Food Shelf Life Stability

Chemical, Biochemical, and Microbiological Changes

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Food Shelf Life Stability

Chemical, Biochemical, and Microbiological Changes

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Preface

Shelf life stability of raw or processed foods is a measure of how long food products retain optimal quality. The factors that determine shelf life and how they can be used to extend it are issues discussed throughout this book. Increasingly, as maintenance of food quality increases and transportation improves, foods can be produced at much greater distances from their point of consumption. Large and valuable quantities of fresh foods are frequently handled, stored, and transported over long distances when quality can deteriorate due to physical and chemical changes and the action of biochemical systems, even when microbial counts are low. Consumers are better educated about healthy foods and are more careful in selecting products with unexpired dates of freshnes.

This book is divided into three distinct sections dealing mainly with the physical, chemical, or biochemical factors that influence shelf life. In Chapter 1 the effects of water activity and plasticization on the physicochemical properties of food materials as well as on physical, chemical, biochemical, and microbial changes are described. Chapter 2 discusses how mechanical and temperature (both high and low) changes impact the shelf life stability of fruits and vegetables. Chapter 3 covers the role of irradiation in extending the shelf life of foods by reducing spoilage organisms in dairy and meat products or by altering the postharvest ripening and senescence of fruits and vegetables. Chapter 4 includes a broad discussion on how packaging affects shelf life. Professor Marvin A. Tung, one of the co-authors, unfortunately died very suddenly at the end of 1999 and this chapter is a fitting tribute to Professor Tung who has contributed much to our understanding of the importance of packaging.

Section 2 includes four chapters each discussing how chemical factors can be applied to extend the shelf life of foods. Chapter 5 provides an overview of the benefits of controlled and modified atmosphere packaging on the shelf life of fruits, vegetables, grains, and oilseeds. Chapter 6 describes the importance of antioxidants in retarding the development of rancidity, with a particular focus on the search for new and effective natural antioxidants. Chapter 7 includes a detailed examination of the role emulsifiers and stabilizers play in enhancing food products. Chapter 8 describes the success and versatility of sulfites as effective antimicrobial agents as well as their ability to control enzymic and nonenzymic spoilage.

Section 3 examines the biochemical factors affecting shelf life. Chapter 9 reviews the role of oxidative enzymes in foods, lipoxygenases, peroxidases, and polyphenol oxidases and methods of control. Chapter 10 is on biotechnology and focuses on traditional and new technologies used to extend shelf life of foods, paying particular attention to quality traits.

We hope that this book will be a useful reference for teachers, students, and researchers in food science. In a world where the population is increasing, while at the same time land resources are dwindling, methods for extending the shelf life of foods are an important way of maximizing our limited food resources.

N.A.M. Eskin and David S. Robinson

The Editors

N.A. Michael Eskin is a Professor of Food Chemistry at the University of Manitoba, Winnipeg, Canada. He is the author and co-author of 8 books and over 90 research publications. Professor Eskin serves on the editorial board of three international journals and is also an Associate Editor of the *Journal of the American Oil Chemists' Society.* He is a Fellow of the Institute of Food Science and Technology in the U.K. and of the Canadian Institute of Food Science and Technology. In addition to his scientific work, he is a satirist and a contributor to Sesame Street Canada.

David S. Robinson recently retired as Professor and Head of the Procter Department of Food Science at Leeds University in the U.K. He has researched extensively on oxidative enzymes in foods as well as published many papers in this area. Professor Robinson authored several books including *Food Biochemistry & Nutritional Value* and *Oxidative Enzymes in Foods*. Professor Robinson is a Fellow of the Institute of Food Science and Technology.

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Dedication

This book is dedicated to the memory
of Professor Marvin Tung

Section I

Physical Factors

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Yrjö H. Roos

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INTRODUCTION

Water is the most important diluent of water soluble food components and plasticizer (softener) of various water miscible polymeric compounds as well as often the main food component. Chemical reactions, enzymatic changes, and microbial growth may occur readily in foods with high water contents when their occurrence is not restricted by environmental factors such as pH or temperature. Water has several effects on food stability, palatability, and overall quality. The physicochemical state of water is related to water activity, a_w, which is a measure of water availability for the growth of various microorganisms[1-3] and physicochemical stability[4,5] of high-moisture foods. Water as a plasticizer has an additional effect on the shelf life of low- and intermediate-moisture foods.[6]

Rates of deteriorative changes and microbial growth at normal food storage conditions often depend on water content and a_w. Food deterioration due to microbial growth is not likely to occur at $a_w < 0.6$.[2] However, chemical reactions and enzymatic changes may occur at considerably lower water activities. Typical deteriorative changes of low-moisture foods include enzyme-catalyzed changes, nonenzymatic browning, and oxidation. Enzymatic changes and nonenzymatic browning have been found to occur above a critical water content and to show a rate maximum at an intermediate-moisture level which is followed by a decrease at higher water activities.[4] Oxidation may have a high rate at low water contents, the rate may go through a minimum with an increase in a_w, and then it may decrease at higher water activities.[4]

Low-moisture nonfat food solids are often a mixture of disordered molecules; i.e., they exist in an amorphous, metastable, non-equilibrium state. The amorphous solids may exist in a highly viscous, solid, glassy state or in a supercooled, viscous liquid state. The change between these states occurs over a transition temperature range that is referred to as *glass transition*. The glass transition can be observed from changes in heat capacity, dielectric properties, various mechanical properties, volume, and molecular mobility.[6-8] Introduction of the polymer science principles to the characterization of food materials,[6,9-14] and especially water plasticization, has improved understanding of the physicochemical principles that affect relaxation times and rates of mechanical changes in low- and intermediate-moisture and frozen foods.[5,6,9,14-16] Unfortunately, combined effects of glass transition, water content, and temperature on the kinetics of various chemical reactions and deterioration are not well established.[3,6,7,17-19] However, attempts to establish methods for the prediction of the physical state and rates of deteriorative changes of amorphous foods, based on the T_g concept, have been made.[6,9,14,19] A major assumption related to shelf life and quality is that stability is maintained in the glassy state and various changes may occur above T_g with rates determined by the temperature difference, $T - T_g$.[5,6,9] Unfortunately, it cannot be over-emphasized that the transition occurs over a temperature range and a single transition temperature is not always well defined.[20]

This chapter defines water activity and plasticization and describes their effects on physicochemical properties of food materials and effects of water on the physical state, and physical, chemical, biochemical, and microbial changes. The shelf life stability of high-moisture foods is discussed, but the emphasis is on various effects of a_w, glass transition, and water plasticization on temperature-, water content-, and

time-dependent phenomena affecting shelf life and quality of low- and intermediate-moisture foods.

WATER ACTIVITY

The purest forms of water in foods are crystalline ice and gaseous vapor. Depression of the vapor pressure of water by solutes is probably one of the most important factors that affect the properties of water in foods. It can be shown that water activity, a_w, is the ratio of the vapor pressure in a solution or a food material, p_w, and that of pure water at the same temperature, p_w^0, (Eq. 1.1). Therefore, the equilibrium or steady state a_w is related to equilibrium relative humidity (ERH) of the surrounding atmosphere by Eq. (1.2), and a_w can be considered to be a temperature-dependent property of water which is used to characterize the equilibrium or steady state of water within a food material.

$$a_w = \frac{p_w}{p_w^0} \tag{1.1}$$

$$ERH = a_w \times 100\,\% \tag{1.2}$$

Water activity, temperature, and pH are the most important factors that control rates of deteriorative changes and the growth of microorganisms in foods.[21] These parameters are often referred to as *hurdles*.[22] Other important hurdles include redox potential, modified atmosphere, oxygen tension, pressure, radiation, competitive flora, microstructure, and preservatives.[22] Reduction of a_w often affects microbial growth, the predominant microbial culture, and it increases shelf life as a result of the reduced availability of water for the microbial growth. According to Hocking and Christian,[23] a_w and pH, alone or in combination, often determine whether foods are subject to bacterial or fungal spoilage.

Water Activity of Foods

Most fresh foods can be considered as high-moisture foods and their shelf life is largely controlled by the growth of microorganisms. High-moisture foods have an a_w of 0.90 to 0.999 and they usually contain more than 50% w/w water. These foods include fresh meat and seafood, various dairy products, and fruits and vegetables as well as beverages. Most bacteria, molds, and yeasts are likely to grow in high-moisture foods. However, the types of spoilage microorganisms and their species are highly dependent on both a_w and pH[23] as well as other hurdles.

Intermediate moisture foods (IMF) have an a_w of 0.60 to 0.90 and the water content is 10 to 50%. These foods include many traditional low-moisture foods, such as grains, nuts, and dehydrated fruits, but also a number of processed foods. Brimelow[24] classified IMF products into those *consumed as is*, those *consumed after rehydration*, and those *consumed after dehydration*. All of these categories had examples of traditional and novel foods. Traditional as is consumed products included salted, cured meats, salted fish, Parma ham, dried fruits, some cheeses, and jams. Pet

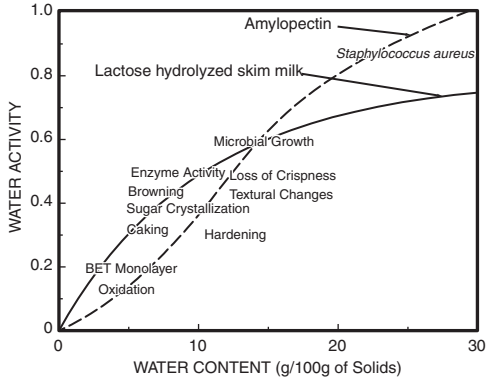

FIGURE 1.1 Critical water activity and water content ranges for various changes and microbial growth occurring in food materials. The sorption isotherms of lactose hydrolyzed skim milk and amylopectin are shown as examples of extreme values for water activities and water contents.

foods and some novel fruit products were classified as consumed as is novel foods. Examples of traditional and novel IMF products consumed after rehydration were jellies, meat-filled pasta, and condensed milk and soup, sauce, and meal concentrates, respectively. The traditional and novel IMF products consumed after dehydration included some fruit cakes/pies/puddings and pop-tarts, respectively.

Although microbial spoilage is prevented at a_w below 0.60,[2] low-moisture foods may exhibit deleterious changes, such as structural transformations, enzymatic changes, browning, and oxidation, depending on a_w, temperature, and, therefore, extent of water plasticization. As shown in Figure 1.1, critical a_w values can be defined for various changes and microbial growth resulting in loss of stability.[25] However, the critical values are specific for each food material and they may be dependent on food composition and plasticization behavior.

Water Sorption

Water activity of high-moisture foods and several IMF products is relatively constant and dependent on composition, especially solids content, and the type of water soluble components. However, the a_w of low-moisture foods and many IMF products is dependent on storage relative humidity and temperature. Steady state relationships between a_w and water content at a constant temperature are described by sorption isotherms. Typical sorption isotherms of food materials are sigmoid curves, which exhibit hysteresis between the adsorption and desorption isotherm, as shown in Figure 1.2. Determination of sorption isotherms is necessary for the determination of stability at various storage conditions and requirements for packaging materials to ensure product shelf life.[25]

The most common method to obtain sorption isotherms is the determination of steady state water contents for food materials at constant relative humidity and temperature conditions, e.g., equilibration of samples over saturated salt solutions in vacuum desiccators.[5] Prediction of water sorption isotherms is then based on the

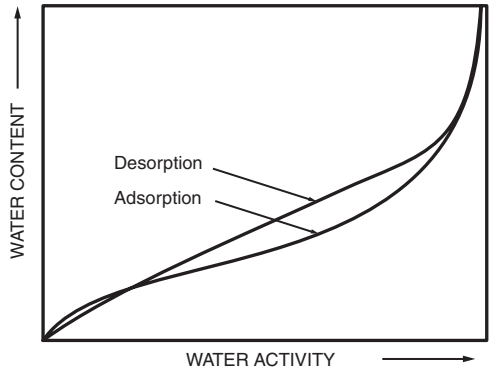

FIGURE 1.2 A schematic representation of sorption isotherms typical of food materials. A hysteresis is often obtained between the adsorption and desorption isotherm.

determination of sufficient experimental data and fitting sorption models to the data. A number of empirical and theoretical sorption models are available.[26,27] Some of the models have proved to be useful in predicting water sorption by food materials, particularly the Guggenheim-Anderson-DeBoer (GAB) model.[27]

The well-known Brunauer-Emmett-Teller (BET) sorption model by Brunauer et al.[28] has been applied to obtain the BET monolayer water content of foods.[29] The BET monolayer value expresses the amount of water that theoretically may form a layer of water molecules with the thickness of one molecule on the adsorbing surface. The BET model is given by Eq. (1.3), where m is water content (g/100 g of solids), m_m is the monolayer value, and K is a constant.

$$\frac{m}{m_m} = \frac{a_w}{(1-a_w)\left[1+(K-1)a_w\right]} \tag{1.3}$$

The BET model can also be written into the linearized form, as suggested by Eq. (1.4).

$$\frac{a_w}{m(1-a_w)} = \frac{1}{m_m K} + \frac{K-1}{m_m K} a_w \tag{1.4}$$

The applicability of the BET model is limited because it has proved to fit water sorption data only over the narrow a_w range from 0.1 to 0.5.[29] However, the BET monolayer value (Figure 1.1) has been suggested to be an optimal water content for stability of low-moisture foods[4,30] and correlate with an optimum a_w allowing the longest shelf life.[25]

The GAB adsorption model was introduced by van den Berg.[27,31] The GAB model given by Eq. (1.5) is derived from the BET model, but it has an additional parameter, C.

$$\frac{m}{m_m} = \frac{K' C a_w}{(1-Ca_w)\left[1+(K'-1)Ca_w\right]} \tag{1.5}$$

The GAB model has been shown to fit experimental sorption data for almost all food materials and cover the whole a_w range.[27] The model is applicable to predict water sorption for most foods and it can also be used to calculate the GAB monolayer value. However, the BET and GAB monolayer values are not equal and neither of these values can be considered as a real "stability" water content. Both the BET and GAB monolayer values are dependent on temperature and they do not reflect stability-related changes in the physical state of foods.[6,32] An alternative approach is to determine *critical*, stability controlling *water content* and *water activity*, based on the determination of the steady state water content and a_w that depress the glass transition temperature to ambient or storage temperature.[5,32]

Temperature Dependence of Water Activity and Sorption

The temperature dependence of the vapor pressure of water may be assumed to follow the Clausius-Clapeyron relationship. Therefore, it can be shown that the temperature dependence of a_w also follows the Clausius-Clapeyron relationship[29] according to Eq. (1.6) where the a_w is a_{w1} and a_{w2} at temperatures T_1 and T_2, respectively, Q_s is the heat of sorption, and R is the gas constant (8.14 J/g mol).

$$\ln \frac{a_{w2}}{a_{w1}} = -\frac{Q_S}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{1.6}$$

The temperature dependence of a_w suggests that if the storage temperature of a food at a constant water content, e.g., in a sealed package, increases, the a_w of the food also increases. If the a_w of the food is kept constant, an increase in temperature results in a decrease in water content. The temperature dependence of water sorption is described in Figure 1.3. The heat of sorption increases with decreasing water content as more energy is needed to remove water molecules associated with the food solids.

PHYSICAL STATE AND WATER PLASTICIZATION

State Transitions

The key factors controlling quality changes and stability in food processing and storage are temperature, time, and water content. High-moisture foods contain excess water that provides an excellent media for diffusion and reactions. Thus, a_w cannot be used to control stability and the shelf life is determined mainly by pH, storage temperature, and protective packaging.

Food materials at low water contents and in the freeze-concentrated frozen state form metastable amorphous matrices. Although the stability is determined by temperature and water content, it is often greatly related to the physical state of the amorphous matrix of food solids and plasticizing water.[6] The stability in the glassy state is based on restricted rotational mobility of molecules, while changes may occur in the supercooled liquid state above the glass transition temperature where

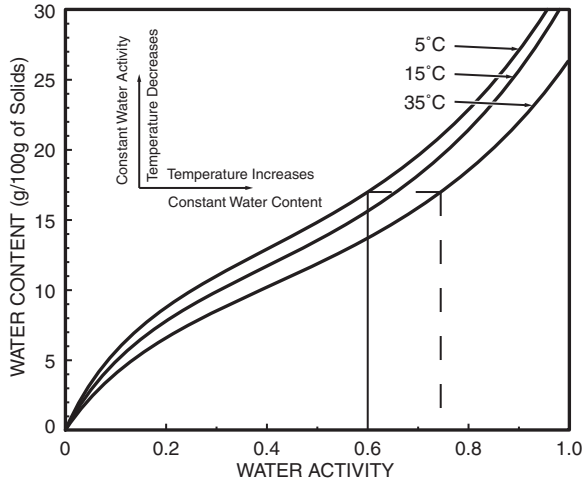

FIGURE 1.3 Temperature dependence of water sorption. The isotherms shown are those of amylopectin.[69]

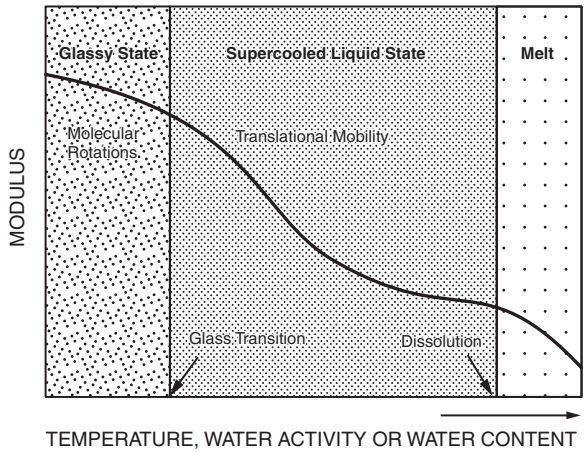

FIGURE 1.4 A schematic representation of changes in modulus and the physical state occurring in amorphous food materials as a function of temperature, water activity, or water content.

translational mobility of molecules is possible.[5,6] The significant change in molecular mobility is observed as the material is transformed from the supercooled solid into the liquid-like state over the T_g temperature range (Figure 1.4). Relationships between the physical state, mobility, and food texture have been reported, but very little and controversial data exist on molecular mobility, reaction rates, and microbial growth in concentrated and frozen food systems.

The T_g often refers to the onset or midpoint temperature of the glass transition temperature range, as determined using differential scanning calorimetry (DSC). For synthetic polymers it is the most important factor controlling mechanical properties.[33] Amorphous or partially amorphous structures in foods are formed in various processes that allow a sufficiently short time for removal of water or cooling of concentrated solids to produce the supercooled liquid or glassy state. These processes include baking, concentration, drum-drying, freeze-drying, spray-drying, and extrusion.[5-7] The amorphous food materials exhibit time-dependent changes as they approach an equilibrium state. These transformations are characterized by changes in mechanical properties, i.e., various collapse phenomena observed from a change in structure or viscous flow resulting in stickiness, caking, and loss of porosity,[6,7,10,34-36,38] and changes in diffusion, i.e., crystallization of amorphous sugars, flavor retention and release, and, possibly, reaction kinetics.[5-7,10,15,16,37-39]

Effects of Water on the Physical State

The T_g values of food materials vary from that of pure water at about –135°C[40-43] to those of polysaccharides, such as starch. Important T_g values of food components are those of sugars,[6,44] oligosaccharides,[45,46] and proteins.[47,48] Unfortunately, T_g values for a number of biopolymers, such as anhydrous polysaccharides and proteins, cannot be measured, as they undergo thermal decomposition at temperatures below T_g. These materials and other nonfat food solids generally become plasticized by water.[6,9,45,46,48-50] Water plasticization can be observed from the depression of the glass transition temperature with increasing water content, which also improves detectability of the transition. Therefore, measured T_g values for starch at various water contents have been reported, but not for, e.g., anhydrous starch or gluten.[49,50]

Prediction of the T_g depression as a result of water plasticization is useful in evaluation of effects of food composition on T_g, as glass transition-related changes often affect shelf life and quality. The Gordon-Taylor equation[51] has proved to be particularly useful in fitting experimental data on T_g and composition of amorphous carbohydrates, proteins, and foods.[5,46,48,52] The Gordon-Taylor equation [Eq. (1.7)] uses component T_g values, T_{g1} and T_{g2}, and weight fractions, w_1 and w_2, for solids and water, respectively, and a constant k to obtain the T_g of the mixture. The T_{g2} = –135°C is often used for amorphous water.[5,6,46,53]

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \tag{1.7}$$

Water Activity and Physical State

The effect of water and a_w on the physical state of food solids is often observed from structural changes that occur above a critical a_w or water content,[5,32,54] as was described in Figure 1.1. Roos[55] established a linear relationship between a_w and T_g. The linearity often applies over the a_w range of 0.1 to 0.8, but the true relationship over the whole a_w range seems to be sigmoid.[56] The relationship between T_g and a_w

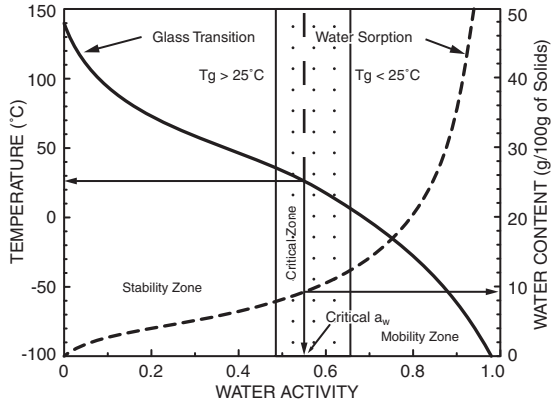

FIGURE 1.5 "Stability diagram" showing critical values for water content and water activity, a_w, that depress the glass transition temperature, T_g, to ambient temperature (25°C). The data are those of a 20 DE (dextrose equivalent) maltodextrin.[32]

at a constant temperature provides a simple method for prediction of storage relative humidity (RH) effects on the T_g. Such prediction is useful in the evaluation of stability of various low- and intermediate-moisture foods, e.g., food powders, low-moisture cereals, and snack foods, on the basis of the food material science concept.

