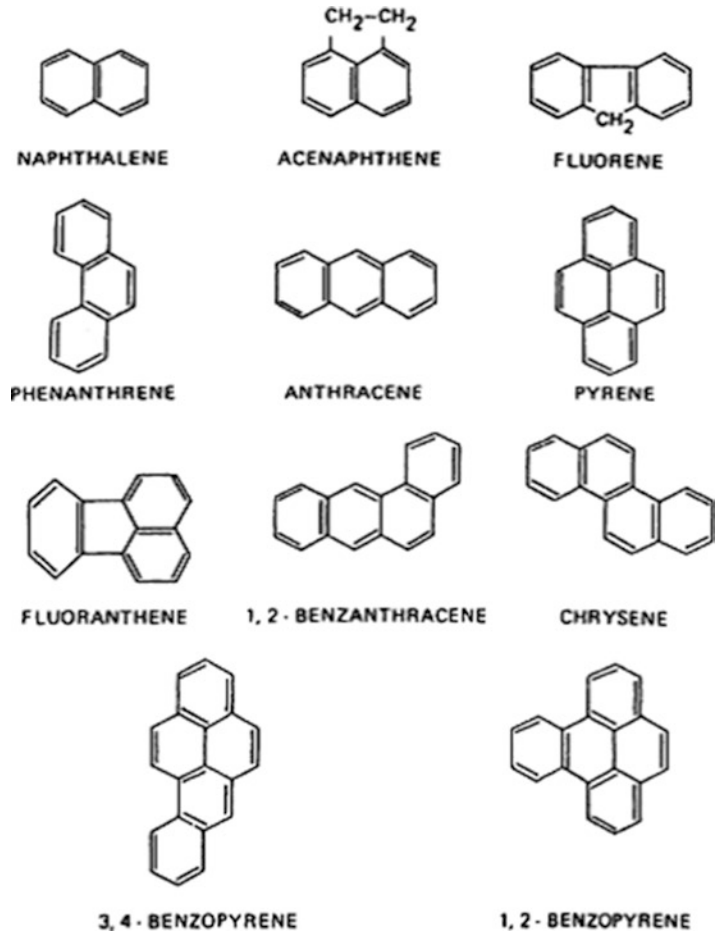


Fig. 15.15 Chemical structure of some polycyclic aromatic hydrocarbons

Table 15.18 Aromatic polycyclic hydrocarbons in wood smoke and in wood smoke vapor phase

Hydrocarbon	Amount, µg/45 kg sawdust whole smoke	vapor phase
Phenanthrene	51.5	28.4
Anthracene	3.8	1.9
Pyrene	5.5	4.1
Fluoranthene	5.7	4.2
1,2-Benzanthracene	7.0	4.3
Chrysene	2.6	0.3
3,4-Benzopyrene	1.2	0.4
1,2-Benzopyrene	0.9	Trace

Source: From K.S. Rhee and L.J. Bratzler, Polycyclic Hydrocarbon Composition of Wood Smoke, *J. Food Sci.,* Vol. 33, pp. 626–632, 1968

foods during smoking. Howard and Fazio (1969) reported the levels of aromatic polycyclic hydrocarbons in foods, and results for smoked foods are listed in Table 15.19. These compounds have also been found in unsmoked foods, as is shown in Table 15.20. Higher levels than those found in smoked food may occur as a result of barbecuing or charcoal broiling. Roasting of coffee and nuts results in formation of PAHs. The levels present in roasted coffee increase with more intense roasting; this is shown in Table 15.21, which is based on results obtained by Fritz (1968). PAHs occur in vegetables (Grimmer and Hildebrand 1965), and the levels are thought to be related to the leaf area and the relative level of atmospheric pollution.

Surprisingly, the largest proportion of the total human intake of PAHs does not come from smoked or roasted foods, but from other common products. Bartle (1991) has stated that cereals are likely to be a greater hazard, especially in the

Table 15.19 Polycyclic aromatic hydrocarbons found in smoked food products (ppb)

Food Product	Benzo (a)-anthracene	Benzo (a)-pyrene	Benzo (e)-pyrene	Benzo (g,h,i,)-perylene	Fluoranthene	Pyrene	4-Methyl-pyrene
Beef, chipped	0.4				0.6	0.5	
Cheese, Gouda					2.8	2.6	
Fish							
Herring					3.0	2.2	
Herring (dried)	1.7	1.0	1.2	1.0	1.8	1.8	
Salmon	0.5		0.4		3.2	2.0	
Sturgeon		0.8			2.4	4.4	
White					4.6	4.0	
Ham	2.8	3.2	1.2	1.4	14.0	11.2	2.0
Frankfurters					6.4	3.8	
Pork roll					3.1	2.5	

Source: From J.W. Howard and T. Fazio, A Review of Polycyclic Aromatic Hydrocarbons in Foods, *Agr. Food Chem.,* Vol. 17, pp. 527–531, 1969, American Chemical Society

Table 15.20 Polycyclic aromatic hydrocarbons in unsmoked food products

Food Product	Fluoranthene (ppb)	Pyrene (PPb)
Cheese, cheddar	0.8	0.7
Fish, haddock	1.6	0.8
Fish, herring (salted)	0.8	1.0
Fish, salmon (canned)	1.8	1.4

Source: From J.W. Howard and T. Fazio, A Review of Polycyclic Aromatic Hydrocarbons in Foods, *Agr. Food Chem.,* Vol. 17, pp. 527–531, 1969, American Chemical Society

Table 15.21 Polycyclic Aromatic Hydrocarbons in Coffee (μg/kg)

Compound	Heavy roasting	Normal roasting
Anthracene	6.2	1.5
Phenanthrene	74.0	28.0
Pyrene	28.0	3.5
Fluoranthene	34.0	3.9
1,2-Benzanthracene	14.2	1.5
Chrysene	14.8	–
3,4-Benzopyrene	5.8	0.3
1,2-Benzopyrene	7.0	0.7
Perylene	0.6	–
11, 12-Benzfluoranthene	1.8	–
Anthanthrene	0.9	–
1, 12-Benzperylene	2.2	–
3,4-Benzfluoranthene	1.2	–
Coronene	0.9	–
Indenopyrene	0.7	–

Source: From W. Fritz, Formation of Carcinogenic Hydrocarbons During Thermal Treatment of Foods, *Nahrung,* Vol. 12, pp. 799–804, 1968

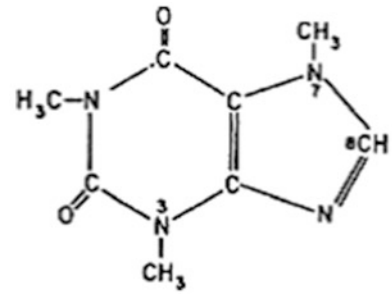


Fig. 15.16 The structure of caffeine 1,3,7- trimethylxanthine

form of flour, than smoked or barbecued foods. Although cereal has a much lower PAH content than smoked or roasted foods do, cereal is consumed in much greater amounts.

Caffeine

Caffeine is a naturally occurring chemical, 1,3,7-trimethylxanthine (Fig. 15.16), which is found in the leaves, seeds, and fruits of more than 63 species of plants growing all over the world. It occurs as a constituent of coffee, tea, cocoa, and chocolate, and is an additive in soft drinks and other foods. Because humans have used it for thousands of years, caffeine has GRAS status in the United States. Roberts and Barone (1983) have estimated that daily caffeine consumption in the United States is 206 mg per person. Caffeine

shows a number of physiological effects (Von Borstel 1983) and, as a result, its regulatory status has been under review (Miles 1983). Complicating the matter is the fact that caffeine is both a naturally occurring chemical as well as a food additive. Caffeine stimulates the central nervous system, can help people stay awake, and can relieve headaches. Adverse effects may include sleep disturbance, depression, and stomach upsets. Large overdoses of caffeine may be fatal (Leviton 1983). Caffeine is a good example of a widely occurring natural toxicant that has been part of our food supply for centuries.