Roos[32] used sorption models and the Gordon-Taylor equation for the description of water plasticization. The models were fitted to experimental data and used to show the T_g and water sorption isotherm in a single plot. The information was used to locate critical values for a_w and water content, defined as those decreasing the T_g to ambient temperature,[5,32,52] as shown in Figure 1.5. However, the T_g is not a well-defined parameter,[57] as it is dependent on the method of observation and its definition. Therefore, it should be noticed that the stability and shelf life of food materials are not governed by a single T_g, a_w, or water content value, but the rate of changes and decrease in shelf life are likely to increase over a transition range as shown in Figures 1.5 and 1.6.

State Diagrams

State diagrams are simplified phase diagrams that describe the concentration dependence of the glass transition temperature of a food component or a food system.[6] State diagrams are effective tools in establishing relationships between the physical state of food materials, temperature, and water content. State diagrams show the glass transition temperature as a function of water content and the effect of ice formation on T_g and on the equilibrium ice melting temperature, T_m (Figure 1.7). State diagrams may also show solubility as a function of temperature and information on various changes that may occur due to the metastable state of amorphous food solids, as they approach the equilibrium state. In food formulation and design, state diagrams allow evaluation of the effects of food composition and water content on the physical state and physicochemical properties during processing and storage.[53]

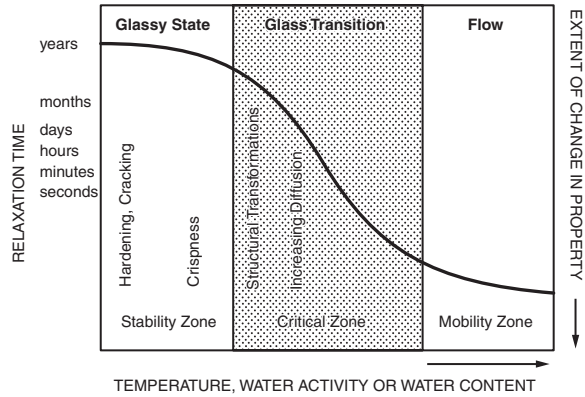

FIGURE 1.6 A schematic representation of relaxation times resulting in time-dependent changes in mechanical properties and diffusion, described by the extent of change in property, in amorphous food materials as a function of temperature, water activity, or water content.

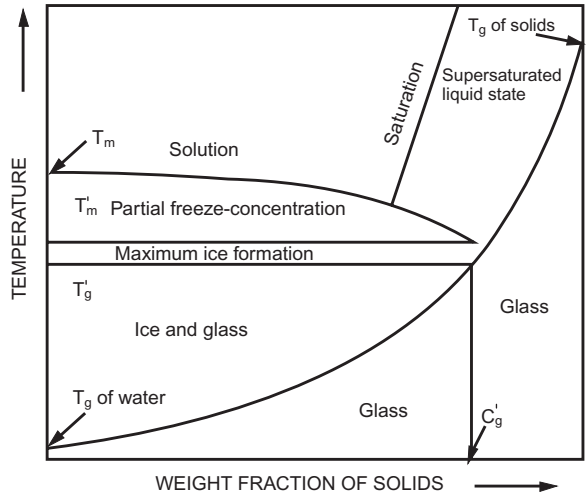

FIGURE 1.7 A typical state diagram of food materials showing the composition dependence of the glass transition temperature, T_g, equilibrium ice melting temperature, T_m, the effect of maximum freeze-concentration on the observed glass transition, T'_g, corresponding solute concentration C'_g, and the onset of ice melting in the maximally freeze-concentrated material, T'_m.

PHYSICAL STABILITY

Relaxation Times and Mechanical Properties

Mechanical properties of amorphous food materials are affected by glass transition which may change textural characteristics. Rates of changes in mechanical properties can be analyzed in terms of relaxation times. Williams et al.[58] reported that a single

empirical relationship describes the temperature dependence of mechanical properties of amorphous polymers above T_g. The ratio, a_T, of relaxation times, τ and τ_0, of configurational rearrangements at respective temperatures, T and T_0, reflects the temperature dependence of mobility. Literature data for viscosity of a number of amorphous materials showed that a_T followed Eq. (1.8), where C_1 and C_2 are constants, and T_0 is a reference temperature.[58]

$$\log a_T = \frac{-C_1\left(T - T_0\right)}{C_2 + \left(T - T_0\right)} \tag{1.8}$$

Equation (1.8) is known as the Williams-Landel-Ferry (WLF) relationship, which was reported to be applicable over the temperature range from T_g to $T_g + 100°C$. The use of T_0 at $T_g + 50°C$ instead of using $T_0 = T_g$ as the reference temperature was considered preferable, because experimental data on relaxation times at and below T_g are often scarce or nonexisting. However, Williams et al.[58] reported "universal values" for C_1 and C_2, but their use in predicting food behavior at temperatures above T_g has been criticized.[59] Peleg[57,59] has shown that the WLF model may not be useful within the transition range and he has suggested the use of Fermi's distribution function to model the rate dependence on a_w, water content, and temperature of changes occurring over the glass transition. The main difference between the curves obtained is that the WLF prediction has an upward concavity, while Fermi's distribution function gives a downward concavity at and around the transition region.[57]

Viscosity

The WLF relationship relates viscosity or any other temperature-dependent mechanical property to T_g or some other reference temperature, as suggested by Levine and Slade,[6,9-11,14] although extrapolation and use of the relationship within the transition temperature range is not justified.[57] Similarities between the physical properties of various amorphous materials, however, have shown that the use of the WLF model for predicting temperature dependence of viscosity allows establishing diagrams showing isoviscosity states above T_g or at least to describe the dramatic effect of the transition on viscosity or relaxation times of mechanical changes. State diagrams with isoviscosity lines show effects of both temperature and water content on the physical state (Figure 1.8). Such diagrams are useful in estimation of effects of composition on relaxation times, i.e., rates of changes in mechanical properties, at a constant temperature or to establish critical temperatures in food processing (e.g., agglomeration, extrusion, and dehydration) and storage (e.g., storage of low-moisture foods and powders at high relative humidity/temperature environments).[6,53]

Stiffness

The term *collapse*, as defined by Levine and Slade,[10] covers various time-dependent structural transformations that may occur in amorphous food and other biological materials at temperatures above T_g. These changes reflect the effect of the changes in relaxation times of mechanical properties and flow that occur over the T_g tem-

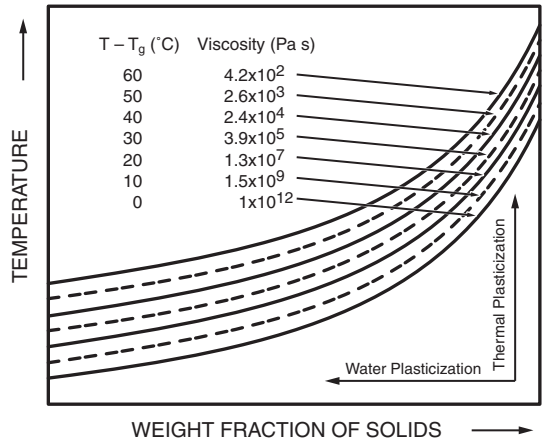

FIGURE 1.8 A state diagram showing the decrease in viscosity occurring in amorphous food materials as they are plasticized by temperature or water above the glass transition temperature or corresponding water content.

perature range. It should be noticed that these changes have been known to occur above a critical water content or a_w during food storage. According to Levine and Slade,[10] collapse phenomena may include or have an effect on stickiness and caking of food powders, plating of particles on amorphous granulas, crystallization of component compounds, structural collapse of dehydrated structures, release and oxidation of encapsulated lipids and flavors, enzymatic activity, nonenzymatic browning, graining of boiled sweets, sugar bloom in chocolate, ice recrystallization, and solute crystallization during frozen storage.

Peleg[60] used *stiffness* as a general term referring to the response of food materials to an external stress. A single model [Eq. (1.9)] based on Fermi's distribution model, where X is a_w, T, or water content, m, could be used for modeling their effects on stiffness. The stiffness parameter, Y, as a function of a_w, T, or m can also be related to its value at a reference state, Y_s, and a constant, a_X, which is a measure of the broadness of the transition. The reference value, X_s, obtained from $b = -X_s/a_X$, indicates the value for a_w, T, or m, that decreases Y to 50% below Y_s. Peleg[20,57,60] emphasized that Eq. (1.9) predicts a change in Y, which may be any property that is related to stiffness, including instrumental and sensory measures of mechanical properties, e.g., crispness, within the T_g range. The stiffness parameters when plotted with critical a_w, m, or T values provide valuable information on the extent of changes occurring at and above the glass transition.

$$\ln\left(\frac{Y_s}{Y} - 1\right) = b + \frac{1}{a_X} X \tag{1.9}$$

Peleg[57] has also shown that the "stiffness equation" can be combined with the Gordon-Taylor equation to establish three-dimensional relationships between water content, temperature, and a change in the stiffness parameter.

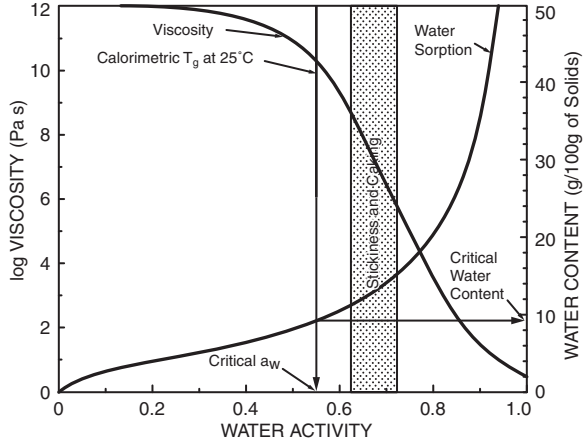

FIGURE 1.9 A hypothetical representation of changes in viscosity and water content in amorphous food materials as a function of water activity, a_w. The critical a_w depresses the glass transition temperature, T_g, to ambient temperature (25°C).

Stickiness, Caking, and Collapse

Stickiness and caking of food powders and collapse of structure have been shown to be related to glass transition with rates governed by the temperature difference, $T - T_g$.[6,9,10,15,16,61] Downton et al.[62] proposed that particles of amorphous powders stick together if sufficient liquid can flow to build strong enough bridges between the particles. Sufficient flow occurred when the particle surface viscosity was decreased to a critical value of about 10^7 Pa s. The main cause of stickiness is plasticization of particle surfaces by water which allows interparticle binding and formation of clusters.[63] Stickiness may be considered to be a time-dependent property of amorphous food solids. The viscosity in the glassy state is extremely high and contact time between particles must be very long to result in bridging. The dramatic decrease in viscosity over the glass transition temperature range, as shown in Figure 1.9, obviously reduces the contact time and causes interparticle fusion resulting in stickiness and caking.[5,62]

Collapse of structure refers to viscous flow occurring as the material cannot support its own weight. Structural collapse is a phenomenon that causes loss of quality of dehydrated, especially freeze-dried, foods.[34,38] Tsourouflis et al.[34] found that the decrease in viscosity and flow were dependent on water content and temperature. Studies of Tsourouflis et al.[34] and To and Flink[35,36] suggested that there is a critical viscosity above which time to collapse may substantially increase. It was later shown that collapse was observed above T_g when the relaxation time for collapse became sufficiently short and of practical importance.[64]

Crispness

Crispness is essential to the quality of various low-moisture cereal and snack foods. Crispness of low-moisture foods is affected by water content and it may be lost as

a result of plasticization of the physical structure by temperature or water.[54,65] A critical a_w at which crispness is lost has been found to be specific for each product, but a change often occurs over the a_w range of 0.35 and 0.50.[54,66] Loss of crispness is obviously a result of extensive water plasticization above the critical water content or a_w that is sufficient to depress the T_g of the material to below ambient temperature, as described in Figures 1.4 and 1.9.

Crystallization Phenomena

Crystallization of amorphous food components, e.g., sugar crystallization and starch retrogradation are probably the most dramatic time-dependent changes that affect structural properties and quality of low-moisture and cereal foods.[5,6,9,10,15,16,38,67] Makower and Dye[68] found that amorphous glucose and sucrose were stable at 25°C when relative humidities were lower than 5 and 12% corresponding to a_w of 0.05 and 0.12, respectively. At higher storage humidities, water sorption resulted in crystallization and release of sorbed water. Water plasticization and depression of T_g to below ambient temperature are responsible for crystallization of amorphous sugars in foods as a result of increased free volume and molecular mobility, decreased viscosity, and enhanced diffusion as shown in Figure 1.10.[5,15,16] Crystallization seems to initiate at T_g or corresponding a_w and proceed with a rate determined by the temperature difference $T - T_g$ to a maximum extent also defined by the $T - T_g$. Crystallization in gelatinized starch, which is typical of starch-containing foods and at least partially responsible for bread staling, is also governed by T_g.[69,70]

The kinetics of crystallization of sugars and starch components at a constant temperature above T_g can be related to water content and a_w, which define $T - T_g$.[16,70] Foods that contain mixtures of sugars have a more complicated crystallization

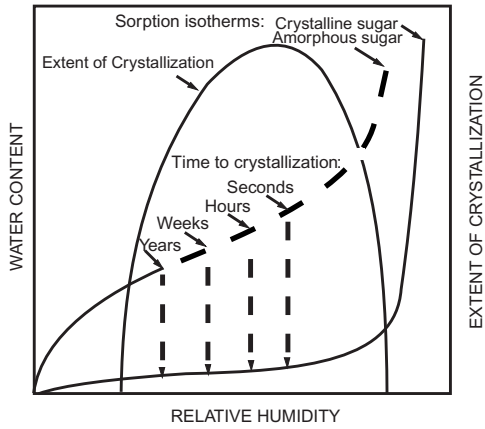

FIGURE 1.10 The difference in water sorption between amorphous and crystalline sugars. Water sorption above a critical relative humidity depresses the glass transition of the material to below ambient temperature and causes time-dependent crystallization and loss of adsorbed water. The extent of crystallization has a maximum at an intermediate relative humidity.

behavior. Iglesias and Chirife[39] studied crystallization kinetics of sucrose in amorphous food models in the presence of polysaccharides. Amorphous sucrose adsorbed high amounts of water, which was lost rapidly. Materials with added polysaccharides showed a fairly rapid water uptake. However, the loss of adsorbed water occurred more slowly and the rate decreased with an increase in the polysaccharide content suggesting an inhibitory effect on crystallization.

CHEMICAL AND MICROBIAL STABILITY

Kinetics of chemical reactions and quality changes is an important determinant of food shelf life. The rate of a chemical reaction defines the change of concentration at a given time. Extended shelf life can be based only on slow reaction rates and manipulation of rate affecting factors to a desired level. The order of a chemical reaction is defined by Eq. (1.10), which states that the change in concentration, C, of a chemical compound (or quality factor) during a chemical reaction at time, t, is defined by the initial concentration, the reaction rate constant, k, and the order of the reaction, n.

$$-\frac{dC}{dt} = kC^n \tag{1.10}$$

The reaction rate constant is a measure of the reactivity and defines the change in concentration of the reactant or quality factor as a function of time.

Temperature Dependence of Reaction Rates and Quality Changes

An empirical approach in studies of temperature-dependent kinetics of reaction rates and quality changes is determination of the rate, k_T, at a temperature, T, and the rate, k_{T+10}, at T + 10 which allows definition of the ratio of the rates known as the Q_{10} value. The Q_{10} value defines that an increase in temperature by 10° increases the rate by the Q_{10} factor.

The temperature dependence of reactions and changes affecting shelf life of foods often follow the Arrhenius-type temperature dependence. The Arrhenius relationship is given in Eq. (1.11), where k is the rate constant, k_0 is the frequency factor, E_a is activation energy, R is the gas constant, and T is absolute temperature. The Arrhenius relationship defines that a plot of k against 1/T gives a straight line with the slope E_a/R.

$$k = k_0 e^{-\frac{E_a}{RT}} \tag{1.11}$$

The Arrhenius relationship is probably the most important relationship used to model temperature dependence of various quality changes in foods. However, there are important factors that may result in deviation from the Arrhenius kinetics as well as cause unexpected changes in product shelf life. According to Labuza and Riboh[71]

nonlinearities in Arrhenius plots of reaction rates may result from (1) first-order phase transitions (e.g., melting of solid fat, which may increase mobility of potential reactants in the resultant liquid phase); (2) crystallization of amorphous sugars may release water and affect the proportion of reactants in the solute-water phase; (3) freeze-concentration of solutes in frozen foods may increase concentration of reactants in the unfrozen solute matrix; (4) reactions with different activation energies may predominate at different temperatures; (5) an increase in a_w with increasing temperature may enhance reactions; (6) partition of reactants between oil and water phases may vary with temperature depending on phase transitions and solubility; (7) solubility of gases, especially of oxygen, in water decreases with increasing temperature; (8) reaction rates may depend on pH, which also depends on temperature; (9) loss of water at high temperatures may alter reaction rates; and (10) protein denaturation at high temperatures may affect their susceptibility to chemical reactions.

It has been well established that water as a plasticizer has a significant effect on molecular mobility and probably on rates of quality changes above a critical, temperature-dependent a_w or water content. A chemical reaction requires sufficient mobility of reactants and products in addition to the driving force, e.g., temperature or concentration, of the reaction or change in quality. Slade and Levine[6] suggested that diffusion in amorphous foods is related to viscosity and, therefore, governed by the glass transition. According to this assumption, the rate of a reaction is controlled by viscosity and diffusion and it may be assumed that below T_g the rate of a reaction can be extremely slow. At temperatures above T_g, diffusivity increases as viscosity decreases and in some cases the temperature dependence of the reaction rate may follow the WLF-type temperature dependence. It is likely that in low-moisture and frozen foods, a change in the rate constant of a diffusion-controlled reaction or quality change occurs in the vicinity of the T_g (Figure 1.11). However, the true rate constants of deteriorative reactions at temperatures typical of food storage are relatively low and only minor changes of activation energies can be observed as food materials are transformed from the solid, glassy state into the supercooled liquid state.

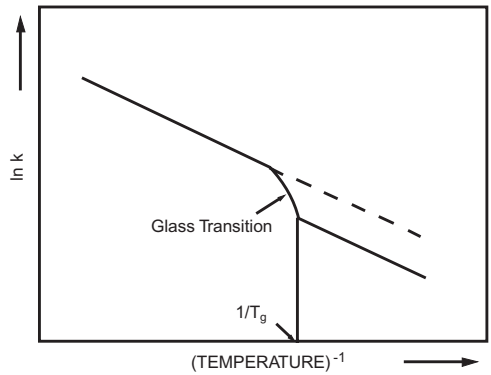

FIGURE 1.11 The effect of glass transition on the temperature dependence of reaction rate constants, k, of diffusion-controlled reactions, as may be observed from the Arrhenius plots.

The effect of a glass transition on reaction rates or quality changes can be observed from experimental data. The change in diffusivity, D, above T_g may be dramatic and substantially larger than it would be according to the Arrhenius-type temperature dependence, but the difference in the observed and the true reaction rate constants has been found to be relatively small and the apparent change in the rate constant occurs within a relatively narrow temperature range above T_g.[5] In practice, rates of diffusion-controlled reactions have been determined for foods above T_g and the rates have followed the Arrhenius-type temperature dependence.[72] It should be noticed that the change in diffusion at a constant temperature as a result of water plasticization may be even more dramatic when observed as a function of a_w or water content than as a function of temperature. This may reflect a change in diffusivity as the concentration of reactants at low water contents is seldom a limitation for the reaction.