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Appendix A: Moisture Analysis

Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of different reasons:

- *Legal and Labeling Requirements.* There are legal limits to the maximum or minimum amount of water that must be present in certain types of food.
- *Economic*. The cost of many foods depends on the amount of water they contain - water is an inexpensive ingredient, and manufacturers often try to incorporate as much as possible in a food, without exceeding some maximum legal requirement.
- *Microbial Stability.* The propensity of microorganisms to grow in foods depends on their water content. For this reason many foods are dried below some critical moisture content.
- *Food Quality.* The texture, taste, appearance and stability of foods depends on the amount of water they contain.
- *Food Processing Operations.* A knowledge of the moisture content is often necessary to predict the behavior of foods during processing, *e.g.* mixing, drying, flow through a pipe or packaging.

It is therefore important for food scientists to be able to reliably measure moisture contents. A number of analytical techniques have been developed for this purpose, which vary in their accuracy, cost, speed, sensitivity, specificity, ease of operation, *etc*. The choice of an analytical procedure for a particular application depends on the nature of the food being analyzed and the reason the information is needed.

Properties of Water in Foods

The moisture content of a food material is defined through the following equation:

$$\%\text{Moisture} = (m_w/m_{sample}) \times 100$$

Where m_w is the mass of the water and m_{sample} is the mass of the sample. The mass of water is related to the number of water molecules (n_W) by the following expression: $m_w = n_w M_w / N_A$, where M_w is the molecular weight of water (18.0 g per mole) and N_A is Avadagro's number (6.02×10^{23} molecules per mole). In principle, the moisture content of a food can therefore be determined accurately by measuring the number or mass of water molecules present in a known mass of sample. It is not possible to directly measure the number of water molecules present in a sample because of the huge number of molecules involved. A number of analytical techniques commonly used to determine the moisture content of foods are based on determinations of the mass of water present in a known mass of sample.

J.M. deMan et al., *Principles of Food Chemistry*, Food Science Text Series,
https://doi.org/10.1007/978-3-319-63607-8

Nevertheless, as we will see later, there are a number of practical problems associated with these techniques that make highly accurate determinations of moisture content difficult or that limit their use for certain applications. For these reasons, a number of other analytical methods have been developed to measure the moisture content of foods that do not rely on direct measurement of the mass of water in a food. Instead, these techniques are based on the fact that the water in a food can be distinguished from the other components in some measurable way.

An appreciation of the principles, advantages and limitations of the various analytical techniques developed to determine the moisture content of foods depends on an understanding of the molecular characteristics of water. A water molecule consists of an oxygen atom covalently bound to two hydrogen atoms (H_2O). Each of the hydrogen atoms has a small positive charge (δ+), while the oxygen atom has two lone pairs of electrons that each has a small negative charge (δ−). Consequently, water molecules are capable of forming relatively strong hydrogen bonds ($O\text{-}H^{\delta+} \bullet {}^{\delta-}O$) with four neighboring water molecules. The strength and directionality of these hydrogen bonds are the origin of many of the unique physicochemical properties of water. The development of analytical techniques to determine the moisture content of foods depends on being able to distinguish water (the "analyte") from the other components in the food (the "matrix"). The characteristics of water that are most commonly used to achieve this are: its relatively low boiling point; its high polarity; its ability to undergo unique chemical reactions with certain reagents; its unique electromagnetic absorption spectra; and, its characteristic physical properties (density, compressibility, electrical conductivity and refractive index).