If a change in a quality factor is diffusion-controlled and dependent on viscosity, the observed rate constant may become a function of the true rate constant and diffusivity.[73] In some studies, the observed rate constants of diffusion-controlled reactions and quality changes in amorphous food matrices have been assumed to be proportional to $1/\eta$, which has led to the direct application of the WLF relationship in the form of Eq. (1.12), where k' is the observed rate constant and k'_s is the observed rate constant at a reference temperature, T_s.

$$\log \frac{k'_s}{k'} = \frac{-C_1\left(T - T_S\right)}{C_2 + \left(T - T_S\right)} \tag{1.12}$$

Application of Eq. (1.12) to model rates of nonenzymatic browning at several water contents has failed in most cases.[74,75] The use of Eq. (1.12) may be justified when the material has a constant water content and the constants C_1 and C_2 have been derived from experimental data.[72]

Rates of diffusion-controlled reactions are likely to be affected by temperature and water content in addition to glass transition.[73] Nelson[72] studied effects of T_g on kinetic phenomena, including crystallization of amorphous sugars and rates of chemical changes in food materials. She found that deteriorative reactions occurred at temperatures below T_g, but a large increase in the rates of several reactions in the vicinity of T_g was evident. These findings were in agreement with rates of nonenzymatic browning obtained for various amorphous foods and food models.[76] Nelson[72] concluded that the proper application of the WLF model in predicting kinetic data involves determination of the WLF coefficients. The coefficients, C_1 and C_2, are dependent on the system and also on its water content. However, the rates of chemical changes in rubbery matrices, instead of following the WLF model, often followed the Arrhenius-type temperature dependence.[19,72]

Effects of Water and Glass Transition on Reaction Rates

A number of studies have reported kinetic data for observed reaction rates in low-moisture foods. These data have often been reported as a function of a_w or water

content at a constant temperature. The change in the physical state due to glass transition or other phase transitions has been considered in only a few studies. These studies have revealed that reaction rates are increased above glass transition, but some reactions have occurred also at temperatures below T_g.[72,74]

The relative rate of deteriorative changes is traditionally related to water content and a_w with the assumption that stability of low-moisture foods can be maintained at water contents below the BET monolayer value.[4,30,77] Duckworth[78] used wide-line nuclear magnetic resonance spectroscopy to determine "mobilization points" for solutes at a constant temperature in low-moisture food matrices. The mobilization point was found to be peculiar to the system and the level of hydration needed to achieve mobility was solute-dependent. Duckworth[78] also found that no solute mobilization occurred below the BET monolayer value, and results for a system that contained reactants of the nonenzymatic browning reaction suggested that browning initiated at the mobilization point. An increase in the reaction rate was apparent with increasing a_w and the rate maximum occurred at the a_w corresponding to the hydration level allowing complete mobilization. Extensive water contents resulted in dilution and reaction rates were reduced. Molecular mobility in low-moisture foods is obviously important in defining rates at which reactants may diffuse within the solid matrix. According to Duckworth,[78] mobilization of solutes required sufficient amounts of hydrated reactants. The theory assumed that food materials with water contents less than the BET monolayer value were composed of a hydrated matrix with undissolved reactants and reactions did not occur. Above the monolayer value, some of the reactants were dissolved, which allowed mobility in a saturated solution and an increasing rate with increasing water content. However, Duckworth[78] did not consider that the systems studied were amorphous and the solutes were in the dissolved, but solid, state. It may be assumed that the mobility of the reactants at low water contents was restricted by the high viscosity of the glassy state. Reaction rates at low water contents but at a constant temperature may be considered to be restricted by diffusional limitations,[18] as the transport of the reactants as well as transport of the products become the rate controlling factors.

It is likely that most of the nonfat solids in low-moisture foods are amorphous and therefore mobility may be achieved by plasticization.[79] Such plasticization may result from an increase in temperature (thermal plasticization) or addition of plasticizers such as glycerol or water (water plasticization). Roozen et al.[80] used ESR to study molecular motions of dissolved probes in malto-oligosaccharides and maltodextrins with various water contents as a function of temperature. The rotational motions were detected from rotational correlation time, τ_c which was related to the rotational diffusion coefficient. Roozen et al.[80] found that τ_c, decreased linearly with increasing temperature at temperatures below T_g, suggesting that temperature-dependent molecular motions occurred in the glassy state. However, a dramatic decrease of the rotational correlation time occurred over the T_g temperature range, which indicated the dramatic effect of T_g on molecular mobility. Roozen et al.[80] also noticed the decrease of the temperature at which the change in molecular mobility occurred with increasing water content as a result of water plasticization. The dramatic increase of molecular mobility above, but in the vicinity of T_g, obviously has an effect on rates of quality changes in low-moisture foods.

Nonenzymic Browning

Nonenzymic browning is a series of condensations that can be considered to be bimolecular.[18] The initial reactants of nonenzymatic browning in foods are often a reducing sugar and an amino acid or amino group. The reaction produces flavors in foods during processing, but it decreases food quality during storage.

Eichner and Karel[81] studied the effect of mobility, viscosity, and the glassy state on the rate of browning in glucose-glycine-glycerol-water model systems. They found a decrease in the browning rate especially at low water contents when the amount of glycerol in the system was low. The decrease was assumed to result from decreased mobility of the reactants and reaction products when the sugar solution was reported to be in the glassy state. Eichner and Karel[81] found that the addition of glycerol improved the mobility of the reactants as a result of plasticization and the rate of the reaction was increased. Flink et al.[82] studied the browning rate of nonfat milk powder, which was humidified at 0, 11, and 32% RH at 37°C and stored at various temperatures. They observed that the rate of browning was low below a critical temperature, above which the rate of the reaction increased substantially. They also observed that the browning rate was dependent on water content and the critical temperature for the reaction decreased with increasing initial a_w. The results of the study of Flink et al.[82] suggested that the rate of browning at temperatures below T_g was low and the increase in browning rate above the critical temperature occurred as a result of plasticization and increasing molecular mobility above glass transition.

Nonenzymic browning rates have been reported for several foods that are likely to exist in the glassy state at low a_w or water contents and to exhibit increasing browning rates above some critical a_w values or water contents. Karmas et al.[76] derived T_g values for cabbage, carrots, onions, and potatoes and analyzed their browning rates as a function of $T - T_g$. The results showed that nonenzymic browning was not likely to occur below T_g. Browning occurred above a critical $T - T_g$, which was dependent on water content. It would be expected that the true rate constant of the browning reaction is affected by both water content and temperature. A material with a high water content has a low T_g and browning may occur at a relatively low temperature. However, the true rate constant decreases with decreasing temperature, which also decreases the observed rate constant.

Karmas et al.[76] reported browning data as a function of temperature for several model systems that had various initial water activities at room temperature. Arrhenius plots for the materials were nonlinear with two changes. These changes were observed to occur in the vicinity of T_g and at about $T_g + 10°C$ above T_g. Karmas et al.[76] found that the activation energies for the reaction below T_g (30 to 90 kJ/mol) were lower than above T_g. The activation energies above T_g (65 to 190 kJ/mol) were typical of the nonenzymic browning reaction. However, those within the glass transition temperature range (250 to 400 kJ/mol) were substantially higher than values commonly obtained for the reaction. Karmas et al.[76] pointed out that the step change in the Arrhenius plots was similar to those found for diffusion in polymers. Roos and Himberg[75] found that browning in food models occurred at temperatures below T_g which agreed with the results of Karmas et al.[76] The rate of browning increased both with increasing temperature and increasing $T - T_g$.

Reactions in amorphous foods are complex and they may be controlled by several factors, including T_g, and the use of a single relationship, whether Arrhenius or WLF, to model temperature dependence of food deterioration is often inadequate.[73] It should also be noticed that the rate constant is not likely to follow the WLF-type temperature dependence unless the reaction is fully diffusion-controlled and the diffusion coefficient follows the WLF relationship. This is probably the situation in milk powders, as an obvious increase in the rate constant is observed at the T_g of amorphous lactose.[83] However, Roos et al.[83] found that the amount of water produced in the nonenzymic browning reaction may be significant and enhance the reaction as a result of additional plasticization.

The above studies on relationships between T_g and the rate of the nonenzymic browning reaction have suggested that the reaction may become diffusion-controlled and that the rate may be affected by T_g. However, the rate constant is dependent on a number of other factors that include temperature, water content, and structural transformations.[74] The size of the reactants may also be an important factor that affects rates of diffusion-controlled reactions.[74] It may be assumed that the rate of diffusion decreases with increasing size of the diffusant. The temperature and water content have the most important effects due to the fact that an increasing temperature increases the true rate constant and it is also dependent on water content.

Other Reactions

Water plasticization may also have an effect on rates of vitamin destruction during food storage. Dennison et al.[84] studied the effect of a_w on thiamine and riboflavin retention in a dehydrated food system. The product was a starch-based food model that probably had a high T_g. They found that the retention of the vitamins was high after a storage period of 8 months at 20 and 30°C and $a_w < 0.65$. A substantial loss of thiamine and riboflavin was noticed at 45 and 37°C and $a_w > 0.24$, respectively. It may be assumed that water and thermal plasticization occurred in the freeze-dried model and caused the observed degradation of the nutrients.

Nelson[72] observed rates of ascorbic acid degradation within freeze-dried non-crystallizing maltodextrin matrices at various temperatures and water contents. The reaction may occur through a number of pathways, but Nelson[72] assumed that the reaction involved diffusion of small molecules such as oxygen in the system studied. Nelson[72] found that the rate of ascorbic acid degradation generally increased with increasing temperature. The reaction also occurred at temperatures below T_g, probably because of the small size of the diffusing oxygen molecules. Application of the Arrhenius model to describe the temperature dependence showed a change in the activation energy of the reaction in the vicinity of T_g for a system that had a relatively low water content. The material with higher water contents had Arrhenius plots with a continuous line or no data were obtained below T_g. Nelson[72] found that the kinetics of the reaction were affected by structural changes. The WLF relationship failed to describe the temperature dependence of the reaction. Bell and Hageman[85] found that aspartame degradation in a poly(vinylpyrrolidone) (PVP) matrix also occurred below T_g and that the rate at room temperature was more dependent on a_w than on the state of the system.

Effects of Structural Transformations on Stability

Collapse

Collapse in low-moisture foods may significantly affect effective diffusion coefficients, mainly as a result of the loss of porosity and formation of a dense structure. Karmas et al.[76] related some of the differences in observed rates of the nonenzymatic browning to collapse of the matrix. Collapse was also observed by Nelson[72] to affect the rate of ascorbic acid degradation in maltodextrin matrices.

The formation of glassy carbohydrate matrices is of great importance in flavor encapsulation and in the protection of emulsified lipids in food powders from oxidation.[87] Flink[38] pointed out that the encapsulated compounds are stable as long as the physical structure of the encapsulating matrix remains unaltered. Encapsulated compounds may be released due to collapse, which results in loss of flavors and exposure of lipids to oxygen. However, Flink[38] pointed out that collapsed structures may hold encapsulated compounds and protect them from release because of the high viscosity of a collapsed media. Labrousse et al.[88] found that an encapsulated lipid was partially released during collapse, but during the collapse reencapsulation occurred and assured stability of the lipid. However, differences in the rates of diffusion within glassy carbohydrate matrices and supercooled, liquid-like, viscous matrices have not been reported. Therefore, it may be difficult to evaluate various effects of collapse on the release of encapsulated compounds or effects of diffusion on quality changes in collapsed matrices.

Crystallization of Food Components

Crystallization of amorphous sugars is known to result in serious quality losses in food powders. The crystallization of amorphous lactose in dehydrated milk products has been observed to result in acceleration of the nonenzymatic browning reaction as well as other deteriorative changes and caking. King[89] reported that crystallization of lactose coincides with an increase in free fat, which presumably facilitates lipid oxidation in milk powders. Crystallization of amorphous lactose also occurs in other dairy powders, such as whey powder above a critical a_w.[90] Lactose crystallization in dairy powders results in increasing rates of non-enzymatic browning and loss of lysine.[91,92] Saltmarch et al.[92] found that the rate of browning at 45°C increased rapidly above 0.33 a_w and showed a maximum between 0.44 and 0.53 a_w. The rate maximum for browning occurred at a lower a_w than was found for other foods. Crystallization of lactose occurs within the reported a_w range and the a_w which allows crystallization decreases with increasing temperature. Saltmarch et al.[92] found that the rate maximum was coincident with extensive lactose crystallization which was observed from scanning electron micrographs.

Crystallization in low-moisture carbohydrate matrices which contain encapsulated volatiles or lipids results in a complete loss of flavor and release of lipids from the matrix. Flink and Karel[93] studied the effect of crystallization on volatile retention in amorphous lactose. Crystallization was observed from the loss of adsorbed water at relative humidities, which allowed sufficient water adsorption to induce crystallization. At low relative humidities, the encapsulated compound was retained at water

contents below the BET monolayer value and probably also below T_g. The retention was high until the water content reached its maximum value. At higher relative humidities, the amount of adsorbed water decreased and the rate of the volatile loss increased. The results showed that crystallization of amorphous lactose resulted in loss of both adsorbed water and encapsulated volatiles. It is obvious that the crystalline structure was not able to entrap volatile compounds.

The effect of glass transition on the rate of oxidation of methyl linoleate that was encapsulated in an amorphous lactose matrix was studied by Shimada et al.[94] They observed that oxidation did not occur in encapsulated methyl linoleate. Lactose crystallization was observed at temperatures above T_g and the rate increased with increasing $T - T_g$. Shimada et al.[94] did not observe oxidation above T_g until crystallization released the encapsulated compound. Methyl linoleate that was released from amorphous lactose became accessible to atmospheric oxygen and oxidized rapidly. It is obvious that nonencapsulated lipids are susceptible to oxidation in low-moisture foods. Encapsulated lipids in foods may become protected from oxidation, but crystallization of the encapsulating matrix releases such compounds and causes rapid deterioration.

Biochemical Stability

Enzyme activity has been found to be related to hydration.[95,96] At low water activities enzymatic activity is generally not observed, as water cannot enhance diffusion of substrates to enzyme molecules.[95] The water activity dependence applies both to hydrolases and oxidases, unless the substrates are non-aqueous liquids allowing changes to occur at low a_w.[95] Obviously, enzymatic activity depends on diffusion of substrates and products as well as enzyme molecules and it may depend on the physical state of the material as well as a_w. It has also been found that the limiting a_w for enzyme activity may decrease with an increase in temperature, probably because of an increase in molecular mobility.[97]

A change in heat capacity and an increase in motional freedom of enzyme molecules have coincided with the onset of enzyme activity,[98] which suggests that a relationship may exist between enzyme activity and glass transition. Silver and Karel[99] studied the effect of water activity on sucrose inversion by invertase. The rate of the reaction increased with increasing a_w and the rate followed first-order kinetics. The samples were freeze-dried and the sucrose was likely to exist in the amorphous state. This was also noticed from the fact that the onset of hydrolysis occurred at water activities below the suggested mobilization point of 0.81 a_w for crystalline sucrose.[78] Silver and Karel[99] observed a continuous decrease in the activation energy with increasing a_w, which was concluded to suggest that the reaction was diffusion-controlled. Drapron[97] stated that not only a_w, but also the ability of water to give a certain mobility to enzymes and substrates, is important to enzyme activity. He assumed that the amount of water needed increases with increasing molecular size due to impaired diffusion. However, lipase activity was not related to the mobility provided by water. Interestingly, Drapron[97] pointed out that in β-amylolysis the a_w at which the reaction started was lower at 30°C than at 20°C. He assumed that the mobility of the components increased with temperature.

It should be noticed that proteins in foods are also plasticized by water. Protein denaturation occurs in the presence of water, but interestingly, although addition of polyhydroxy compounds tends to decrease the denaturation temperature, an increase in the denaturation temperature of globular proteins has been found to occur with increasing T_g of the polyhydroxy additive.[86]

Microbial Stability

Microbial growth requires a minimum a_w, in addition to optimal pH, temperature, and other factors that may influence the growth of microorganisms.[21] Water activity is considered as one of various hurdles that can be varied to provide stability and safety in foods.[22,100] The minimum requirement for microbial growth is a_w 0.62 which allows growth of xerophilic yeasts.[1,101] An increasing a_w allows the growth of molds, other yeast, and finally bacteria at high water activities. The most important a_w value for the safety of food materials is probably 0.86 which allows the growth of *Staphylococcus aureus*,[1,2,102] a well-known pathogen. Minimum a_w values for the growth of microorganisms are given in Table 1.1. It should be noticed that microorganisms may also have maximum a_w values above which their growth is declined.

Microbial stability is often the most important criterion in food preservation. The a_w limits for growth of various microorganisms (Figure 1.12), although being slightly dependent on growth media, are well established and successfully used in food development and manufacturing.[1,104] Gould and Christian[104] recognized the possible secondary influence of high viscosity and diffusional factors on the growth of microorganisms. Slade and Levine[6] have emphasized the effects of water dynamics, which are based on the theories of food polymer science, on the growth of microorganisms and criticized the use of the a_w concept in predicting microbial stability. Slade and Levine[6] suggested that germination of mold spores is a mechanical relaxation process that is governed by the translational mobility of water. The effect of glass transition on the heat resistance of bacterial spores was studied by Sapru and Labuza.[103] They found that the inactivation of spores followed the WLF relationship, which fitted to the data above T_g better than the Arrhenius relationship. Sapru and Labuza[103] also found that the heat resistance of bacterial spores increased with increasing T_g of the spores. These findings emphasize the importance of water plasticization for microbial growth and heat inactivation. However, the growth of microorganisms has been observed to occur within glassy food materials[101] and other factors in food formulation, including a_w and pH, should be considered in addition to the physical state for the increase of microbial stability.

Chirife and Buera[3,101] showed that dehydrated fruits and vegetables have large $T - T_g$ values over the a_w range of microbial growth and the physical state has little influence on observed microbial growth. Using wheat flour as an example, they demonstrated that growth of microorganisms may occur even at conditions which support the glassy state. However, it should be remembered that a_w is a property of a water-solute system while glass transition of the same system is usually measured as the behavior of the water-plasticized solids. Obviously, the possible effect of glass transition on the growth of microorganisms in foods remains questionable. Rigorous studies are needed to establish possible relationships between a_w, physical state, and

TABLE 1.1
Minimum Water Activities (a_w) for the Growth of Various Microorganisms[2,105]

Bacteria	Minimum a_w	Molds	Minimum a_w	Yeasts	Minimum a_w
Aeromonas hydrophila	0.970	*Alternaria citri*	0.84	*Debaryomyces hansenii*	0.83
Bacillus cereus	0.930	*Aspergillus candidus*	0.75	*Saccharomyces bailii*	0.80
Bacillus stearothermophilus	0.930	*Aspergillus flavus*	0.78	*Saccharomyces cerevisiae*	0.90
Bacillus subtilis	0.900	*Aspergillus fumigatus*	0.82	*Saccharomyces rouxii*	0.62
Campylobacter jejuni	0.990	*Aspergillus niger*	0.77		
Clostridium botulinum A	0.940	*Aspergillus ochraceous*	0.77		
Clostridium botulinum B	0.940	*Aspergillus restrictus*	0.75		
Clostridium botulinum E	0.965	*Aspergillus versicolor*	0.78		
Clostridium botulinum G	0.965	*Botrytis cinerea*	0.83		
Clostridium perfringens	0.945	*Chrysoporium fastidium*	0.69		
Enterobacter aerogenes	0.940	*Etemascus albus*	0.70		
Escherichia coli	0.935	*Erotum chevalieri*	0.71		

Halobacterium halobium	0.750	*Erotum echinulatum*	0.62
Lactobacillus plantarum	0.940	*Erotum repens*	0.71
Lactobacillus viridescens	0.950	*Erotum rubrum*	0.70
Listeria monocytogenes	0.920	*Mucor plumeus*	0.93
Pediococcus cerevisiae	0.940	*Paecilomyces variotii*	0.84
Salmonella spp.	0.940	*Penicillium chrysogenum*	0.79
Shigella spp.	0.960	*Penicillium citrinum*	0.80
Staphylococcus aureus (anaerobic)	0.910	*Penicillium cylopium*	0.81
Staphylococcus aureus (aerobic)	0.860	*Penicillium expansum*	0.83
Vibrio parahaemolyticus	0.936	*Penicillium islandicum*	0.83
Yersinia enterocolitica	0.960	*Penicillium patulum*	0.81
		Penicillium viridicatum	0.81
		Rhizopus nigricans	0.93
		Rhizoctonia solani	0.96
		Stachybotrys atra	0.94
		Xeromyces bisporus	0.61
		Wallemia sebi	0.75

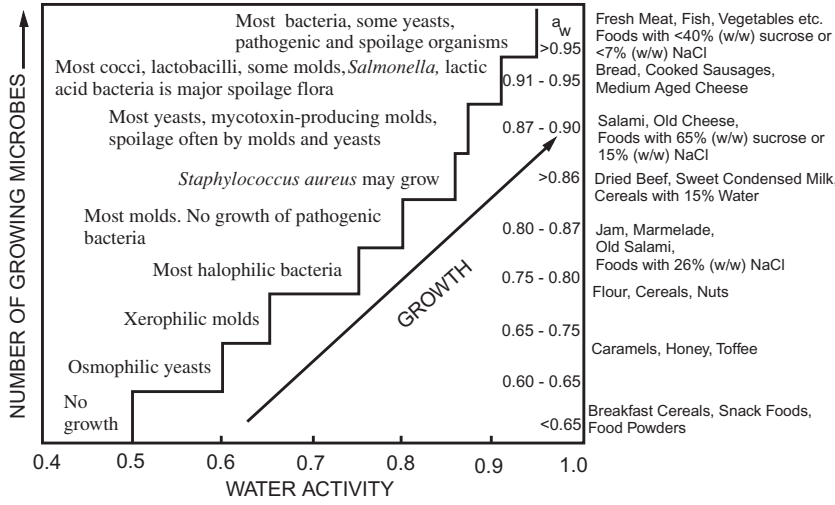

FIGURE 1.12 Minimum water activity, a_w, ranges for the growth of microorganisms in foods and examples of various food materials having a_w within the minimum range.

microbial growth in food materials which may also be affected by phase separation and heterogeneity of the system.

Control of Stability by Water Activity and Composition

Knowledge of the physical state and physicochemical properties of food components is advantageous in food design and formulation. The information on factors affecting rates of various kinetic processes can be used to manipulate and control rates of changes that occur during food processing and to develop food products that are less sensitive to detrimental changes during storage. It is obvious that the main factors that control stability of low-moisture foods are a_w and composition. Low-moisture foods are considered to be stable when they are stored in cool and dry conditions. An increase in temperature or water content may result in a significant change in the rates of deteriorative changes. Knowledge of the effects of temperature and water on the physical state and diffusion in amorphous food matrices may be used to establish relationships between food composition and storage conditions.