Despite having the same chemical formula (H_2O) the water molecules in a food may be present in a variety of different molecular environments depending on their interaction with the surrounding molecules. The water molecules in these different environments normally have different physiochemical properties:

- *Bulk water*. Bulk water is free from any other constituents, so that each water molecule is surrounded only by other water molecules. It therefore has physicochemical properties that are the same as those of pure water, *e.g.,* melting point, boiling point, density, compressibility, heat of vaporization, electromagnetic absorption spectra.
- *Capillary or trapped water.* Capillary water is held in narrow channels between certain food components because of capillary forces. Trapped water is held within spaces within a food that are surrounded by a physical barrier that prevents the water molecules from easily escaping, *e.g.,* an emulsion droplet or a biological cell. The majority of this type of water is involved in normal water-water bonding and so it has physicochemical properties similar to that of bulk water.
- *Physically bound water.* A significant fraction of the water molecules in many foods are not completely surrounded by other water molecules, but are in molecular contact with other food constituents, *e.g.* proteins, carbohydrates or minerals. The bonds between water molecules and these constituents are often significantly different from normal water-water bonds and so this type of water has different physicochemical properties than bulk water *e.g.,* melting point, boiling point, density, compressibility, heat of vaporization, electromagnetic absorption spectra.
- *Chemically bound water.* Some of the water molecules present in a food may be chemically bonded to other molecules as water of crystallization or as hydrates, *e.g.* $NaSO_4 \bullet 10H_20$. These bonds are much stronger than the normal water-water bond and therefore chemically bound water has very different physicochemical properties to bulk water, *e.g.,* lower melting point, higher boiling point, higher density, lower compressibility, higher heat of vaporization, different electromagnetic absorption spectra.

Foods are heterogeneous materials that contain different proportions of chemically bound, physically bound, capillary, trapped or bulk water. In addition, foods may contain water that is present in different physical states: gas, liquid or solid. The fact that water molecules can exist in a number of different molecular environments, with different

physicochemical properties, can be problematic for the food analyst trying to accurately determine the moisture content of foods. Many analytical procedures developed to measure moisture content are more sensitive to water in certain types of molecular environment than to water in other types of molecular environment. This means that the measured value of the moisture content of a particular food may depend on the experimental technique used to carry out the measurement. Sometimes food analysts are interested in determining the amounts of water in specific molecular environments (*e.g.,* physically bound water), rather than the total water content. For example, the rate of microbial growth in a food depends on the amount of bulk water present in a food, and not necessarily on the total amount of water present. There are analytical techniques available that can provide some information about the relative fractions of water in different molecular environments (*e.g.,* DSC, NMR, vapor pressure).

Sample Preparation

Selection of a representative sample, and prevention of changes in the properties of the sample prior to analysis, are two major potential sources of error in any food analysis procedure. When determining the moisture content of a food it is important to prevent any loss or gain of water. For this reason, exposure of a sample to the atmosphere, and excessive temperature fluctuations, should be minimized. When samples are stored in containers it is common practice to fill the container to the top to prevent a large headspace, because this reduces changes in the sample due to equilibration with its environment. The following section provides an overview on the topic with some key methods. Please note these are some key methods but not all the methods available.

Evaporation Methods

Principles

These methods rely on measuring the mass of water in a known mass of sample. The moisture content is determined by measuring the mass of a food before and after the water is removed by evaporation:

$$\%Moisture = \frac{M_{INITIAL} - M_{DRIED}}{M_{INITIAL}} \times 100$$

Here, M_{INITIAL} and M_{DRIED} are the mass of the sample before and after drying, respectively. The basic principle of this technique is that water has a lower boiling point than the other major components within foods, *e.g.,* lipids, proteins, carbohydrates and minerals. Sometimes a related parameter, known as the *total solids,* is reported as a measure of the moisture content. The total solids content is a measure of the amount of material remaining after all the water has been evaporated:

$$\%Total\ Solids = \frac{M_{DRIED}}{M_{INITIAL}} \times 100$$

Thus, %Total solids = (100 − %Moisture). To obtain an accurate measurement of the moisture content or total solids of a food using evaporation methods it is necessary to remove all of the water molecules that were originally present in the food, without changing the mass of the food matrix. This is often extremely difficult to achieve in practice because the high temperatures or long times required to remove all of the water molecules would lead to changes in the mass of the food matrix, *e.g.,* due to volatilization or chemical changes of some components. For this reason, the drying conditions used in evaporation methods are usually standardized in terms of temperature and time so as to obtain results that are as accurate and reproducible as possible given the practical constraints. Using a standard method of sample preparation and analysis helps to minimize sample-to-sample variations within and between laboratories.

Evaporation Devices The thermal energy used to evaporate the water from a food sample can be provided directly (*e.g.,* transfer of heat from an oven to a food) or indirectly (*e.g.,* conversion of electromagnetic radiation incident upon a food into heat due to absorption of energy by the water molecules).

Convection and Forced Draft Ovens

Weighed samples are placed in an oven for a specified time and temperature (*e.g.* 3 h at 100 °C) and their dried mass is determined, or they are

dried until they reach constant mass. The thermal energy used to evaporate the water is applied directly to the sample *via* the shelf and air that surround it. There are often considerable temperature variations within convection ovens, and so precise measurements are carried out using *forced draft ovens* that circulate the air so as to achieve a more uniform temperature distribution within the oven. Samples that contain significant quantities of carbohydrates that might undergo chemical changes or volatile materials other than water should not be dried in a convection or forced draft oven. Many official methods of analysis are based on forced draft ovens.

Vacuum Oven

Weighed samples are placed under reduced pressure (typically 25–100 mmHg) in a vacuum oven for a specified time and temperature and their dried mass is determined. The thermal energy used to evaporate the water is applied directly to the sample *via* the metallic shelf that it sits upon. There is an air inlet and outlet to carry the moisture lost from the sample out of the vacuum oven, which prevents the accumulation of moisture within the oven. The boiling point of water is reduced when it is placed under vacuum. Drying foods in a vacuum oven therefore has a number of advantages over conventional oven drying techniques. If the sample is heated at the same temperature, drying can be carried out much quicker. Alternatively, lower temperatures can be used to remove the moisture (*e.g.* 70 °C instead of 100 °C), and so problems associated with degradation of heat labile substances can be reduced. A number of vacuum oven methods are officially recognized.

Microwave Oven

Weighed samples are placed in a microwave oven for a specified time and power-level and their dried mass is weighed. Alternatively, weighed samples may be dried until they reach a constant final mass - analytical microwave ovens containing balances to continuously monitor the weight of a food during drying are commercially available. The water molecules in the food evaporate because they absorb microwave energy, which causes them to become thermally excited. The major advantage of microwave methods over other drying methods is that they are simple to use and rapid to carry out. Nevertheless, care must be taken to standardize the drying procedure and ensure that the microwave energy is applied evenly across the sample. A number of microwave oven drying methods are officially recognized.

Infrared Lamp Drying

The sample to be analyzed is placed under an infrared lamp and its mass is recorded as a function of time. The water molecules in the food evaporate because they absorb infrared energy, which causes them to become thermally excited. One of the major advantages of infrared drying methods is that moisture contents can be determined rapidly using inexpensive equipment, *e.g.,* 10–25 min. This is because the IR energy penetrates into the sample, rather than having to be conducted and convected inwards from the surface of the sample. To obtain reproducible measurements it is important to control the distance between the sample and the IR lamp and the dimensions of the sample. IR drying methods are not officially recognized for moisture content determinations because it is difficult to standardize the procedure. Even so, it is widely used in industry because of its speed and ease of use.