Traditional shelf life predictions of low-moisture foods have been based on the information on rates of deteriorative changes and loss of nutrients at various temperatures and water contents. The main assumption for stability is often that water contents close to the BET monolayer value allow maximum stability. An increase in water content at a constant storage temperature results in rapid deterioration as reaction rates increase at intermediate water contents. It may be assumed that the rates of a number of deteriorative changes in low-moisture foods are affected by diffusional limitations. Reaction rates in the solid, glassy state at low water contents approach zero, but an increase in temperature or in water content probably increases diffusion and therefore the reaction rate. It is probable that at temperatures above

T_g reaction rates at a constant water content follow the Arrhenius-type temperature dependence.

Sorption isotherms of low-moisture foods provide important information that can be used in predicting shelf life. The relationship between a_w and water content can be directly used in evaluating effects of water content on stability. It is obvious that the relationships between mechanical properties, temperature, and water content of amorphous foods provide improved criteria for shelf life predictions. The sorption isotherm combined with water plasticization data allows the establishment of critical values for water content that can be related to stability. Water contents that are higher than the critical values decrease the T_g of the material to below ambient temperature, which results in stickiness, caking, and probable crystallization of amorphous components. The critical values may be directly used in establishing criteria for maximum water vapor permeability values for packaging materials that can maintain stability and reduce water plasticization during storage.

Stability Maps

The effect of a_w on the relative rates of deteriorative changes has often been described using stability maps which show the relative rate of enzymatic changes, non-enzymatic browning, lipid oxidation, microbial growth, and overall stability as a function of water activity.[4] The rate of the various reactions may also be related to the physical state, molecular mobility, water plasticization, and glass transition of amorphous food solids, as shown in Figure 1.13. Structural transformations, as well as diffusion-controlled deteriorative reactions and those affected by crystallization phenomena occur at increasing rates with increasing a_w above the critical a_w. It is likely that water contents lower than the critical water content are needed for maximum stability. Stability maps are applicable for evaluating storage stability of low- and intermediate-moisture foods. Water activities of most fresh foods are high and their stability is based on other hurdles such as low pH and low storage temperature. However, water is the predominant component of most foods and the physicochemical properties of water are the main factors that control their behavior during processing and storage by contributing to the amount of heat required for heating or refrigeration and by being the main solvent and plasticizer of the nonfat components.

The assumption that stability is related to glass transition allows establishment of the stability map that describes the effect of water on changes that depend on the relaxation times of mechanical changes in foods. Figure 1.13 relates deteriorative changes, which are governed by T_g, to water activity. In addition state diagrams and sorption isotherms are useful as stability maps. They may also be used to obtain material-specific data for the glass transition temperature at various water contents and relationships between water content and water activity.

FUTURE RESEARCH

Characterization of the physical state of food materials and application of the polymer science theories to the description of food properties and various kinetic phenomena have significantly contributed to the present understanding of food stability.

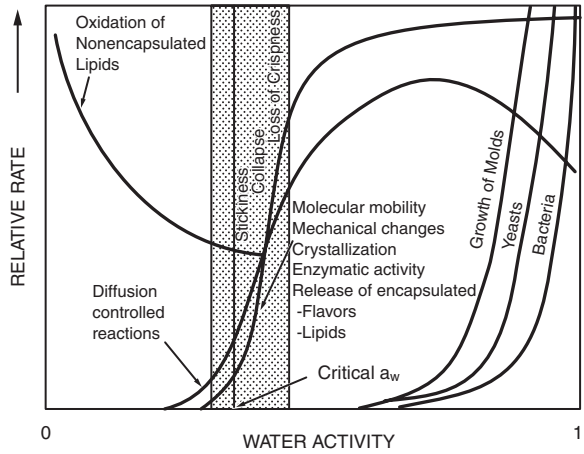

FIGURE 1.13 A food stability "map" showing the effect of water activity on various time-dependent changes and rates of diffusion-controlled reactions as well as the growth of microorganisms in food materials.

Knowledge of material properties is extremely useful in the production of encapsulated flavors, extruded products, confectionery, development of totally new products, such as dehydrated enzymes or starters, and avoiding quality changes that may result from mechanical changes, e.g., loss of crispness and recrystallization phenomena. The temperature-, water content-, and time-dependent changes, which have been problems in manufacturing and storage of food powders and other low-moisture foods, can be reduced by avoiding exceeding their critical values based on the T_g determination or by compositional adjustments that provide sufficiently high values for critical a_w and T_g. The most important applications of producing high quality dehydrated foods include reduced collapse and improved flavor retention in dehydration processes. The kinetics of enzyme activity are important to food quality and applying the knowledge in food industry may allow the design of improved products with extended shelf lives or even improved retention of activity, e.g., in enzyme preparations. The applicability of the various kinetic models (Arrhenius and WLF) should be tested with more data on reaction kinetics. Establishing relationships between the physical state and kinetics of quality changes in relation to T_g and other relaxations even in the glassy state are particularly important.

Relevant research needs to be undertaken to establish clear relationships between changes observed by various techniques and to evaluate them in terms of a_w and molecular mobility. A combined use of knowledge of both a_w and water plasticization is advantageous. The a_w concept is a more traditional approach to understand quality changes in foods. Water activity has been successfully used in setting limits for microbial stability in foods and qualitative characterization of relative rates of deteriorative reactions in low-moisture foods. The combined use of a_w and glass transition theory has a high practical applicability as it provides criteria for critical a_w and, together with sorption isotherm, for water content at a constant temperature. Water activity and glass transition alone do not explain why various changes in texture or rates of deteriorative

reactions occur above the critical water activity or water content. New information is needed to characterize such changes as component crystallization and chemical and enzymatic reactions occurring during processing and storage of low-moisture foods.

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2 Mechanical and Temperature Effects on Shelf Life Stability of Fruits and Vegetables

Lope G. Tabil, Jr. and Shahab Sokhansanj

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INTRODUCTION

Agricultural products both of plant (e.g., fruits, vegetables, and root crops) and animal origins (e.g., meat and fish) are highly perishable. They have a high water content ranging from between 60 and 95%. Even after harvest, fruits and vegetables continue with their metabolic processes such as respiration and transpiration, utilizing food and water reserves. After slaughter, meat and fish muscles undergo glycolysis and pH drop, and are subject to microbial attack. When food and water reserves are used up, the produce irreversibly deteriorates. Many of these changes are due to physiological processes, and are accelerated by such external factors as storage temperature, humidity, mechanical injury, and attack of pests and diseases. Abnormal physiological deterioration occurs when fresh produce is subjected to extremes of temperature, atmospheric modification, or contamination.[1]

Rough handling of fresh produce is a serious problem in the postharvest system. The shelf life of agricultural produce may be shortened due to unfavorable handling temperature and humidity, together with mechanical injury and infestation of pests and diseases. Fruits and vegetables are highly susceptible to mechanical damage during harvesting and handling. As such, this chapter will focus on fruits and vegetables, discussing how postharvest handling affects shelf life and measures to minimize the deteriorative effects of mechanical injury are given.

Fruits are commonly derived from an ovary and the surrounding parts, while vegetables are derived from different plant parts. Vegetables can be grouped into three categories, namely: (1) seeds and pods; (2) bulbs, roots, and tubers; and (3) flowers, buds, stems, and leaves.[2] Examples of the different derivation of fruits and vegetables; from plant parts are: potato — swollen stem tuber; sweet potato — root tuber from fibrous root; carrot — root tuber from main taproot; taro — compressed stem tuber (corm); cauliflower — massed flower head; pineapple — fused mass comprising of unfertilized flower parts and main flower stalk; cabbage and lettuce — whole above-ground vegetative growth; spinach — leaves; leeks — swollen leaf-base; mango and avocado — fleshy fruit with single seed; tomato, citrus, banana, and pepper — fleshy fruit, with several seeds; okra, green bean, and yard-long bean — immature green pods with partly developed seed; green pea — immature seed.[1] Consequently, fruits and vegetables have different physical and chemical characteristics and responses vary to postharvest conditions and treatments to which they are subjected. Handling of crops to maintain quality and lengthen their storage life is specific according to the commodity, time, and environmental conditions.

STORAGE LIFE OF AGRICULTURAL PRODUCTS

The following metabolic processes and events affect the postharvest storage life of agricultural produce and are important controlling factors in postharvest handling.

Respiration

This is a basic reaction of plants before and after harvest. Harvested plant materials continue to respire and use the stored starch or sugar to maintain their structural integrity. Since the stored food is not replaced, senescence follows and the produce eventually decays. Respiration causes weight loss, excessive CO_2 build-up in storage, and triggers a host of other metabolic reactions such as ethylene synthesis, color, texture, and flavor changes.

Transpiration

Harvested produce rapidly lose water from their surfaces in a process known as transpiration. Transpiration is a major component of weight loss in fruits and vegetables. A 5 to 10% weight loss will cause significant wilting, shriveling, poor texture, and poor taste.[3] Moisture loss occurs rapidly in a warm, dry environment especially among injured products, and it is affected by commodity characteristics such as surface area to volume ratio, presence of waxy substances in the skin, tenderness

of the skin, and presence of protrusions on the skin surface. Texture of the produce is adversely affected by excessive water loss, rendering the product unmarketable.

Ripening

This is a natural development process that mature fruits undergo, giving them desirable texture, taste, and flavor. However, it is followed by senescence and breakdown of the fruit. Ripening of fruits often leads to increased susceptibility to mechanical injury and attack of pests and diseases.

Postharvest Damage

This consists of any loss in quantity or quality of the produce after harvest that may be caused by mechanical injury or injury from temperature effects. Losses by mechanical injury begin from the time of harvest until the produce reaches the consumers' table. Bruises, cuts, splits, and cracks are some of the manifestations of mechanical injury. These trigger an increase of physiological processes, which can be disastrous to the produce. Injury from temperature effects is caused by exposing the produce to extremes of temperature during postharvest handling. Heat, freezing, and chilling injury can be prevented if appropriate environments are provided during handling. These injuries cause a reduced shelf life of crops, especially fruits and vegetables. Insects usually infest the crop while in the field, depositing their larva or egg on the produce, while fungi and bacteria infect the produce causing diseases. Pests and diseases can be controlled by quarantine treatments employing physical and chemical methods or a combination of the two.

POSTHARVEST HANDLING

Handling operations vary according to the produce. Some fruits, such as citrus, are waxed to improve their appearance and prolong storage life by serving as a fungicide carrier. Ethylene may also be applied to fruits to induce ripening among climacteric fruits such as bananas, and enhance uniform color and improve marketability among fruits. The following operations can subject agricultural products to mechanical and temperature stresses.

Harvesting

Harvesting is the collection of the produce that may involve picking, cutting, digging, or shaking, and presents an opportunity for the crop to be mechanically damaged. After harvesting, the produce may be thrown or dumped in the container and may come in contact with the hard surface and sharp edges of the container or collide with other products. Some trimming and sorting are done on the produce during harvest. Injury may result when under-trimmed products collide with each other and excessive water loss may occur if products are over-trimmed. Tomatoes are sorted according to color or presence of defects, while cabbages, carrots, radishes, and some leafy vegetables are trimmed or topped in the field.

In developed countries, harvesting of selected vegetable and fruit crops has been mechanized. Losses due to mechanical damage can be high and these have been associated with poorly designed equipment. Harvesting of potatoes is mostly done by mechanical harvesters. Increasing mechanization for transport, grading, and marketing had been practiced in recent years. There have been reports of damage to tubers due to the design of potato harvesters or improper operation. Horvath[4] observed that the size and temperature of the tubers are two important contributory factors causing damage of potatoes during harvest. Impact injury may be inflicted on mechanically harvested fruits when they are dislodged from the tree.[5]

Field Packaging and Transport to the Packinghouse

The harvested crops are placed in a variety of containers such as baskets, sacks, wooden boxes, and plastic crates for transport to the packinghouse. Some containers are either too flexible or their inside surfaces are too rough, that they often do more harm rather than protect the commodity from handling damage. In some instances, the products may be packed too tightly or too loosely causing compression damage and bruising. Also, the filled container may be subjected to rough handling. When transport to the packinghouse is not immediately available, produce-filled containers often wait under the sun.

Produce Preparation for Packaging

Inside the packinghouse the produce is initially sorted, cleaned, graded, subjected to quarantine treatments, and, in some cases, waxed before packaging. Mechanical injury is the main cause of quality loss in packinghouses. Quarantine treatments should be properly tested and approved before their application in the packinghouse. These may involve the application of heat, chemicals, or a combination of both. Temperature stress or heat damage may also occur, when the commodities are exposed to heat treatment. Each time a product item comes in contact with another item or with the equipment in the packinghouse, damage may be inflicted. As an example, the following sequence of events has been identified to cause cumulative damage or bruising on apples as they go through transport from storage to packinghouse: flotation out of the storage bin; accumulation of water at the line input; elimination of trash and undersized fruit; manual grading; washing in brushes; dewatering on sponge rolls; waxing; drying on a roller conveyor; manual inspection; singulation; sizing; accumulation; packing or bagging; and stacking on a shipping pallet.[6]

Precooling

Precooling is the immediate removal of field heat from the commodity. High ambient temperatures are favorable for the deterioration of the produce since respiration and transpiration rates are high. Heat is removed from the commodity as a preparatory step in the refrigerated storage and transport of the product. The shelf life of the produce is considerably shortened if it is not promptly cooled because respiration and transpiration continue after harvest.

Packaging

The produce is placed in an appropriate packaging material for further processing, marketing as fresh produce, or storage. Several types of packaging materials are available varying in cost, rigidity and flexibility, water resistance, and ability to control atmosphere.

Produce Handling and Transport

As the produce moves from one point to another during marketing, packaging plays an important role in protecting them from damage during handling and transport. During transport mechanical damage is inflicted on the commodity by the vibrating vehicle, sudden stops and starts, or rough roads. Furthermore, some vehicles may not possess refrigeration systems.

POSTHARVEST DISORDERS

Disorders in marketable agricultural produce fall into two groups, namely, disorders resulting from internal changes such as senescence, and those resulting from external factors such as unfavorable environments or physical injury.[7] The latter results in greater losses during marketing of the produce than those originating internally. Care in handling the produce during and after harvest should be exercised so that quality is maintained. However, injury may be inflicted on the commodity and this may be caused by impact, compression, abrasion, puncturing, tearing, or a combination of any of the above during handling and transport. Mechanical damage due to improper handling of fruits and vegetables can be grouped into five types: (1) impact, (2) compression, (3) abrasion, (4) puncture, and (5) tears.[7]

Impact

This damage occurs when the produce hits a hard surface, with force sufficient to damage or separate the cells. Externally this is manifested by a bruise or a crack. Commodities like fruits and vegetables sustain impact damage during harvesting when the produce is dropped from the harvester to the storage bin, box, or crate. It can also occur as the commodity moves inside the packinghouse. Danger of impact can extend to trailers or rail cars while loading or unloading, and during sudden stops.

Compression

Placing too much of the produce in a container with limited capacity results in compression damage. Stacking containers one over the other may damage the produce at the bottom due to compression, especially if the containers are not rigid enough. Compression damage is caused by excessive static load on the produce.

Abrasion

This can occur when the produce rubs against each other when packed closely or conveyed at high speeds, or rubs against surfaces such as unlined containers, conveyors, and bins.

Puncture

The produce may be punctured because of the presence of stem or pedicel, which has not been detached at harvest. Punctures can also be inflicted by fingernails, protruding sharp objects, and surfaces of containers.

Tears

Tearing is common to leafy vegetables resulting in excessive water loss and increased respiration leading to desiccation, discoloration or decay of the affected leaf.

Temperature Effects

Fresh produce may not only suffer mechanical damage caused by physical handling, but disorders may result from undesirable storage temperatures. Certain fruits and vegetables are sensitive to temperature variations during handling and storage. Low temperature results in chilling or freezing injury, which may be manifested by a water-soaked appearance, pitting, discoloration or failure to develop the proper ripening pigments, and the development of strong off-odors. On the other hand, heat injury is sustained by a produce due to exposure to the sun after harvest, or with any warm surface like the soil or a wall heated by the sun. Weight loss, softening, discoloration, and eventual desiccation of the affected tissue are common. The causes and effects of injuries sustained by fruits and vegetables are summarized in Table 2.1.

MECHANICAL INJURY

During the postharvest life of a produce, handling events contribute to cumulative damage decreasing its quality and storage life. In a baseline study for tangerines, it was reported that "non-handled" fruits (those that were carefully picked and packed) incurred only 1% loss due to decay compared with the normally "handled" fruits, which had 20% loss due to decay after 2 weeks at 21°C.[8] A third of the damage was inflicted during manual picking and the majority was inflicted by the polisher brushes in the packinghouse.

Mechanical causes of failure in fruits and vegetables can be classified as cracking or splitting, slip, and bruising.[9] In the case of potatoes, cracking may occur either at the center of the tuber due to tensile and shear stresses, or around the outside of the tuber due to tensile hoop stresses. Tensile hoop stress on tomatoes can cause splitting. Failure by slip is due to compression, but actual failure takes place by shearing with two parts of the produce sliding past each other, along a plane 45° to the compressive load. Crushing has been observed in products subjected to impact and compression such as potatoes, unripe plantains, pineapples, unripe papayas, and unripe pears. Bruising results when the cells below the skin or rind are ruptured and the cellular contents are subjected to enzymatic oxidation. This is observed in potatoes, apples, and pears. The incidence and cause of mechanical injury on selected fruits and vegetables is shown in Table 2.2.

TABLE 2.1
Damage that May Be Sustained by Produce During Postharvest Handling

Source/Type	Cause	Effect
From injuries		
Cuts, punctures	Sharp objects piercing package	Deep punctures or cuts in produce leading to water loss and rapid decay
	Splinters in bamboo or wooden containers	
	Staples/nails protruding in containers	
	Poor harvesting practices	
Impact	Throwing or dropping of produce or filled packages	Bursting of packaging, bruising of contents
	Sudden starts/stops of vehicles	Splitting of produce
	Speeding vehicles on rough road	
	Underpacking of containers	
Compression (squeezing/squashing)	Flimsy or oversized containers stocked too high	Bruising or crushing of contents
	Poor stowage and collapse of containers during transport	
	Overpacking of containers	
Vibration (shaking)	Vibration of vehicle itself and rough roads	Wooden boxes come apart damaging produce
From environment		
Heat damage	Exposure of harvested produce or packages to external heat, e.g., direct sunlight or storage near heating system	Overripening or softening of fruits, wilting/shrinkage, and development of off-flavor
	Natural buildup of internal heat of produce due to poor ventilation within the package, in storage, or in vehicle	Decay develops rapidly Cardboard cartons may become dry and brittle, easily damaged on impact
Chilling or freezing damage	Low or sub-zero ambient temperatures	Damage to chilling-sensitive produce
	Exposure of sensitive produce to temperature below chilling or freezing tolerance level during storage	Breakdown of frozen produce on thawing, plastic containers become brittle and may crack
Moisture and free-water damage	Exposure to rain or high humidity condensation on packages and produce moved from cold storage to damp atmosphere at ambient temperature	Softening and collapse of stacked cardboard containers Squashing of produce in collapsed containers
	Packing of wet produce in cardboard containers	Decay promoted in damaged produce
Damage from light	Plastic sacks and crates not treated with UV inhibitor eventually breaks up when exposed to direct sunlight	Disintegration of plastic sacks damages produce when moved Fracturing of plastic crates can cut or bruise produce

TABLE 2.1 (continued)
Damage that May Be Sustained by Produce During Postharvest Handling

Source/Type	Cause	Effect
Damage from other causes		
Chemical contamination	Contamination of containers stored near chemicals	Flavor contamination with surface damage and discoloration of produce in contact with container
	Damage of produce by containers treated with preservatives, e.g., boxes made from wood treated with pentachlorphenate (PCP)	Decay of produce owing to contaminating molds; wood-rotting mold causes collapse of boxes
Insect damage	Insects present in packed produce	Consumer resistance and legal problems from presence of insects in packed produce
	Wood-boring insects in wooden boxes	Spread of wood-destroying insects in infected boxes
Damage due to disease	Fungal and bacterial spores	Molding and decay of produce
Human and animal damage	Contamination and eating by rodents and birds	Rejection of damaged produce by buyers or inspectors
	Pilferage by humans	Loss of income through loss of produce

Data from *Prevention of Post-Harvest Food Losses: Fruits, Vegetables and Root Crops, A Training Manual,* FAO Training Series No. 17/2, Food and Agriculture Organization of the United Nations, Rome, Italy, 1989.

INCIDENCE OF CUTS, PUNCTURES, AND CRACKS DURING POSTHARVEST HANDLING

One of the most common forms of mechanical damage is impact, which is manifested as cracks. Dropping or throwing the produce into the container, dropping the packed produce during handling, collision with other products, and vibration during transport are some of the instances when impact damage is inflicted on the commodity. Cuts, punctures, and wounds result from harvesting (cutting, digging, or lifting), using containers with sharp edges, packing produce with their stem or pedicel, and rough handling.