Advantages and Disadvantages

- *Advantages:* Precise; Relatively cheap; Easy to use; Officially sanctioned for many applications; Many samples can be analyzed simultaneously
- *Disadvantages:* Destructive; Unsuitable for some types of food; Time consuming

Distillation Methods

Principles

Distillation methods are based on *direct* measurement of the amount of water removed from a food sample by evaporation: %Moisture = 100 ($M_{WATER}/M_{INITIAL}$). In contrast, evaporation methods are based on *indirect* measurement of the amount of water removed from a food sample by evaporation:

%Moisture = 100 ($M_{INITIAL} - M_{DRIED}$)/$M_{INITIAL}$. Basically, distillation methods involve heating a weighed food sample ($M_{INITIAL}$) in the presence of an organic solvent that is immiscible with water. The water in the sample evaporates and is collected in a graduated glass tube where its mass is determined (M_{WATER}).

Dean and Stark Method

Distillation methods are best illustrated by examining a specific example: the Dean and Stark method. A known weight of food is placed in a flask with an organic solvent such as xylene or toluene. The organic solvent must be insoluble with water; have a higher boiling point than water; be less dense than water; and be safe to use. The flask containing the sample and the organic solvent is attached to a condenser by a side arm and the mixture is heated. The water in the sample evaporates and moves up into the condenser where it is cooled and converted back into liquid water, which then trickles into the graduated tube. When no more water is collected in the graduated tube, distillation is stopped and the volume of water is read from the tube.

Practical Considerations

There are a number of practical factors that can lead to erroneous results: (1) emulsions can sometimes form between the water and the solvent which are difficult to separate; (2) water droplets can adhere to the inside of the glassware, (3) decomposition of thermally labile samples can occur at the elevated temperatures used.

Advantages and Disadvantages

- *Advantages:* Suitable for application to foods with low moisture contents; Suitable for application to foods containing volatile oils, such as herbs or spices, since the oils remain dissolved in the organic solvent, and therefore do not interfere with the measurement of the water; Equipment is relatively cheap, easy to setup and operate; Distillation methods have been officially sanctioned for a number of food applications.
- *Disadvantages:* Destructive; Relatively time-consuming; Involves the use of flammable solvents; Not applicable to some types of foods.

Chemical Reaction Methods

Reactions between water and certain chemical reagents can be used as a basis for determining the concentration of moisture in foods. In these methods a chemical reagent is added to the food that reacts specifically with water to produce a measurable change in the properties of the system, *e.g.,* mass, volume, pressure, pH, color, conductivity. Measurable changes in the system are correlated to the moisture content using calibration curves. To make accurate measurements it is important that the chemical reagent reacts with all of the water molecules present, but not with any of the other components in the food matrix. A method that are commonly used in the food industry are the *Karl-Fisher titration*

Karl-Fisher Method

The Karl-Fisher titration is often used for determining the moisture content of foods that have low water contents (*e.g.* dried fruits and vegetables, confectionary, coffee, oils and fats). It is based on the following reaction:

$$2H_2O + SO_2 + I_2 = H_2SO_4 + 2HI$$

This reaction was originally used because HI is colorless, whereas I_2 is a dark reddish brown color, hence there is a measurable change in color when water reacts with the added chemical reagents. Sulfur dioxide and iodine are gaseous and would normally be lost from solution. For this reason, the above reaction has been modified by adding solvents (*e.g.,* C_5H_5N) that keep the S_2O and I_2 in solution, although the basic principles of the method are the same. The food to be analyzed is placed in a beaker containing solvent and is then titrated with *Karl Fisher reagent* (a solution that contains iodine). While any water remains in the sample the iodine reacts with it and the solution remains colorless (HI), but once all the water has been used up any additional iodine is observed as a dark red brown color (I_2). The volume of iodine solution required to titrate

the water is measured and can be related to the moisture content using a pre-prepared calibration curve. The precision of the technique can be improved by using electrical methods to follow the end-point of the reaction, rather than observing a color change. Relatively inexpensive commercial instruments have been developed which are based on the Karl-Fisher titration, and some of these are fully automated to make them less labor intensive.