It was reported that damage has already occurred to 58% of the "breaker" tomatoes in the field before they were harvested (picked by hand), in a study conducted in North Carolina.[11] The major source of damage in the postharvest handling system was the practice of overfilling crates of tomatoes (33% damage) at the ends of rows where crates await shipment. Specific damages reported were crushing, puncture, abrasion, and overheating of the fruits. Crushing was a direct result of overfilling the wooden crates. Overheating occurred while the crates remained under the sun (for 1 to 7 h) awaiting transport. Some of the wooden crates

TABLE 2.2
Examples of Incidence of Mechanical Injury on Selected Fruits and Vegetables

Agricultural Product	Type of Injury	Causes
Fruits		
Apples	Bruising	Rough handling
Citrus	Oil spotting — bruise injury	Rough handling
Cranberries	Bruising	Impact or pressure during handling
Grapes	Bruising	Over-packing of containers
Peaches, plums, nectarines, apricots, and cherries	Bruising	Over-packing of containers Vibration during transit
Pears	Friction bruising	Rubbing with hard surfaces
	Impact and pressure bruising	Rough handling
Persimmons	Bruising	Rough handling
Vegetables and Melons		
Artichoke	Scuffing	Rubbing of bulbs against each other and with the container
	Split bracts	Excessive force during harvesting and handling
Asparagus	Broken tips	Rough handling
	Crushed spears	Overpacking of container
Cantaloupes	Bruising and scuffing	Rough handling In-transit damage
Leeks	Butchered lower ends	Excessive trimming
(Head) Lettuce	Crushing	Rough handling
	Bruising	Overfilling of containers
	Torn tissue	Dropping of containers Compression due to stacking of filled containers
Melons, honeydew watermelons	Bruising or cutting	Impact or pressure in handling
	Bruising at the thin blossom-end	Rough handling Loading pattern
Mushrooms	Finger marks, cuts, bruises	Rough handling
Onions	Bruising	Machine harvesting
	Cuts	Rubbing against rough surfaces
Summer squash	Scratches, bruising, and scuffing	Rough handling

Data from Ryall, A. L. and Lipton, W. J., *Handling Transportation and Storage of Fruits and Vegetables,* Vol. 1, AVI Publishing Company, Westport, CT, 1972 and Ryall, A. L. and Pentzer, W. T., *Handling, Transportation and Storage of Fruits and Vegetables,* Vol. 2, AVI Publishing Company, Westport, CT, 1974.

were in a state of disrepair, causing puncture and abrasive injury on the fruits. Down grading of tomatoes in the U.S. is caused mainly by injuries during mechanical harvesting, representing 25% of culls among partially ripe fruits and 21% of culls among mature-green fruits.[12]

From a survey conducted by the National Material Handling Bureau of Australia, 15% of the potatoes were damaged during harvesting and 35 to 57% after storage.[13]

Larsson[14] studied the incidence of mechanical injury of potatoes in Sweden and reported that tuber damage in the packing line was equivalent to or even greater than the damage sustained in the harvesting process. Misener and co-workers[15] conducted a study to identify the incidence and magnitude of mechanical injury sustained by potatoes during commercial handling and packing operations in Eastern Canada. Injury increased incrementally as the tubers traveled through the packing line. The number of drops and cumulative drop heights were significant contributors to the increased damage index. Shatter bruise (cracking) appears as a break in the surface of potato that may penetrate deeply into the tuber. Mechanical injury in potatoes refers to cutting, breaking, or any other damage mechanically inflicted on the tuber.[16] Shatter bruise is an external damage that can be detected upon close inspection. It occurs more frequently as tuber turgidity increases, but generally decreases with maturity. Loading and unloading during storage and transportation were singled out by Meyer and co-workers[16] as the likely causes of shatter bruise in potatoes. Hand loading is less injurious to tubers than dump loading. Shatter bruises of potatoes may also occur in the bottom of a rail car due to the combined effects of compression and vibration. Grant and co-workers[17] reported that the relatively high frequency of shatter bruise in potatoes occurs during transport, while the amount of severely damaged tubers due to transport shock and vibration is relatively low. Bruising is aggravated by low temperatures during handling.

In root and bulb crops, which are manually harvested, mechanical injury will likely result from digging tools or lifting of the root. Mechanical injury to carrots is inflicted during harvesting. Apeland[18] reported that as much as 30% of carrots may be broken during mechanical harvesting and about 13% could be split. Tucker[19] observed that roots damaged before storage are predisposed to rotting and weight loss during storage. Losses of carrots during refrigerated storage in Taiwan were mainly due to bacterial infections that enter through cracks and broken taproots.[20]

Physical damage of lettuce transported in mechanically refrigerated trucks was a major problem in the produce market,[21] where as much as 63% of the heads showed damage. In Thailand, Ketsa[22] investigated the effect of prepackaging on the physical damage to fresh lettuce heads, namely, by (a) wrapping untrimmed heads in newspaper and packing in bamboo baskets; (b) wrapping trimmed heads in newspaper and packing in collapsible plastic crates with lids; or (c) wrapping trimmed heads in stretch film and packing in collapsible plastic crates. After refrigerated transport for 12 h, damage was 28.1, 23.1, and 19.5%, respectively, with the heads wrapped in plastic retaining the greatest degree of freshness.

Since apples have an abundance of air-filled interstitial spaces in their flesh, they are not prone to cracks during handling. Only about 1 to 4% of the apples are cut and 3% are punctured in a study of commercial packing lines conducted by Brown and co-workers.[6] On the other hand, produce with less air-filled interstitial spaces incur injury appearing as cracks, breaks, or splits as in the case of potatoes. In papayas, physical damage is in the form of cuts and punctures, arising from the improper use of harvesting equipment, and contact of harvested fruits with rough surfaces, fingernails, and adjacent fruit stems.[23] Crushing of fruits such as papayas and bananas during rough handling is common because of increased softening of the fruits during ripening.

INCIDENCE OF ABRASION, BRUISING, COMPRESSION, AND VIBRATION DAMAGE

Bruising can cause the browning of tissues of such fruits as apples, pears, peaches, apricots, cherries, grapes, and bananas, resulting from enzymatic oxidation of cellular contents.[24,25] Bruise damage results when the items hit each other or come in contact with hard surfaces of machinery, containers, or handling equipment. Conditions of impact that will promote bruising depend on each fruit's tissue structure.[26] Produce with dense tissue and with less air-filled interstitial space are susceptible to deep bruises that may not be detectable on the surface and often develop into cone-shaped and radial fractures as described among peaches[27] and potatoes.[13] On the other hand, produce with a high volume of air-filled interstitial spaces appear to distort in an elastic manner at the contact surface until cell breakage occurs and is typically found in apples.[28,29] Usually the elastic region is continuously re-established further into the fruit until all the impact energy is either dissipated during cell breakage or stored by elastic membrane distention. The incidence of bruising in apples during picking, bin filling, handling, and transport to the packinghouse was reported to be between 1.46 to 2.32 bruises per fruit.[30] The lower value resulted from gently placing the fruits in bins, handling the bins by standard fork-lift, and transporting the bins by tri-axle fifth wheel trailer. The higher bruise value resulted when the filled bins were hauled by a bin carrier in the orchard and transported by truck. It was reported that 12% of apples in Korea were injured by bruising and puncture during picking.[31] It was reported that over 95% of apples on the retail market in Denmark are bruised,[32] while 85% of apples are reportedly bruised upon reaching retail outlets in Korea.[31] Brown and co-workers[6] evaluated commercial packinghouses in the 1986–87 packing season in Michigan and found that nearly all the apples sustained bruise damage averaging over 5 bruises per fruit. Causes of bruising were: (a) inadequate cushioning in the packing line; (b) excessive impact at transfer points with energies estimated to be approximately 0.2 to 0.3 Joules; (c) lack of deceleration devices (curtains made of vertical hanging belting, rubber or fabric, and brushes to slow and control the apples); (d) excessive operating speeds of equipment; and (e) non-uniform flow of apples. Brown and co-workers[33] studied the packing line movement of apples using instrumented spheres (IS) to reduce impact damage. It was found that sizing chains, steel and plastic rollers, belts, sizer cups, and similar hard surfaces where drops exceeded 12.7 mm had impact levels exceeding 30 g (1 g = 9.81 m/s^2) as recorded by the IS. Pason and co-workers[29] observed that "McIntosh" apples are most sensitive to impact compared to other varieties such as "Golden Delicious", "Red Delicious", and "Northern Spy". Even a 2-mm drop onto a steel surface can initiate bruise damage on "McIntosh" apples one day after harvest. Bruising can also be a problem in transporting prepackaged apples. Factors affecting bruising during transport are the quality of the road, shipment distance, and type of packaging system used.[34]

Peaches and pears are also susceptible to bruising by impact. Mature and older peaches (those that have been stored) are more susceptible to impact damage and may sustain a larger bruise volume than less mature and fresh peaches.[35] Peaches of medium maturity should be picked to minimize damage during postharvest handling

and distribution. Miller and Delwiche[36] reported that bruising due to physical injury during harvest is the most common problem among peaches, where as much as 10 to 30% of the fruit may suffer bruises. In Denmark, about 20% of peaches and nectarines at retail are bruised.[32] Bruises cannot be identified immediately but appear later as brown discolorations in the injured flesh due to enzymatic browning reaction. Pears are also susceptible to bruising by impact. After sizing, 82% of "Bartlett" pears have accumulated bruises due to impact.[37] Bruising in pears caused by impact was recorded to be 14% at the pickers' bag, 26% at the field bin, and 38% after dump. Mitchell[37] found that all fruits can be bruised if dropped from a height of 16 in., 78% can be bruised at a drop height of 12 in., 56% at 9 in., 44% at 6 in., and 40% at 4 in. Chen and co-workers[38] reported that different varieties of pears differ in firmness, susceptibility to impact, compression damage, and response to storage at 0°C and subsequent ripening at 20°C. Asian pears are bruised more easily than European pears because they do not change texture after picking and storage at 0°C for several months. Pears often show friction discoloration that appears as diffused brown skin discoloration especially at high points on irregular fruit surfaces.[39]

Excessive compressive forces from the adjacent potato tubers in a pile may cause a flattened or sunken area known as a *pressure bruise*. A disorder that cannot be detected on the surface also resulting from rough handling is *blackspot*, which is characterized by the discoloration of potato tissue just beneath the skin. Bruising in potatoes occurs when tubers are stored and stacked at heights of 3 to 4 m,[40] which causes an excessive compressive load at the bottom of the pile. The tissue that is bruised is more or less badly deformed and damaged depending on the severity of the defect. The relationship of stack height and the incidence of bruising is shown on Table 2.3, where incidence of bruising appears to be less when air during storage is humidified at 90 to 95% relative humidity. The unsuitability of potatoes for industrial processing is caused mainly by bruise damage, i.e., blue discoloration and hypodermic damage or *blackspot*.

Deterioration through mechanical injury is the most serious problem among bananas. Thus, careful attention is required in handling during packaging, transport, storage, and marketing. Impact damage on bananas is not discrete because it occurs first to the latex vessels running longitudinally in the banana skin.[41] Consequently, bananas are very prone to develop a dark brown surface coloration that has an effect on perceived quality disproportionate to the volume of bruised flesh which more truly reflects eating quality. Abrasion and bruising are also observed in papayas, and is manifested as latex stain and dark green stains, respectively, which will rapidly soften and decay.[23] Causes of these injuries are the incorrect use of harvest instruments, contact with rough surfaces, dropping of fruits, and excessive movement of fruits during transport.

Mature green tomatoes can be bruised significantly (5 to 45%) after dropping the fruits on their opposite sides at a height of 20 cm.[42] Tomatoes in the breaker stage were susceptible to bruise with 15 to 75% incidence of internal bruising. "Spartan Banner" onions were subjected by Timm and co-workers[43] to drop tests. It was observed that dropping the bulbs by only 5 mm onto a flat steel surface resulted to a bruising incidence of 20%, while drops at 17 mm caused bruises in all bulbs. In packing lines, several drops are experienced by the bulb. Onions are

TABLE 2.3
Bruising of Potato Tubers (cv Bintje) at Different Heights, with or without Air Humidification at a Storage Temperature of 3 to 5°C

Height above the Ground, m	Bruising,[a] % of tubers	
	With Air Humidification	Without Air Humidification
0.25	24	64
0.50	23	51
1.00	19	39
1.50	12	32
2.00	7	11
2.50	2	10
3.00	0	1
Average	12	30

[a] Percentage of tubers with moderate and severe bruises.

From Meijers, C.P., *Post-Harvest Behaviour, Store Design, Storage Practice, Handling*, Rastovski, A. and van Es, A., Eds., Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands, 162-168, 1987. With Permission.

susceptible to bruising when sorted immediately after harvest. Sorting is best done after the curing period. Excessive compression pressure on onions is manifested not only as bruises but crushing of the bulbs when they are packed and stacked in flexible packages like gunny bags. Maini and co-workers[44] reported that 7% of the bulbs were crushed when bags were stacked 5 layers high, and 28% were crushed when bags were stacked 7 layers high.

Strawberries are also sensitive to handling operations during and after harvest because of their very tender skin. Picking operations have been known to inflect the major portion of physical damage. They have a short shelf life because of their susceptibility to attack by pathogens and high rates of respiration (20 to 40 mg CO_2/kg h at 20°C).[45] It was reported that between 66 and 99% of the semi-colored to ripe strawberry fruits were bruised after a journey of 1600 km and six handling operations in Australia.[46] In Denmark, more than 20% of the strawberries were found to be bruised upon reaching the retail outlets.[32] In the U.S., 70 and 48% of the strawberry shipments in the New York market monitored between 1972 and 1984 incurred bruise damage and "soft leaky fruit" disorder, respectively.[47] "Soft leaky fruit" disorder is the result of over-maturity, high temperature, rots, freezing, or mechanical injury. Bruising in strawberries appears as discoloration similar to apples. When a strawberry fruit is bruised, cells burst and the sap is released into the air-filled interstitial spaces.[46]

A study on transporting ripe tomatoes was conducted by Itokawa and co-workers[48] in Japan. During conventional transport using a small size truck, the vertical vibration acceleration level was reportedly less than 15 m/s^2 and the frequency range was less than 30 Hz exerted on the corrugated cardboard boxes.

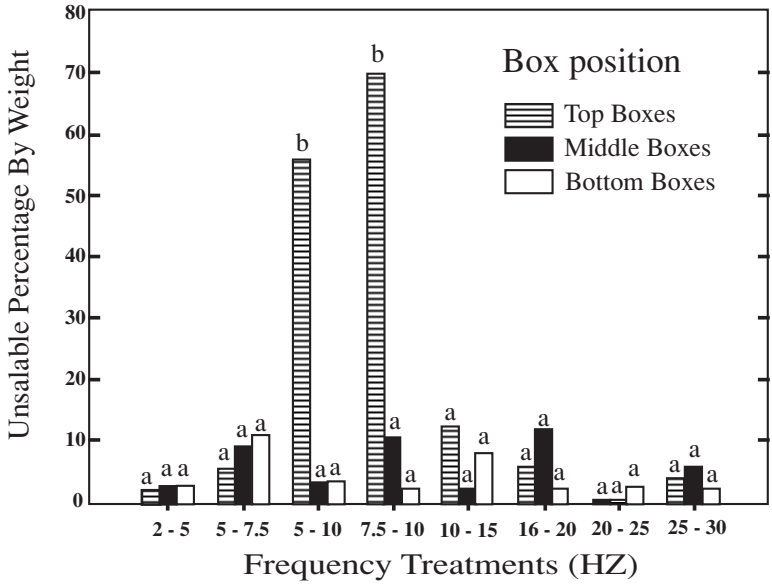

FIGURE 2.1 Percent unsalable strawberries after vibration at different frequencies. Location or frequency treatments with the same letter above the bar are not significantly different. (From Fischer, D., Craig, W. L., Watada, A. E., Douglas, W. and Ashby, B. H., *Appl. Eng. Agric*., 8, 366, 1992. With permission.)

Tomatoes in plastic containers were subjected to dynamic compression due to the vibrations. The compressive stress on the bottom was 2 to 3 times that of the static conditions at the resonance frequency. Damage of the vibrated ripe tomatoes in corrugated cardboard boxes occurred under vertical acceleration of 10 m/s^2 in 30 min. A high percentage of unmarketable weight loss (more than 55% in top boxes), after subjecting strawberries to vibration frequencies between 7.5 to 10 Hz, is shown in Figure 2.1. Fischer and co-workers[49] reported that vibration frequency of 5 to 10 Hz caused the most damage to strawberries and table grapes with the fruits in top boxes sustaining the most damage.

EFFECTS OF MECHANICAL INJURY ON THE QUALITY AND SHELF LIFE OF THE PRODUCE

Depending on the physical characteristics of the produce, mechanical damage may cause severe deterioration of quality and shortened shelf life. Injured fruits and vegetables increase their respiratory activity. Transpiration also increases because the exposed surfaces of the produce caused by cuts, punctures, cracks, abrasion, and tears serve as avenues of water loss. Through the exposed surfaces, microorganisms can infect the product and accelerate its deterioration. Since the cell contents are exposed, oxidation and a host of other reactions occur, impairing the sensory quality and shortening the shelf life of the produce. Due to damaged tissues, ethylene production also increases, and this triggers other deteriorative changes leading to

senescence and decay. Pathogens enter at the site where the fruit or vegetable is severed. An example is stem-end rot caused by *Diploidia matalensis* occurring in citrus fruits, papayas, and mangos, and by *Botrytis cinerea* or *Penicilium expansum* among fleshy-stemmed varieties of apples and pears.[50] Additional openings like cuts, cracks, and abrasions also act as points of entry of the pathogens. The presence of moisture on the wound surface is ideal for fungal spore germination given the right atmosphere. Even for pathogens that can penetrate the skin of the produce, the presence of wounds or cuts accelerates their penetration and infection. In stone fruits, the conidia of *Monolinia fructicola* gain access through fresh wounds, providing the needed moisture and nutrients for their growth.[51]

Mechanical harvesting, which is more injurious than manual harvesting, causes more bruises and excessive pressure that stimulates latent infections without necessarily rupturing the rind. According to Rippon,[52] *blue mold* in apples and *soft rot* in potatoes, which are both initiated within the lenticels, result from crushing of cells around the lenticels due to excessive pressure. Cuts, skinnings, wounds, and cracks are avenues of water loss in potato tubers causing shrinkage and weight loss. Storage of potato tubers that are mechanically damaged is not advisable. Sorting of severely damaged tubers has to be carried out before storage, and curing will take care of healing the rest of the tubers with minor injuries like cuts and abrasion.

Sommer[51] outlined the following events after the inflection of mechanical injury:

> Cells ruptured by the cut are killed and cellular contents are mixed and exposed in the wound area. Enzymes, such as polyphenol oxidases, which are compartmentalized when the cell is alive, are mixed with the polyphenols in the cell sap. Browning in the wound results from enzymatic oxidation of phenolic compounds. Living cells near the injury are stimulated to become very active metabolically even though they do not themselves show signs of major injury. Repair is set in motion by these stressed but unbroken cells. Polyphenol synthesis may lead to the accumulation of greater quantities of those already present. New compounds, often similar to those that appear following infection, may appear in the wound area.

Klein[53] reported in the case of apples that both aerobic and anaerobic respiration rates do not increase after impact and compression loading. Even then, it was observed that carbon dioxide production increased. It was explained that this phenomenon results from enzymatic reactions following the destruction of apple tissue and the release of the contents of the ruptured cells. Monitoring CO_2 production, when compared with controls, is one way of detecting the level of mechanical injury in the commodity.