Physical Methods

A number of analytical methods have been developed to determine the moisture content of foods that are based on the fact that water has appreciably different bulk physical characteristics than the food matrix, *e.g.* density, electrical conductivity or refractive index. These methods are usually only suitable for analysis of foods in which the composition of the food matrix does not change significantly, but the ratio of water-to-food matrix changes. For example, the water content of oil-in-water emulsions can be determined by measuring their density or electrical conductivity because the density and electrical conductivity of water are significantly higher than those of oil. If the composition of the food matrix changes as well as the water content, then it may not be possible to accurately determine the moisture content of the food because more than one food composition may give the same value for the physical property being measured. In these cases, it may be possible to use a combination of two or more physical methods to determine the composition of the food, *e.g.,* density measurements in combination with electrical conductivity measurements.

Spectroscopic Methods

Spectroscopic methods utilize the interaction of electromagnetic radiation with materials to obtain information about their composition. Three of the more established methods are TD-NMR,NIR and mid-IR.

TD-NMR (Time Domain) is also called Pulsed NMR TD-NMR is a highly powerful technique, It is an alternative to typical NMR, commonly used in structural analysis. In TD-NMR, it is possible to use permanent magnets as it works at low magnetic fields. Permanent magnets are simple, inexpensive and do not need extensive cooling by liquid gases. However, the low magnetic field results in a low resolution. This is inadequate for obtaining Fourier-transformation frequency spectra. Hence TD-NMR receives the data from the analysis of dependence of signal intensity on time. The signal time-dependency is either fitted directly with appropriate equations or relaxation time constants are obtained from it. Figure X illustrates the various regions of the electromagnetic spectra. Two regions are used for the determination of moisture: the Near Infrared, described as NIR and the mid-Infrared region noted as IR. The NIR region encompasses the region from 2500 to 700 nm. NIR measurements are made with either a filter based instrument on an FT-NIR instrument. In NIR the first second and third overtones of the OH stretch are seen at 1450, 970 and 760 nm respectively. The position of these bands are temperature and hydrogen bonding Furthermore there are combination bands at 1940 and 1190 nm from OH stretching and bending. Generally, the band at 1940 nm is used for low moisture foods while a band 1450 for high moisture foods. In the case of FTIR which can be defined as the region from about 600–4000 wavenumbers peaks at 3300 wavenumbers (broad OH) and 11,650 wavenumbers (HOH bend) are used.

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Appendix B: Units and Conversion Factors

The International System (SI) of the Units rests upon seven base units and two supplementary units as shown in Table B.1. From the base units, derived units can be obtained to express various quantities such as area, power, force, etc. Some of these have special names as listed in Table B.2. Multiples and submultiples are obtained by using prefixes as shown in Table B.3.

Older units in the metric system and the avoirdupois system are still widely used in the literature, and the information supplied in this appendix is given for convenience in converting these units, Table B.4.

Temperature

0 °C = 273 K
Celsius was formerly called Centigrade
100 °C = (100 × 1.8) + 32 °F = 212 °F
0 °C = 32 °F
°F = (°C × 1.8) + 32
°C = (°F – 32) ÷ 1.8

Table B.1 Base units and supplementary units

Quantity	Unit	Symbol
Base units		
Length	meter	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol
Supplementary units		
Plane angle	radian	rad
Solid angle	steradian	sr

Table B.2 Derived units with special names

Quality	Unit	Symbol	Formula
Force	newton	N	$kg.m/s^2$
Energy	joule	J	N.m
Power	watt	W	J/s
Pressure	pascal	Pa	N/m^2
Electrical potential	volt	V	W/A
Electrical resistance	ohm	Ω	V/A
Electrica conductance	siemens	S	1/Ω
Electrical charge	coulomb	C	A•s
Electrical capacitance	farad	F	C/V
Magnetic flux	weber	Wb	V•s
Magnetic flux density	tesla	T	Wb/m^2
Inductance	henry	H	Wb/A
Frequency	hertz	Hz	2π/s
Illumination	lux	lx	$cd•sr/m^2$
Luminous flux	lumen	lm	cd•sr