The development of "spongy tissue" in mangos, characterized by soft pulp, pale color, with acidic and off-flavors, is a direct effect of mechanical damage according to Salunkhe and Desai.[54] "Wound pathogens" can gain entry through injuries inflicted during harvesting and handling. Stem-end rot in mangos results from an injury created by severing the fruit from the tree. Friction discoloration is common among pears. Enzymatic browning, which is catalyzed by polyphenol oxidase,[55] appears when injury exposes the cellular contents to oxygen.[39] According to Meheriuk and McPhee,[56] immature fruits or fruits of advanced maturity are more susceptible to

TABLE 2.4
Effects of Mechanical Injury on Tomatoes

Mechanical Injury	Stage of Ripeness	Effect
Bruising	All stage of ripeness or maturity	Random and uneven ripening; uneven appearance and quality Water soaking Metabolic and morphologic breakdown of bruised tissue[62,63]
Impact-Bruising	Mature-green	High respiratory activity High ethylene production Ripening of damage areas Decrease in titrable acidity[64]
	Light-pink	Increase in off-flavors Less tomato-like flavor[65]

discoloration than fruits harvested at maturity. For cucumbers, mechanical damage has been known to render pickled products unacceptable for marketing. It causes physiological breakdown during storage and processing which is typified by sloughing off of the placental tissue.[57] Tissue softening in cucumber involves the induction of cell wall-degrading enzymes,[58] anodic peroxidase enzymes,[59] and changes in sugar composition of cell-wall polysaccharides.[60] Mechanical injury due to rough handling of citrus fruits is seen as discoloration in the rind due to oil spotting and oleocellosis. Following the breaking of oil cells in the peel, the oil is exuded, damaging the surrounding cells and serving as the entry point for microorganisms.[61] Although not a serious reason for quality loss among tomatoes, mechanical injury often causes subsequent deteriorative changes such as shrinkage, attack of microorganisms, and decay. Effects of mechanical injury during handling of tomatoes are summarized in Table 2.4. Ceponis and Butterfield[66] reported that predominant decay organisms infect tomatoes at mechanical injury sites. Sargent and co-workers[42] showed that internal bruising caused by handling impacts disrupts the normal ripening of the tomato locular gel. Bruising in potatoes is manifested as *blackspot* that appears days after the injurious load has been applied. For this to occur, the tuber tissue (cell walls and cell membranes) must be ruptured and oxygen must react with certain chemical compounds in the cytoplasm. Upon cell rupture, certain cytoplasmic phenols are freed and oxidized by the enzyme phenol oxidases, to form blue-black melanin pigments via the red colored dopachrome.[40] Gray and Hughes[67] gave the following series of reactions after a detrimental load was inflicted on the tuber:

tyrosine → 3,4-dihydroxy-phenylalanine (DOPA) → phenylalanine,
3-4-quinone → 2-carboxy-2,3-dihydro-5,6-dihydroxyindole →
2-carboxy-2,3-dihydroxy-indole-5,6-quinone →
5,6-dihydroxy-indole-5,6-quinone → melanin

The first two reactions are catalyzed by polyphenol oxidases, and the remainder can be non-enzymatic. This discoloration was initiately thought to arise solely from

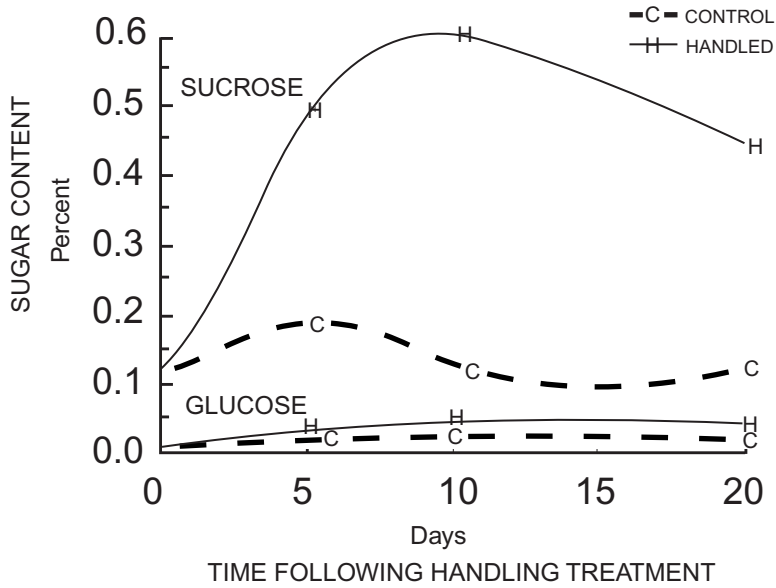

FIGURE 2.2 Sucrose and glucose concentrations in control and handled norchip tubers (7 months in storage 9°C, 90% RH) as influenced by increasing times between handling and sugar analysis (0, 5, 10, and 20 days). (From Orr, P. H., *ASAE Paper No. 85-6024*, American Society of Agricultural Engineers, St. Joseph, MI, 1985. With permission.)

the amino acid tyrosine. Phenols including chlorogenic and caffeic acid are now involved.[40] High storage temperatures after the inflection of the bruise accelerates the development of this undesirable coloration. The undesirable color of potato chips after months in storage is due to the rough handling of the tubers upon loading to and unloading from the storage.[68] Usually in dry handling of potatoes, bulk scoops, hoppers, and multi-tiered conveyors are employed. Potato-on-potato tumbling can inflict damage to the tubers and it has been claimed[68] that a rapid increase of sucrose in the handled samples was induced by stress on the tubers (Figure 2.2). Increased sucrose concentration that is detrimental to chip color developed in five days after handling. Mechanical stress on the potato tubers can also stimulate the synthesis of glycoalkaloids (an antinutrient) in the peel and the flesh.[69] High temperatures promote faster glycoalkaloid synthesis than low temperatures. Its synthesis after mechanical injury was reportedly accomplished within 15 days. Cutting of tubers resulted to the highest glycoalkaloid content, followed by puncturing, dropping, hammering, and bruising.

During harvest, peas are subjected to bruising and undergo tenderization. It was shown that even slight damage of peas during vining leads to marked changes in their metabolism.[70] Rapid development of off-flavors of vined peas may arise after mechanical shelling. Due to bruising sustained by peas during vining, respiration is affected, resulting in decreased amounts of CO_2, aldehyde, and alcohol produced.[71] Textural changes often accompany bruising. Delays between harvesting (vining) and processing must be avoided to maintain the quality of the produce. Physical damage

and pathogen infection of the lettuce heads increase ethylene production. Coupled with a high temperature especially at over 5°C, russet spotting can develop during storage and transport. Russet spotting occurs on the lower midribs of outer leaves consisting of clusters of olive-brown spots.[72] Ethylene production can also be induced during cold storage by fuel emission, particularly from propane, from forklift trucks, or in retail storage rooms and in home refrigerators containing ripening fruit.[73]

MEASURES TO MINIMIZE MECHANICAL INJURY

Careful Handling

Care should be exercised in handling fresh produce. Staff should be trained on the proper techniques of harvesting, in placing the produce in the container, as well as in the loading and unloading of containers for transport and storage. Loading aids such as trolleys, roller conveyors, pallet, or forklift trucks should be used to reduce handling of individual packages. Handling bananas by the bunch should be avoided as this predisposes the fruits to mechanical injury. Likewise, handling of dehanded bananas in sacks or placement of these fruits directly in the truck or ship's container should be avoided. Bananas are dehanded and packed in boxes for transport. However, for local markets, locally available containers can be used with adequate lining materials, such as bamboo baskets lined with newspaper. During the manual harvesting of fruits and vegetables such as citrus and mangos, harvesting aids like clippers, shears, or picking poles with nets and cutting knives are employed to avoid cuts and punctures. Pulling or plucking fruits and root crops by hand may cause bruises and cuts at the stem end. Leafy vegetables should be packed loosely in the container as they are very prone to damage. Bins used in harvesting fruits should be lined. A 35 to 40% reduction was observed when the bins used in harvesting apples were lined with foamed plastics.[30] For delicate fruits like strawberries and starfruits, or carambolas, transfer from one container to another during picking and handling should be avoided.[74] Potatoes taken out of bulk storage must be handled gently and appropriate equipment must be used. Mechanical injury results when potatoes are loaded and unloaded from the store. One likely solution to this problem, if economics will allow, is to use palletized boxes for storage so that the handling equipment will not be in direct contact with the tubers, and the tubers will not have the opportunity to tumble upon each other. Fluming (wet handling) was suggested[75] as a possible means of handling potatoes to minimize tuber damage. The technical determinants of damage in potatoes and the measures that can be taken to minimize such damage are given in Table 2.5.

Bruising often occurs in the packinghouse when the produce is sorted, cleaned, graded, and packaged. Packing lines should be laid out in such a way so that the number of transfer points and drops that the produce will undergo is minimized. As much as 51% reduction of bruising in apples can be realized if cushioning materials are placed on hard surfaces.[77] Schulte and co-workers[29] showed that the addition of 6.35-mm-thick Poron cushion on surfaces where apples fall can increase the maximum drop height to 226 mm before bruising occurred. Gan-Mor and Mizrach[78] introduced a new material to cushion impact and reduce fruit bruising due to impact

TABLE 2.5
Technical Determinants of the Extent of Mechanical Damage of Potatoes and the Corresponding Measures to Minimize Them

Factor	Effect on Mechanical Damage	Measure to Minimize Effect of the Factor
Falling height	Increase with increased falling height	Falling height should not exceed 40 cm.
Conveyor belt speed	Increase with increased belt speed	Belt speed should not exceed 0.5 m/s
Impact surface	Hard surfaces cause more damage on potatoes	Use of padding like plastic or rubber foam
Tuber weight	Heavier tubers sustain more damage than lighter ones	Limit the number of possible falls Avoid right angle bends in conveyor transfers and sharp edges
Tuber specific gravity	High specific gravity tubers sustain more blue discoloration	Efficient organization of unloading, transport, grading, and dispatch
Tuber temperature	At low temperature (4 to 6°C), tubers are more susceptible to blue discoloration than at higher temperatures (15 to 20°C)	Tubers are heated at the end of storage season

Data from de Haan, P. H., Damage to potatoes, in *Storage of Potatoes: Post-Harvest Behavior, Store Design, Storage Practice, Handling,* Rastovski, A. and van Es, A., Eds., Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, 1987, 371-380.

in the packing line by utilizing thin steel plates in place of soft pads (which are subjected to rapid abrasion). Brushing can also bruise fruits that are sensitive, like the incidence of rind pitting on "Meyer" lemons reported by Wild.[79] Reduction of brushing times is needed to avoid bruising the rinds of citrus fruits. To prevent mechanical injury in tomatoes, the following were recommended: (a) better crop management; (b) use of improved crates for transporting; (c) filling crates at a proper level; (d) reducing exposure to excessive temperatures; (e) more care in handling of tomatoes in the system; (f) improving tomato grading techniques; and (g) better and consolidated management of the postharvest handling system.[11]

Proper Packaging

Preventing the occurrence of vibration damage is the function of properly designed packaging. If commodities are loosely packed in a container, their tendency is to bounce and impact with one another or against the package or container walls leading to bruising. Vibration damage can be reduced by attenuating the resonant amplitude levels, rather than eliminating resonant frequencies from the system.[3] If the natural frequency band of the containers in the stack is removed from the natural frequency band of the fruits inside, then the acceleration level input to the fruits may be significantly reduced. Pason and co-workers[34] found that less bruise damage was sustained by apples packed in cellmaster cartons with polystyrene soft cell trays, whereas damage was higher in traymaster cartons with paper pulp trays. Bruise

damage was intermediate in bagmaster cartons where apples were packed in polyethylene bags.

When packaging perishable produce, two important principles should be observed: individual specimens should not move with respect to one another or the walls of the package to avoid damage due to vibration, and the package should be full without overfilling or tight packing, which increases compression and impact bruising.[2] To cushion the specimen from damage, liners, tray cups of paper or polystyrene foam, and nets have been employed. If packed in woven jute sacks or nets, commodities like root crops and onions should be stacked in unit loads on pallets or pallet boxes. The packaging material chosen should provide adequate ventilation and protection to the packaged produce.

Curing of Root Crops and Tubers

Curing is an important postharvest operation among root crops like Irish potatoes, sweet potatoes, and onions. It is carried out in order to heal harvest-inflicted injuries and also to close the neck of onions and garlic. Breaks in the periderm in injured root crops can serve as an entrance for pathogens, induce water loss, and increase metabolic activity. Following wounding of the tuber tissue, suberization of the cells on the surface of the wound takes place, which involves the deposition of suberin along the cell walls.[80] Suberin, which serves as a barrier to water loss and microorganisms, is an insoluble polymeric material attached to the cell walls of periderms, including wound periderm among aerial plants.[81] Van Es and Hartmans[80] outlined the following event of wound healing:

> The wound periderm is formed at the onset of primary suberization. A few cell layers away from the wound surface, new cell walls are formed parallel to the wound surface. This gives rise to a phellogen (cork cambium), which serves as the basis for wound periderm formation: the phellogen cells form a number of phellem cells parallel to the wound surface. These phellem cells subsequently become suberized and this is known as secondary suberization.

For conditions in the Netherlands, Meijers[82] recommended a curing temperature of 12 to 18°C and a relative humidity of 80 to 95% for 10 to 14 days. Wound healing is faster when temperatures are high. No wound tissue appears to be formed at a relative humidity of 30%. While in saturated atmospheres, curing is delayed due to the proliferation of the cells on the surface of the wound.[82] Wound healing for sweet potatoes occurs in two stages.[83] The first stage is the deposition of suberin on the cell walls adjacent to the lesion, followed by the formation of the wound periderm. Temperatures around 30°C and relative humidities of 85 to 95% are optimal for wound healing. The curing period used to be 14 days, but the present recommendation for sweet potatoes is 4 to 7 days.[84] The range of 4 to 7 days is allowed for different responses to the curing process dependent on environmental conditions and germplasm variability. Studies carried out by Walter and Schadel[85] showed that the initial response to wounding was the deposition of lignin-like materials rather than suberin. Walter and co-workers[83] confirmed the lignification phase of wound healing,

TABLE 2.6
Recommended Temperature and Relative Humidity for Curing Roots and Tubers

Crop	Temperature, °C	Relative Humidity, %	Time,[a] days
Irish potato	13–17	Above 85	7–15
Sweet potato	27–33	Above 90	5–7
Yam[b]	32–40	Above 90	1–4
Taro (dasheen)	30–35	Above 95	4–7
Cassava	30–35	Above 80	4–7

[a] In practice, at least 7 days should be allowed for curing.
[b] *Dioscorea alata* and *D. rotundota*.

Data from *Prevention of Post-Harvest Food Losses: Fruits, Vegetables and Root Crops, A Training Manual,* FAO Training Series No. 17/2, Food and Agriculture Organization of the United Nations, Rome, Italy, 1989.

and this occurred at maximum rates within 5 days. They inferred that this combination of lignified tissues and wound periderm formed after a 5-day curing period was an effective barrier against the entry of pathogens. Storage stability of sweet potatoes, however, may not only rely on whether wound healing has occurred, but rather it is an interaction of several other factors. Table 2.6 shows the recommended conditions for curing of different root crops and tubers.

Warming to the Desired Temperature Before Handling

Wouters and co-workers[86] suggested warming the tubers to above 10°C before handling, as an effective way of reducing the susceptibility of the potatoes to bruise damage, especially when the dry matter content of the tubers is low. They correlated bruise susceptibility to the rupture force obtained from a puncturing test.

Pre- and Postharvest Chemical Treatment

Preharvest sprays to control infection caused by mechanical injury are generally not encouraged because only a small portion of the fungicide is bound to the harvested product, and this may be removed during washing. Nevertheless, Rippon[52] believed that this may be a desirable approach to the problem of the high incidence of superficial injuries and attendant decay from mechanical harvesting. Chemical treatment to control infections should be applied soon after harvest to avert the penetration of pathogens into the host tissue. According to Eckert,[87] the maximum period between harvest and successful treatment varies: 10 h for peaches inoculated with *Rhizopus* spp. and held at 25°C, and 24 h for oranges inoculated with green mold (*Pennicilium digitatum*) and held at 24°C. The maximum time to control *Phoma*

spp. in potatoes with gaseous *sec*-butylamine is 2 weeks.[88] Rind staining, which is manifested as brown patches on the peel of oranges and lemons, possibly results from mechanical abrasion occurring 12 to 24 h after packing.[89] The incidence of rind staining can be reduced by preharvest sprays of dilute gibberelic acid.[90]

Retardation of Softening

Softening associated with ripening make fruits susceptible to bruising and mechanical injury. Fruits are often harvested when they are still in the mature green stage to prevent mechanical damage. Apple softening can be retarded by 2,5-norbornadiene (2,5-NBD), which acts by competing with ethylene for binding sites and inhibits its action in plant tissues.[91] When applied to "Delicious" apples, as a gas in either closed or flowing system, 2,5-NBD prevented the softening of apples stored for 30 days at 25°C with either 2000 or 4000 µL 2,5-NBD/L of air. The firmness of fruits was comparable for fruits held for 30 days at 5°C and loss of soluble solids and starch was similar to that for the refrigerated apples.

Damage Detection

For better quality control of fresh produce during storage and marketing, detection of damage is important for it prevents if not minimizes the spread of diseases and pests, and the downgrading of the product. Conventional damage detection is carried out by visual inspection, which is time consuming and labor-intensive. For a long time, researchers have tried to develop automated systems for detection of damage and sorting. Among these are spectral analysis of acoustical signal which was tested on apples,[92-95] carrots,[96] peaches,[36] radishes,[97] citrus,[98] and prunes;[99] delayed light emission (DLE) on cucumbers;[57,100,101] automated color vision system that was tested on fresh market peaches;[102] and nuclear magnetic resonance (NMR) which was tested on "Bartlett" pears.[103]

TEMPERATURE EFFECTS

The relationships between temperature, respiration, transpiration, and other deteriorative changes are well documented. Chilling and freezing injury occurs at low temperatures and commonly occurs during cold storage, while high temperature injury is likely to occur in tropical areas, or when the produce is exposed to the sun after harvest.

Incidence of Chilling and Freezing Injury

Chilling injury is a major problem in postharvest handling of susceptible plant materials because it precludes the storage of many commodities at low temperatures. As such, special transport and storage facilities are needed for effective handling of fruits and vegetables in developing countries. For crops that are not traded in large volumes, a major problem is encountered because there is a tendency to mix the

commodities in one refrigerated vehicle or storage. Chilling injury occurs during the cold treatment of citrus (exposure at low temperature for a prescribed period), e.g., grapefruit, but is one of the approved quarantine procedures for the control of Mediterranean fruit fly.[104] There are 10 visual symptoms associated with chilling injury, namely:

1. surface lesions — pitting, large sunken areas, and discoloration
2. water-soaking of tissues — disruption of cell structure and the accompanying release of substrates favoring growth of pathogens
3. internal discoloration or browning of pulp, vascular strands, and seeds
4. breakdown of tissues
5. failure of fruits to ripen following removal from storage
6. accelerated rate of senescence
7. increased susceptibility to decay
8. shortened shelf life due to one or more of the above responses
9. compositional changes related to flavor and taste
10. loss of growth capacity for stored propagules.[105]

The lowest safe storage temperature from chilling injury and the corresponding symptoms for fruits and vegetables are shown in Table 2.7. Surface pitting, necrotic areas, and external discoloration are apparent symptoms of chilling injury. Cucumbers show surface pitting when stored at 10°C or below.[132] Similarly, chilling injury in green beans appears as streaky soft spots and slimy patches.[133] At mild chilling, green bananas develop smoky or dull-yellow appearance[134] and on more severe chilling, the peel turns dark brown or black. In peaches, symptoms of chilling injury include lack of juiciness (mealiness, wooliness, leatheriness), browning of the flesh, increased susceptibility to decay, and loss of ability to ripen.[135] Luza and coworkers[135] reported that 10°C was a much better temperature for short-term storage of peaches than 5°C, although the fruits could be stored longer without development of noticeable symptoms. However, symptoms of chilling injury developed rapidly upon transfer of the fruits previously kept at 5 to 20°C for ripening. Sea transport is ideal for mangos because of its low cost, but it seems the fruits do not respond well to low-temperature storage during the long journeys. Storage of mangos at 8°C can cause chilling injury as evidenced by their failure to ripen.[136] Avocados also failed to ripen normally and develop a distinct climacteric rise signaling the onset of ripening when stored at temperatures of 4 to 5°C.[137] For mature-green guavas, chilling injury occurs at storage temperature of 3.5 to 7°C.[138] Superficial scald, a brown discoloration giving the skin a cooked appearance in some apple cultivars, is also a chilling injury symptom evident after long-term storage at 0 to 4°C.[139,140]

Freezing injury is a separate phenomenon from chilling injury because it results from the freezing of tissues and formation of ice crystals at temperatures below the freezing point.[2] This is common among some vegetables because they are stored at or near freezing temperatures. Freezing injury in broccoli florets yields strong off-odors during cooking and globe artichoke, asparagus, lettuce, celery, and sweet corn have been classified as highly susceptible to freezing injury.[7]

TABLE 2.7
Safe Storage Temperature, Symptoms, and Amelioration of Chilling Injury in Fruits and Vegetables

Commodity	Approx. Lowest Safe Temp. °C	Symptoms of Chilling Injury	Amelioration
Fruits			
Apples	0–4[106]	Brown core, low temperature breakdown, superficial scald,[107] soggy breakdown[106]	Pre-storage high temperature conditioning,[108] intermittent warming,[109] low RH storage,[110] gibberelic acid application,[111] diphenylamine and ethoxyquin treatment[106]
Avocados	5–13[107]	Gray-brown discoloration of flesh, uneven ripening, development of undesirable flavors and odors, pitting, scald-like browning or darkening of skin[112]	Controlled atmosphere storage, vacuum infiltration with $CaCl_2$, hypobaric storage[112]
Bananas (green/ripe)	12–14[107]	Dull, gray-brown skin color, failure to ripen, loss of flavor, hardening of central placenta, increased susceptibility to mechanical injury[112]	Slow step cooling, treating with small quantity of veg. oil or dimethylpolysiloxane, hypobaric storage[112]
Cranberries	0–3[106]	Low temperature breakdown, red and rubbery flesh[106]	Intermittent warming[113]
Grapefruit	10[107]	Brown staining of rind, pitting, watery breakdown[107]	Delayed cooling, CA storage, Benomyl and TBZ treatment, hypobaric storage,[112] film wrapping,[114] Imazalil treatment[115]
Lemons	13–15[107]	Pitting, membrane stain, red blotch[107]	Delayed cooling[116]
Limes	7–10[107]	Pitting[107]	Diphenyl treatment[117]
Lychee	Variable	Dull skin[118]	
Mangos	10–13[107] 13[119]	Gray skin scald, uneven ripening, failure to ripen, poor flavor, failure to color, pitting, increased susceptibility to postharvest decay[112]	Partial ripening prior to storage, hypobaric storage[112]
Oranges	7[107]	Pitting, brown stain, watery breakdown[107]	Intermittent warming,[113] TBZ treatment[120]
Navel	4.5[121]		
Ponkan	2[121]		
Satsuma	1[121]		

TABLE 2.7 (continued)
Safe Storage Temperature, Symptoms, and Amelioration of Chilling Injury in Fruits and Vegetables

Commodity	Approx. Lowest Safe Temp. °C	Symptoms of Chilling Injury	Amelioration
Papaya	7[107]	Pitting, failure to ripen, off-flavor, decay[107]	None
	10–12[122]		
Peaches and nectarines	0–5[106]	Wooliness, mealiness, lack of flavor, flesh browning, lost ability to ripen[106]	CA storage,[123] intermittent warming[124]
Pineapple	15	Blackheart, poor flavor[125]	
	8–10[122]	Uneven ripening, dull color, water-soaked flesh, increased acidity, loss in ascorbic acid content[122]	
Plums	0	Flesh browning, off-flavor[106]	Intermittent warming[126]
Tangerine	5–8[127]		
Vegetables			
Asparagus	0–3[106]	Discoloration, softening of stem tips[106]	
Beans (green)	7[107]	Pitting, russetting[107]	
Lima beans	5–8[119]		
Snap bean	5–8[119]		
cv. Master piece	6[121]		
Cassava	5–8[128]		
Chayote	6.5[121]		
Cowpeas	5–8[119]		
Cucumbers	7[107]	Pitting, water-soaked spots, decay[107]	Intermittent warming[129]
cv. Horai	6[121]		
cv. Pixie	5.5[121]		
Eggplant	6.5[121]	Surface scald[2]	
	13[119]		
Ginger	12–14[128]		
Bitter melon (balsam pear)	7[121]		
Ripe cantaloupe	4–6[119]		
Honeydew melon	7–10[107]	Pitting, bitter flavor[107]	
Muskmelon	10–12[119]		
Oriental pickling melon	6[121]		
Watermelon	5[107]	Pitting, bitter flavor[107]	
	7–10[119]		
Okra	7[107]	Discoloration, water-soaked areas, pitting[107]	
Peppers	5–7[119]	Pitting, water-soaked spots	Intermittent warming[129]
Potatoes	4[107]	Internal discoloration, sweetening[107]	

TABLE 2.7 (continued)
Safe Storage Temperature, Symptoms, and Amelioration of Chilling Injury in Fruits and Vegetables

Commodity	Approx. Lowest Safe Temp. °C	Symptoms of Chilling Injury	Amelioration
Pumpkins	10[107]	Decay[107]	
	12.8–15.6[119]		
Sweet peppers	7[107]	Pitting, *Alternaria* rot[107]	
	6.5[121]		
Sweet potato	13[107]	Internal discoloration, pitting,	
cv. Kokei No.14	8.5[121]	decay[107]	
Taro	13–15[128]		
Tomatoes: mature	13[107]	Water-soaked softening, decay,	Pre-storage high
green	9.5[121]	*Alternaria* rot[107]	temperature
cv. Beiju dark	5.5 [121]		conditioning[130]
pink	13[107]	Poor color, abnormal ripening[107]	Intermittent warming[131]
cv. Beiju ripe			
Yam	13–15[128]		

Incidence of High Temperature Injury

High temperature injury occurs when the packaged produce is exposed to the sun while awaiting transport to the packinghouse; during retail in open markets; when the produce comes in contact with a heated surface; when the produce is packed or transported with lack of cooling and ventilation; and when the produce is subjected to heat treatment for disinfection. An and Paull[141] reported that papayas stored at 32.5°C for 10 days failed to ripen normally, as observed by poor color development, abnormal softening, surface pitting, and occasional off-flavor. On the other hand, fruits stored at 10°C for 14 days exhibited faster ripening rates as evidenced by degreening and softening of the flesh, and no delay in flesh color development. At temperatures above 25°C, banana skin remains greenish but the pulp becomes mushy and has poor flavor.[125]

Storage temperatures above 30°C may result in the failure of tomatoes to ripen normally. Color development of tomatoes arises from lycopene synthesis. Failure to synthesize lycopene often occurs during wholesale and retail operations in tropical countries. Temperatures above 30°C inhibit the synthesis of lycopene and most other polyene pigments but not the synthesis of beta-carotene which is responsible for the yellow color.[142] Ripening and ethylene production are suppressed and heat injury may result when tomatoes are stored at 40°C and scald develops at the stem end of the fruit. At temperatures above 30°C, respiratory metabolism is altered and Inaba and Chachin[143] postulated that this may be associated with changes in membrane properties, which ultimately lead to heat injury at 40°C. Abnormal ripening of tomatoes after transferring to 25°C may occur, since thermal stress accumulates on the fruit and consequently, it alters the mitochondrial properties after 4 to 5 days at 35°C. Sub-optimal temperature during storage will also lead to similar effects.[144] In

lettuce, disorders like rib discoloration and pink rib have been reported.[72] Rib discoloration occurs on the midrib of the outer head leaves usually where the rib curves. It was postulated that high temperature favors rib discoloration.[145] Pink rib is a diffuse pink coloration near the base of the outer leaves of the head. High transit temperature and low O_2 atmospheres were the causes.[145] Temperature and length of storage are two interacting external factors that affect the storage life of asparagus. Lipton[146] reported that temperature of 5°C and above accelerated the lignification of tracheal tubes and increased the alcohol insoluble solids content, two of the criteria used to quantify texture. Likewise, temperature of 5°C and above was reported to promote rapid visual deterioration of asparagus.[146]

Effect of Temperature Stress on Quality and Shelf Life Stability of the Produce

Scientists have tried to find a universal mechanism to explain chilling injury from the varying responses of plants exposed to low temperature. Parkin and co-workers[147] reviewed the following possible mechanisms explaining chilling injury: (a) bulk membrane lipid phase transitions at low temperature resulting in the formation of gel phase lipids leading to a loss of membrane integrity and physiological dysfunction advanced by Lyons;[134] (b) domains of lipids undergoing continual transitions at critical temperature from liquid crystal to gel phase resulting in membrane damage as shown in a model by Quinn;[148] (c) low temperature-induced changes in the levels, kinetic properties, and cold lability (loss of activity) of enzymes;[149] and (d) cellular redistribution of calcium as a primary transducer of chilling injury.[150] Parkin and co-workers[147] speculated that lipid peroxidation may have a role in the development of irreversible injury during low temperature stress, where its effect would be similar to senescent processes of free radical damage to tissue and progressive membrane hardening. Membrane properties are altered at low temperature and Inaba and Crandall[151] reported a correlation between low temperature injury and increased electrolyte leakage.

The major biochemical event that leads to chilling injury is thought to be the solidification of membrane lipid microenvironments (lateral phase separations). Subsequently, this affects ion transport regulation and alters the catalytic properties of membrane-bound enzymes.[152,153] The disruption of membrane function affects cellular metabolism such as diminished regulatory control of cellular energy generation, membrane semipermeability, and metabolite and ion compartmentation.[147] Plant tissues subjected to shear during wounding and freezing respond by oxidizing their lipids.[154] Ethane, a product of lipid oxidation, appears before losses in phospholipids are noted. Lurie and Klein,[130] however, claimed that lipid peroxidation was not involved in chilling injury of tomatoes because of the absence of ethane in fruits that were damaged by chilling injury. One symptom of chilling injury in avocado fruit is the gray or dark brown discoloration in the mesocarp.[155] According to Lieberman and co-workers,[156] this discoloration is due to the accumulation of oxidized phenolic compounds. This symptom was observed to be more severe at the stylar end compared to the pedicel end.[157] Flaccidity and a dull, dark gray-green aspect of the tips and sometimes a portion below the elongation zone are the

symptoms of chilling injury in asparagus spears held at 0°C.[146] Compared to those stored at 2.2°C, storage life of asparagus spears stored at 0°C was shorter due to chilling injury. In peaches, chilling injury results in: mealiness which is characterized by separation of mesocarp parenchyma cells, leading to increased intercellular spaces and accumulation of pectic substances in the intercellular matrix and leatheriness which is characterized by collapse of mesocarp parenchyma cells, increase in intercellular space and accumulation of pectic substances in the intercellular matrix.[135] As internal breakdown progresses, the following changes are observed at the ultrastructural level: dissolution of middle lamella; cell separation; irregular thickening of primary cell wall; and plasmolysis of the mesocarp parenchyma cells.

Tomatoes are susceptible to chilling injury at temperatures below 12 to 13°C, resulting in metabolic disorders and susceptibility to disease.[158] The following have been observed resulting from storage below recommended temperature: (a) inhibition of the normal decrease in acidity during ripening;[159] (b) inhibition of flesh softening and pigment synthesis;[160] (c) decline in reducing sugars;[160] (d) decline in soluble solids;[161] (e) flavor loss;[65,125] (f) loss in ascorbic acid content;[162] and (g) susceptibility to disease-causing microorganisms.[163] Mature green fruit cannot tolerate temperatures below 16°C,[164] but red tomatoes can be stored at 8°C for 3 to 4 weeks. In mature green tomatoes, the major portion of injury was at the subtending locules and on the stem end.[165] The location of the injury corresponded to the regions where ripening was slower. Marangoni and co-workers[153] reported that chilling injury in mature-green tomatoes involves the following events: microvesiculation of the endoplasmic reticulum; loss of ribosomes, chloroplasts, and mitochondrial swelling; loss of starch granules; disorganization of the internal lamellae of chloroplasts; and grana unstacking, as well as plastoglobuli and tonoplast degradation.

MEASURES TO MINIMIZE THE DETRIMENTAL EFFECTS OF TEMPERATURE ON THE PRODUCE

Chilling injury is a time- and temperature-dependent problem that seriously affects marketability of many fruits and vegetables. The nature of the injury is not yet fully understood. Until a theory is formulated to generally explain its occurrence, effective prevention of this defect cannot be prescribed. Measures to ameliorate the injury of agricultural crops vary for different crops. Nevertheless, scientists have proposed several methods for reducing the susceptibility of fruits and vegetables to chilling injury.

High Temperature Conditioning

Klein and co-workers[108] carried out investigations at 38°C with apples (cv. "Anna" and "Granny Smith") for 4 days. They showed that after storage at 0°C, the fruits were firmer than the control and softened more slowly during shelf life at 17°C. The "Anna" variety favorably responded to the pre-storage heating because it usually softens easily upon removal from storage. Retention of firmness in response to heating was found to develop during just 1 month of cold storage compared to 4 months for "Granny Smith," which is known to be a firm cultivar. Softening of

many fruits is due to an increase of soluble pectin and corresponding decrease in insoluble pectin. Klein and co-workers[108] postulated that in the case of "Anna" apples, insoluble pectin was retained during storage and consequently a smaller amount of soluble pectin and calcium pectate was found in the heated fruits. Lurie and co-workers[166] used conditioning at 38°C for 4 days to control superficial scald, a chilling injury symptom on "Granny Smith" apples. This treatment can be used to substitute chemical treatment using diphenylamine (DPA) by slowing down the accumulation of α-farnesene and conjugated trienes in apple cuticle during short-term storage. DPA inhibited the oxidation of α-farnesene without affecting its accumulation.

Tomatoes develop chilling injury when kept below 12 to 13°C. This can be prevented by keeping mature green tomatoes at a temperature of 36 to 40°C for 3 days before storage at 2 to 3°C for 3 weeks.[130] After storage, the fruits had a lower potassium ion (K^+) leakage and a higher phospholipid content than the unheated fruits. The heat-treated tomatoes also ripened normally, although more slowly than freshly harvested fruits. Chilling injury of unheated tomatoes was manifested by failure to turn red and the development of brown areas under the peel. Lurie and Klein[130] postulated that heat stress caused an inhibition of ripening and that this inhibition was maintained at 2.5°C and removed only after transfer to 20°C. They also found that heat stress inhibited the loss of phospholipids leading to a lower rate of potassium leakage and gave protection against chilling injury.

Delayed Cooling

One way of reducing the susceptibility of the produce to chilling injury is to condition the commodity at a temperature higher than the intended storage temperature. Chilling injury in lemons can be prevented by temperature-conditioning the fruits at 10, 15, 21, or 27°C immediately before storage at 1°C. Conditioning at 27°C for 7 days caused the least chilling injury but McDonald[116] did not recommend this because of the large variations in response of lemons harvested in different years and within treatments. The susceptibility of cucumber to chilling injury when stored at 5°C for 4 or 6 days was reduced by conditioning at 12.5°C.[167] Lacatan bananas can be less sensitive to chilling injury by lowering the temperature at steps of 3°C at 12-h intervals.[168] Cold treatment is one of the approved quarantine treatments of pink grapefruit (*Citrus paradisi*) for export to Japan. The approved time-temperature regimes for cold treatment are 0.6°C for 11 days, 1.1°C for 12 days, 1.6°C for 14 days, or 2.2°C for 17 days.[169] Cold treatment for carambola is at 1°C for 15 days.[74] Chalutz and co-workers[170] suggested the conditioning of grapefruit, prior to cold treatments, by storing the fruit at 16°C and relative humidity of 90 ± 5% for 7 days. Miller and co-workers[114] subjected grapefruits that were either waxed or film-wrapped and treated with thiabendazole (TBZ) to high temperature conditioning at 31°C for 3 days. After storage at 1°C for 4 weeks, chilling injury developed in all treatments but was drastically reduced in film-wrapped fruits. TBZ caused a slight reduction on chilling injury symptoms. Rind scald or pitting of conditioned fruit, which are symptoms of chilling injury, were not eliminated but were drastically reduced in film-wrapped fruits conditioned at 7°C or at 16°C.

Intermittent Warming

Interruption of cold storage with warm periods has been shown to be beneficial in extending the storage life of apples, citrus, cranberries, cucumbers, nectarines, and many other fruits by minimizing chilling injury.[113] Wang and Baker[129] found that intermittent warming increased the proportion of unsaturated polar lipids in peaches, cucumbers, and sweet peppers, and lessened the deterioration of these products at low temperatures. Peaches, when warmed intermittently, were found to have reduced incidence of wooliness[171] due to the production of adequate levels of pectolytic enzymes, pectinesterase, and polygalacturonase. Tomatoes have also responded favorably to rewarming at 18°C as shown by the recovery of ultrastructural changes in the mitochondria and plastids.[131]

Chemical Application

Application of fungicides, antioxidants, and growth regulators have been found suitable for reducing chilling injury of some commodities. Valencia oranges often incur chilling injury during long-term storage. Wild and Hood[120] reported that dipping Valencia oranges in a water suspension of the fungicide thiabendazole (TBZ) at 1000 mg/L and a temperature of 53°C for 2 min reduced chilling injury when fruits were subsequently stored at 1°C for 15 weeks. Inducing mechanical injury to the rind will slightly increase the incidence of chilling injury during long-term storage. TBZ had been reported to significantly reduce surface pitting in grapefruits during prolonged storage at 8°C.[172] McDonald and co-workers[115] compared the effectiveness of TBZ and imazalil dips at 53°C for controlling decay and reducing susceptibility to chilling injury of "Marsh" and "Redblush" grapefruit. Dipping at 53°C with fungicide generally reduced the susceptibility of grapefruit to chilling injury. Imazalil was found to be more effective in reducing chilling injury susceptibility and controlling decay than TBZ.[115] Scald in lime could be reduced by 12 to 50% using diphenyl treatment.[117] Gibberellic acid dips or injection of apples was shown to reduce low-temperature breakdown.[111] Antioxidants like diphenylamine and ethoxyquinn are widely used to control superficial scald in apples.[106]

Hypobaric Storage

Hypobaric storage is the storage of the produce under reduced atmospheric pressure. Few fruits are suited for hypobaric storage. Pantastico and co-workers[168] have shown that when *Lacatan* bananas were held at 5°C and 220 mm Hg pressure, symptoms of chilling injury were reduced and the green color was retained for approximately 1 month. At 220 mm Hg pressure and temperature of 4.5°C, limes did not develop symptoms of chilling injury for 4 weeks in storage, while "Marsh" grapefruit showed only slight symptoms of approximately 4.4% after 7 weeks in storage.[117]

Recommended Refrigerated Storage Conditions

Fruits and vegetables should be stored above the critical temperatures at which they show symptoms of chilling injury. Once a produce exhibits chilling injury symptoms,

its quality is impaired and shelf life is shortened as the injury itself is irreversible. Sanford and co-workers[173] reported that weight loss and the incidence of shriveling or splitting in lowbush blueberries are major attributes that contribute to the loss of market value arising from mechanical damage and too low a storage temperature. Ballinger and co-workers[174] recommended that for the fresh market, blueberries should not be exposed to temperatures exceeding 10°C and should be preferably kept at or near 1°C. The major qualitative characteristics that contribute to low commercial yield of fruits are loss of firmness, loss of bloom, and loss of the blue anthocyanin coloration either through leakage from the berry or chemical disruption of the pigment.[173] Sanford and co-workers[173] found 0°C storage to result in optimum conditions of the product.

Short storage life is a problem in raspberries due to their fragile structure and rapid deterioration. Even under recommended storage conditions of –0.5 to 0°C, and 90 to 95% relative humidity, usual shelf life is only 2 to 3 days with a maximum of 1 week.[54] Robbins and co-workers[175] tried to extend the shelf life of red raspberries beyond what was reported by careful attention during harvesting, immediate pre-cooling for 4 h at 0°C and subsequent storage at 0°C and 90 to 95% relative humidity. Harvesting at the red-ripe stage also extended the storability of the fruit. Robbins and Moore[176] recommended a storage temperature of 0°C for maintenance of color. It is also preferred for short storage periods to preserve other quality characteristics such as firmness and flavor components. A temperature between 12 and 13°C is generally considered optimum for storage of mangos,[177] although suitable temperatures such as 10°C and 5°C were also given depending on maturity, ripeness, and cultivar. Medlicott and co-workers[136] reported that storage at 12°C caused limited ripening of the fruits, whereas storage at the higher temperature of 25°C caused no detrimental effect on full ripening after 21 days of storage. On the other hand, Campbell and co-workers[178] reported that carambolas (var. "Arkin" and "Golden Star") can be successfully stored at 5°C for at least 3 weeks without significant changes in soluble solids, weight, or organic acid concentration. The relative humidity of the storage should be kept between 85 to 90% to reduce water loss and prevent shriveling.[74] Rewarming the fruit at 23°C after storage for 6 days may still induce normal ripening without chilling injury.

Appropriate Transport

The following suggestions for transporting fresh produce have been recommended by FAO[1] to minimize heat accumulation and losses due to increased respiratory activity: (1) in transporting fresh produce, closed vehicles without refrigeration should not be used except for local deliveries; (2) open-sided or half-boarded trucks can be fitted with a roof or canvass roofing and siding to protect the produce from the sun and wind; (3) a second white painted roof may be fitted 8 to 10 cm above the main roof to act as radiation shield; (4) for long-distance travel, vehicles without refrigeration should have elaborate air intakes which can be fitted in conjunction with louvers to ensure positive airflow through the load; and (5) transport vehicles like trucks, rail cars, and sea containers should be equipped with refrigeration for long journeys. In Table 2.8, fruits are grouped for compatible refrigerated transport.

TABLE 2.8
Groups of Compatible Fruits for Transport in Mixed Loads

Group I (1.0–1.1°C; 90–95% RH)	Group II (12.8–18.3°C; 85–95% RH)	Group III (2.2–5.0°C; 90–95% RH)
Apples	Avocados	Cranberries
Apricots	Bananas	Lemons
Berries (except cranberries)	Grapefruits	Lychees
Cherries	Guavas	Oranges
Figs	Limes	Tangerines
Peaches	Mangos	
Pears	Olives	
Persimmons	Papayas	
Plums	Pineapples	
Pomegranates		

Data from Lipton, W. J. and Harvey, J. M., Compatibility of vegetables during transport in mixed loads, U.S. Dept. of Agriculture, Agric. Res. Serv. [Rep.] 51, U.S. Department of Agriculture, Agricultural Research Service, Washington, D.C., 1972.

TREATMENTS TO PROLONG SHELF LIFE OF FRESH PRODUCE

Waxing

Waxing is employed to make fruits attractive, prevent water loss and shrinkage, and, consequently, prolong shelf life. Waxes tend to create a condition similar to modified atmospheres. Waxing to prolong storage life of commodities has met with little success because of the difficulties in finding ideal waxing material to coat fruit with a uniform thickness.[180] Various citrus cultivars have responded adversely to waxing[181] because it affects the respiration and composition of the internal atmosphere which leads to off-flavor development. "Murcott" tangerine when waxed develops off-flavors regardless of rootstock origin, maturity at harvest, and storage conditions.[182] Cohen and co-workers[182] reported that weight loss after waxing was reduced but the sensory characteristics of the fruit were adversely affected. They found that waxing tangerine increased its internal carbon dioxide and ethanol and consequently produced off-flavor. In "Meyer" lemons, waxing was found to enhance development of Peteca rind pitting (severe, deep rind pitting) especially when polyethylene-based waxes were used.[79] This response was attributed to the physiological stress produced by increased CO_2 concentration.

Precooling

Many fruits and vegetables benefit from prompt cooling immediately after harvest. Low temperatures slow down metabolic processes such as respiration and transpiration and delay the development of postharvest diseases by inhibiting host ripening,

by prolonging disease resistance associated with immaturity, and by directly inhibiting the pathogen at temperatures unfavorable to its development.[133] Precooling is the first step in the handling of many fruits and vegetables. Hall[183] reported that in a tropical environment, a delay of 2 h between harvesting and cooling can reduce the shelf life of produce by a whole day. Dicecco[184] reported that mushrooms held at 10°C have only 25 to 50% the shelf life of those held at 1°C. Chen and co-workers[185] reported that bamboo shoots in Taiwan produced 14% more finished product when stored at 5°C for 2 days after harvest than when held at room temperature before processing. A 40% reduction in marketability of strawberries was caused by a 4-h delay at a holding temperature of 30°C.[45] If not cooled to –0.5°C within 24 h, "Bartlett" pears develop enzymatic breakdown known as "watery breakdown".[186] However, there are commodities that do not require prompt cooling, such as freestone peaches,[186] apples,[108] tomatoes,[130] and lemons.[116] The commodities do not rapidly deteriorate but develop symptoms of chilling injury upon storage.