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https://doi.org/10.1007/978-3-319-63607-8

Table B.3 Multiples and submultiples

Multiplier	Exponent form	Prefix	SI symbol
1,000,000,000,000	10^{12}	tera	T
1,000,000,000	10^{9}	giga	G
1,000,000	10^{6}	mega	M
1000	10^{3}	kilo	k
100	10^{2}	hecto	h
10	10^{-1}	deca	da
0.1	10^{-1}	deci	d
0.01	10^{-2}	centi	c
0.001	10^{-3}	milli	m
0.000001	10^{-6}	micro	μ
0.000000001	10^{-9}	nano	n
0.000000000001	10^{-12}	pico	p

Table B.4 Conversion factors

	To Convert → Into		Multiply By
0.30480	meters (m)	feet (ft) (= 12 in)	3.28084
0.09290	m^2	ft^2	10.76391
0.02832	m^3	cu ft. (ft^3)	35.31467
28.31685	dm^3—liters (L)	ft^3	0.03531
3.78541	liter (= 1000 cc)	US gal (= 128 US fl. oz)	0.26417
4.54609	liter (= 1000 mL)	Imp gal (= 160 I fl. oz)	0.21997
35.2383	liter (L)	US bushel	0.02838
0.06	m^3/h	L/min	16.66667
1.69901	m^3/h	cu ft./min	0.58858
0.22712	m^3/h	USGPm	4.40287
0.27277	m^3/h	IGPM	3.66615
0.10197	kg (= 1000 g)	Newton (N)	9.80665
0.45359	kg	lb (av) (= 16 oz)	2.20462
0.90718	Metric ton (MT)	Short ton (= 2000 lbs)	1.10231
1.01605	M ton (= 1000 kg)	Long ton (= 2240 lbs)	0.96421
0.01602	kg/dm^3	lb/ft^3	62.42789
0.06895	bar (= 10 N/cm^2)	psi	14.50377
0.001	bar	mbar (= 100 Pascals)	1.0 10^3
0.09807	bar	mH_2O	10.19716
1.33331	mbar	mmHg (torr)	0.75001
33.77125	mbar	inHg (60 °F)	0.02961
4.1868	kJ (kiloJoule)	kcal	0.23885
1.05504	kJ	BTU	0.94783
3.6 10^3	kJ	kWh	0.27778 10^{-3}
0.85985 10^3	kcal	kWh	1.16300 10^{-3}
1.16300 10^{-3}	kW	kcal/h	0.85985 10^3
0.29307 10^{-3}	kW (kJ/s)	BTU/h	3.41219 10^3
0.25199	kcal/h	BTU/h	3.96838
0.746	kW	HP (electr.)	1.34048
0.73550	kW	Metric hp	1.35962
0.0935	foot-candle (ft-c)	lux	10.76
1	centipoise (cp)	mPa.s	1
1	centisokes (cSt)	mm^2/s	1
Multiply By	*Into ← To Convert*		

Appendix C: Greek Alphabet

Greek character	Greek name	Roman equivalent
A α	alpha	A a
B β	beta	B b
Γ γ	gamma	G g
Δ δ	delta	D d
E ε, ∈	epsilon	Ĕ ĕ
Z ζ	zeta	Z z
H η	eta	Ē ē
Θ φ, θ	theta	Th th
I ι	iota	I i
K **ϰ**, κ	kappa	K k
Λ λ	lambda	L l
M μ	mu	M m
N ν	nu	N n
Ξ ξ	xi	X x
O ο	omicron	Ŏ ö
Π **π, ϖ**	pi	P p
P ρ	rho	R r
Σ σ, ς	sigma	S s
T τ	tau	T t
ϒ υ	upsilon	Y y
Φ φ,ϕ	phi	Ph ph
X χ	chi	Ch ch
Ψ ψ	psi	Ps ps
Ω ω	omega	**Ōō**

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https://doi.org/10.1007/978-3-319-63607-8