Methods of precooling are forced-air cooling, hydrocooling, hydro-air cooling, and vacuum cooling, although room cooling and package icing are also common. Choice of the precooling method is dependent on the cooling needs and suitability of a commodity, type of packaging material used for the product, type of market and marketing system, and economic and other considerations. Commodities and packaging materials that cannot withstand soaking and immersion in water are not suitable for hydrocooling, hydro-air cooling, or package icing. Vacuum cooling can be used on commodities that have a favorable surface-to-mass ratio like leafy and flower-type vegetables, celery, some sweet corn, and bell peppers.[186] About 1% weight loss is incurred for each 6°C cooled in vacuum cooling.[187] Generally, room cooling can be used for all kinds of fruits and vegetables including strawberries and grapes, although it is not recommended for commodities that deteriorate rapidly. Yahia and Sanudo[61] recommended forced-air precooling of avocados to temperatures of 8 to 10°C. Prompt cooling of table grapes is crucial in preventing the softening of berries and weight loss of stems, which easily break when handled dry. Table grapes are precooled to about 2°C by forced air cooling,[45] which is also ideal for strawberries because of the short period needed. For sweet corn destined for the fresh market, hydrocooling is the method recommended,[188] where crates packed with 4 to 5 dozen ears pass through a spray of cold water. Half-cooling times of 28 min for sweet corn packed 5-ears deep in wire-bound crates have been reported. Storage life can be extended from 4 to 8 days as a result of precooling when maintained at a temperature of 0°C and relative humidity of 90 to 95%. Contact icing of loaded trucks also provides a means of precooling and minimizing quality losses during transportation. Brusewitz and co-workers[189] studied the different cooling methods to extend the shelf life of peaches. They also studied the effect of picking times on the measured variables like weight loss, impact parameters, and bruising. It was confirmed in their study that cooling at recommended conditions (temperature of 4°C and relative humidity of 92 to 94%) was beneficial for extended storage life and reducing weight loss and bruise damage that limit marketability of the produce. Evidently, peaches need humid cool air coupled with hydrocooling before storage to minimize deterioration. Peaches picked on hot afternoons were affected adversely

compared to those picked on cool mornings, when stored for long periods at below optimum humidity.

Use of Fungicides

To control the spread of pathogens from infected to non-infected crops during storage and transport, fungicides are used. Fungicides prevent fruit rots and decay in vegetables and fruits by controlling or killing *Rhizopus* spp. responsible for decay in stone fruits and berries, *Penicillium* spp. on citrus and pome fruits, and *Erwinia carotovora* (bacterial soft rots) on leafy vegetables and potatoes. Benomyl, thiabendazole (benzimidazole), and O-phenylphenate are the most common fungicides used in citrus. Potassium sorbate added to the standard fungicide treatments of citrus can reduce decay by as much as 35% beyond the level of protection provided by fungicides.[190] Thiabendazole-treated sawdust was reportedly used to pack fresh chestnuts to prolong storage life for at least 2 months.[191] By dipping commodities in hot water with fungicides, diseases can be effectively controlled. Rotting, browning, and weight loss in lychees stored at 20 to 30°C can be controlled by benomyl treatment (0.5% at 52°C for 2 min).[192] Kader[122] recommended a 5-min hot water (51.5°C) dip with 500 ppm of benomyl for mangos, papayas, pineapples, and bananas to prevent anthracnose, the most serious postharvest disease of tropical fruits. Methyl bromide (MB) treatment of nectarines for export to Japan was investigated by Harvey and co-workers[193] for control of codling moth. Treatment with 48 g MB/m^3 for 2 h at 21°C controlled codling moth (*Cydia pomonella* L.) and caused no significant phytotoxic response in the cultivars studied ("Summer Grand", "May Grand", "Fantasia", "Fibre Brite", "Red Diamond," and "Spring Red"). All fumigated nectarines were significantly firmer than those untreated after storage at 2.5°C for 7 days and they ripened satisfactorily. Likewise, soluble solids content was not affected by fumigation. Organic bromide residues were reportedly lower than the U.S. tolerance of 20 ppm. Fumigation with sulfur dioxide (SO_2) has been a standard practice for grapes to control decay caused by *Botrytis* spp. This is effectively controlled by fumigating grapes immediately after harvest at a concentration of 0.2 to 0.5%, and once every week during storage using a concentration of about 0.1%.[45]

Hot Water Treatment

Dipping the produce in hot water is carried out to control surface and skin infections.[2] Hot water treatment (HWT) has been successfully used to control pest and insect infestations in papayas,[194] mangos,[195] and "Brazilian" bananas.[196] Fresh cucumbers sometimes incur extensive heat damage when subjected to HWT causing surface pitting, rapid chlorophyll breakdown, and increased susceptibility to disease. This problem can be overcome if the fruits are first conditioned to 32.5 ± 0.5°C for 24 h[197] before hot water treatment at 45°C from 30 to 60 min., or at 46°C from 30 to 45 min, which is also the range required to kill fruit flies. Two stages of hot water treatment have been developed for papayas.[194] The first stage is the immersion of the fruit in water at 42°C for 30 min, the second stage follows immediately uses water at 49°C for 20 min. After HWT, the fruits are cooled with water spray at 20°C for 20 min

before being held in the cold store at 10°C. The incidence of hard and lumpy fruit is minimized. On the other hand, Harvey and co-workers[23] reported that in the Caribbean, quarantine treatment of papaya using HWT consists of a double dip in water for 20 min at 46°C followed by 20 min at 42°C. A 15 min, 50°C hot water immersion treatment was developed by Armstrong[196] to disinfest "Brazilian" bananas from Mediterranean fruit fly (*Ceratitis capitata* Wiedemann), melon fly (*Dacus cucurbitae* Coquillet) and oriental fruit fly (*Dacus dorsalis* Hendel). No detrimental effects on fruit quality and shelf life were detected. In "Arkin" carambola, conditions for hot water treatment were 43.3 to 43.6°C for 55 or 70 min, or 46.0 to 46.3°C for 35 or 45 min.[198] These conditions did not alter the quality of the fruits, except for the duller color of heat-treated fruits. Sharp[199] reported that HWT was effective in controlling Caribbean fruit fly (*Anastripha suspensa* Loew) in "Tommy Atkins" and "Keitt" Florida mangos. The temperature used was 46.1 to 46.7°C for a duration of 45 to 65 min. For Mexican mango cultivars, a temperature of 46.1°C was used for "Haden", "Oro", and "Ataulfo" with exposure times of 90, 75, and 90 min, respectively.[200,201] "Haden" was reported to be acceptable for 12 days without refrigeration and "Ataulfo" was acceptable for 14 days when stored at 11.1°C.

Mayberry and Hartz[202] carried out studies to extend the storage life of muskmelon for at least 28 days at 3°C by wrapping individual fruits with polyethylene bags and dipping the wrapped fruit in hot water at 60°C for 3 min. Hot water treatment provided adequate control of surface microorganisms and the polyethylene bags helped to minimize weight loss and the lengthened storage life derived from this treatment was beneficial to transport of fruits for export.

Moist Air Heat Treatment

Anthony and co-workers[203] used moist air heat treatment at a temperature of 52°C, relative humidity of 90 to 95% on nectarines which were either unwrapped or wrapped individually in plastic to prevent decay of the fruits. Heat treatments at 52°C for 15, 30, and 45 min slowed softening and ethylene production. Wrapping fruits in plastic reduced ethylene production by 75% and respiration by 12% but did not influence softening. The wrap reduced the incidence of skin browning due to heat treatment.

High-Temperature Forced Air (HFTA) Treatment

Armstrong and co-workers[204] developed a high-temperature forced-air (HFTA) disinfestation treatment against fruit fly for Hawaiian-grown papayas, using four temperature stages. The four-stage treatment forced hot air over papaya at temperatures of 43 ± 1, 45 ± 1, 46.5 ± 1, and 49 ± 0.5°C with a relative humidity of 40 to 60%. Exposure times for the first three stages were 2 h and the last stage was 1 h. Hydrocooling immediately followed until the fruit center temperature was 30°C or less. HFTA treatment did not affect fruit quality and was less injurious to papayas than double-stage hot water treatment or quick run-up vapor heat treatment.[204] Miller and co-workers[205] tried HFTA on "Tommy Atkins" mangos with some success. The treatment consisted of exposing mangos to air at a maximum of 51.5 ± 0.2°C for

125 min with an airflow of 0.4 m^3/s. The quality was not adversely affected with the treatment, although weight loss was higher.

Vapor Heat Treatment

Vapor heat treatment (VHT) is the heating the fruit with warm saturated air at temperatures between 40 and 50°C as a quarantine treatment to kill fruit fly eggs and larvae before shipment to fresh markets.[206] Heat is applied directly to the surface of the fruits by condensation of water vapor. This technology had been adopted for mangos due to the banning of ethylene dibromide (EDB) as a fumigant. Ilangantileke and Maglente,[207] who studied the applicability of VHT in controlling fruit fly in mango cv. Nang Klang Wan, reported that it was effective in preventing the hatching of eggs of *Dacus dorsalis* at time-temperature combinations of 48°C for 30 min or 46°C for 1 h. VHT reduced the firmness of mangos, in all time-temperature combinations and delayed the incidence of fruit decay. There was insignificant weight loss and better peel yellowing and the fruits had acceptable organoleptic properties. VHT has been used commercially in the Philippines to control oriental fruit fly on mangos exported to Japan. Carried out in a VHT chamber, the treatment consists of exposing the fruits to steam at 45.6°C for 10 min, then cooling the fruits by cold water and air to 5°C. Internal breakdown in the flesh of ripened mango was a problem in the initial stages of its use. However, it has been solved by carefully implementing the time-temperature regimes and is now routinely used for quarantine treatment. VHT has been used successfully with Florida grapefruit on a laboratory scale.[205] The treatment conditions were vapor heat for 5 h at 43.5°C and relative humidity of 100%. Reduction of peel pitting after storage at 10°C for 4 weeks and 21°C for 1 week was an obvious benefit of VHT. No peel discoloration or rind breakdown was observed. Quality parameters of "Arkin" carambolas were not affected after vapor heat treatment using temperatures of 43.3 to 43.6°C for 90 or 120 min or 46.0 to 46.3°C for 60 or 90 min. Hallman[198] noticed that VHT caused duller color and weight loss was greater after treatment at 46.0 to 46.3°C.

Refrigeration

Fruits and vegetables are usually stored above their freezing point and at temperatures above chilling injury to maintain their freshness. Recommended storage conditions for potatoes vary according to the end use. Table potatoes are stored at temperatures of 4 to 7°C temperature and relative humidity of 95 to 98%. Processing potatoes, i.e., chip potatoes, are stored at 10 to 12°C and 95 to 98% RH. Seed potatoes are stored at 0 to 2°C at 95 to 98% RH.[128] The recommended airflow is 24 to 32 $m^3/h/ton$, which is sufficient to maintain a good temperature distribution in the bulk store and flush out CO_2. Recommended storage condition of guava for an assured storage time of 2 to 3 weeks is at temperatures between 5 to 10°C and relative humidity of 90%.[208] Vasquez-Ochoa and Colinas-Leon[138] reported that the storage condition for guava is at 7°C and 80% relative humidity which can be stored for 3 weeks at the color-turning stage when harvested. The recommended storage

temperature of carrots is 0.6°C with a relative humidity of above 90%.[209] Storage at high temperature of 21.1 to 26.7°C can cause a weight loss of 10% per day[210] with a marketable shelf-life of only 2 days. At 10°C the weight loss rate is about 1% per day with a marketable shelf life of 2 to 3 weeks. The production of the bitter tasting compounds isocoumarin and eugenin can be minimized at temperatures near 0°C.[7,211] As for most vegetables, at least 90% relative humidity must be maintained during storage to avoid weight loss, which can lengthen the storage life of carrots by up to 50%.[212] The recommended storage and transit temperature of fresh sweet corn is at 0°C and 95 to 98% relative humidity, since its high respiration rate is one of the highest of all fruits and vegetables.[208] A common postharvest handling problem for sweet corn is the loss of sweetness due to a decrease in sugar content, which is four times less at 0°C than at 10°C.[213] Partial dehusking and shrink-wrapping of the produce resulted in lower respiration rates and loss of sweetness.[214]

Blanching is carried out on peas prior to further canning, freezing, or dehydration to inactivate enzymes. Common blanching temperatures are 85 to 96°C, while blanching times are from 2 to 5 min depending upon size and maturity.[71] Blanching may cause losses of as high as 36% of the ascorbic acid of peas. The higher blanching temperatures with shorter blanching times are used to minimize these losses. Damaged peas lose more ascorbic acid during blanching due to oxidation.[215] Other vitamins lost during blanching are thiamin, niacin, and riboflavin. Rapid freezing of peas is important to stop deteriorative changes that may develop after harvesting the crop. Frozen peas are now the common form by which peas can be bought on the retail market. Freezing can be done by individual quick-freezing (IQF) on a fluidized bed freezer.

NEEDS FOR FURTHER RESEARCH AND DEVELOPMENT

Little information is available for the degree of care required when handling fruits and vegetables. The often repeated instruction "to handle agricultural products like eggs" is sometimes irrelevant. According to Liu,[180] due to the different characteristics of fruits and vegetables, care in handling may vary considerably. Handling products more gently than eggs may apply to strawberries and raspberries but not to commodities that possess tough rinds. Extra care and precautions taken in handling are expensive, which may not be necessary for some commodities. Information on the level of compression, abrasion, and impact forces a commodity can withstand and practices that can cause damage is needed. Such information could be used to design appropriate packaging materials, handling equipment, and systems without unnecessarily adding costs, and for instruction of workers in the field and packinghouse for proper care in harvesting and handling without unnecessarily adding costs.

There is also a need to develop rapid and reliable non-destructive methods for detecting damage in order to prevent unnecessary wastage due to sampling and the resultant condemnation of packages. Present sorting or grading operations require substantial manpower in removing injured or diseased products. Moreover, manual sorting or grading is unreliable as it is subject to human errors. Rapid and objective means of detecting damage and sorting are needed to maintain product quality and enhance shelf life.

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3 Irradiation

G. Blank and R. Cumming

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INTRODUCTION

Overall, treatment of food commodities with appropriate levels of ionizing radiation is recognized to have various benefits.[1] From a microbiological standpoint the advantages primarily include extending the shelf life and increasing the safety of foods by eliminating and/or reducing microorganisms and parasites that contribute to spoilage and or disease. In addition, non-microbiological advantages include the delaying of ripening in fruits and vegetables and a decrease in the deterioration of quality in stored tuber and bulb crops by preventing postharvest sprouting. Also, radiation has had a long-standing reputation as an effective method to control pest

and vermin in cereals. In 1981, the United Nation's Food and Agricultural Organization (WHO) endorsed irradiation doses up to 10 kGray, as a major technology for the prevention of foodborne illness and for the reduction in food losses due to spoilage by microorganisms and vermin. Ionizing radiation is now approved for use in more than 41 countries for over 35 specified foods, and the list is growing.[2] Approximately 26 countries currently employ radiation on a commercial scale for food application.[3] Over the last 10 to 15 years there has been a tremendous momentum of research focused on the use of irradiation and foods. Pertinent worldwide data, both published and unpublished, have been reviewed extensively and it has been concluded that foods which are approved for radiation treatment pose no greater health risks with regard to their radiological, toxicological, and microbiological safety and are as wholesome in terms of nutritional adequacy as those treated using conventional intervention strategies.[4-7]

In this chapter the use of radiation will be examined specifically as it relates to the shelf life extension of foods. For the most part this will involve an overview of its use in reducing the level of spoilage microorganisms in food commodities and altering postharvest ripening and senescence of fruits and vegetables. Many pathogenic microorganisms including spore formers such as *Clostridium botulinum* are also known to contribute to food spoilage. Although the presence and survival of pathogens in foods is of great importance from a health hazard standpoint, their involvement will be discussed mainly in reference to food deterioration leading to shortened shelf life. In addition, the use of radiation in combination with various other preservative techniques, including heat and modified atmosphere packaging (MAP), will be reviewed.

IRRADIATION

Irradiation Resistance of Microorganisms

Overwhelming evidence has been brought forward to indicate that the shelf life of most foods can be extended safely by radiation treatment. In part, this is due to a reduction in the original number of spoilage microorganisms associated with the food.[8,9] Factors affecting the radiation resistance of microorganisms in foods are therefore of paramount importance especially considering that maximum dose rates are mainly influenced by sensory attributes. The irradiation resistance or sensitivity of a microorganism is commonly given as the D_{10} dose. This is the dose that is required to kill 90% of a population. The radiation dose unit, previously referred to as the rad, is currently known as the Gray (Gy). The Gray is the absorption of 1 joule of energy/kg irradiated material and is equivalent to 100 rads; 1 krad equals 10 Gy and 1 Mrad equals 10 kGy. The irradiation resistance of spoilage microorganisms, some of which are also pathogenic, varies and is dependent on several factors.[10,11] These factors should be considered prior to the application of treatment in order to maximize preservative benefits. For example, foods containing high populations of radiation resistant bacteria such as *Deionococcus* (previously referred to as *Micrococcus*: *M. radiodurans*) and the psychrothrophic *Moraxella-Acinetobacter* group require relatively high application dosages. Their D_{10} values can be greater than

10 kGy.[12] Yeasts and lactobacilli also display unusual resistance and are particularly troublesome when present initially in large numbers in dried or vacuum/modified atmosphere packaged (MAP) foods.[11,13] Fungal and, in particular, bacterial spores are very radiation resistant and exhibit D_{10} values between 1 and 10 kGy.[12,14-18] In contrast, the majority of spoilage bacteria including *Pseudomanas* are not highly radiation resistant and are easily destroyed by relatively low doses; D_{10} values for most species are usually below 1 kGy.[8,10,19] Most fungi are equally radiosensitive.[20,21]

In most instances the radiosensitivity of microorganisms is inexorably linked to the nature or complexity of the food in which treatment occurs. The pH, fat, protein, and moisture contents or water activities of various foods have been shown repeatedly to either enhance or reduce radiation effects on microorganisms; natural or added preservatives and or inhibitory compounds exert similar effects. Excellent reviews covering this topic are available.[8,11]

Briefly, some additional factors that have been reported to impact on the irradiation resistance of microorganisms in foods include:

1. Nature of the target microbe (bacterium, yeast, mold, virus, parasite; genus, species, strain; Gram positive or negative; sporeformer, vegetative cell; size and arrangement of DNA; age or phase of growth).[11,22-25]
2. Initial number of microorganisms.[12,26,27]
3. Physical state of the food: frozen vs. unfrozen; liquid vs. solid.[22,25-26,28-34]
4. Oxidation-reduction potential in and surrounding the food.[13,35-38]
5. Applied dose.[37,38]

Classification of Radiation Processes and Sources

In the food industry, three principal radiation processes, classified on the basis of applied dosage can be used to prolong shelf life.[39]

1. Radappertization: High dose, also referred to as radiation sterility. Applied dosages of radiation are in the range of 30 to 50 kGy and are designed to eliminate most if not all microorganisms in a food. Results are similar to those achieved when canning low acid foods.
2. Radicidation: Medium dose, typically in the range of 1 to 10 kGy, is applied to foods with the primary intention of eliminating all non-sporeforming pathogens including salmonellae and listeriae. Spoilage microorganism levels are drastically reduced. This process can be considered equivalent to thermal pasteurization. Parasites in meat including *Trichina spiralis* in pork will also be destroyed. This does not apply to viruses. Radicidation doses are frequently applied to frozen food products.
3. Radurization: Low dose. This process can also be considered equivalent to heat pasteurization. It is sometimes referred to as cold pasteurization, implying that the process kills microorganisms without participation of heat. Typical doses from 0.4 to 2.5 kGy are employed during treatment of foods in order to substantially reduce the presence of specific spoilage microorganisms and or to delay ripening and senescence thereby extending its usefulness

or keeping quality. Foods receiving these dosages are usually unfrozen. From a standpoint of shelf life extension of refrigerated foods including meat and fish, the most important and sensitive bacterium is *Pseudomonas*.

Gamma rays, electron beams, and X-rays are the most common sources of ionizing radiation. Of the three, gamma rays from isotopes including ^{137}Cs and in particular ^{60}Co are most frequently used in the food industry.[4,11] The energy that is absorbed per unit time is referred to as the dose rate. Gamma ray sources, in comparison to electron beam generators, provide a relatively low dose rate. For example, average doses of 100 to 10,000 Gy/h are typical.[11] Therefore, products to be gamma radiated generally require a longer exposure time in order to achieve a specified absorbed dose. Electron beams, which are produced by linear accelerators powered by electricity, require considerably shorter exposure times, typically seconds or minutes. However, the effective penetration range of an electron beam depends on its energy level. For treatment with foods, 10 MeV has been set as the upper limit.[2,40] Compared to gamma rays the depth of penetration is usually lower, about 5 cm if treated on one side or 10 cm if treated on both sides.[11] Successful application of electron beam treatment is therefore limited by the thickness of the food to be treated and would be appropriate for topical use especially with fruits. For other whole foods, including eviscerated poultry carcasses, gamma radiation would be more suitable since the higher penetration rays would reach the cavity which is a known repository for both spoilage and pathogenic bacteria.

IRRADIATION TREATMENT OF FOODS

Dairy Products

Cheese

Extending the shelf life and/or sterilization of dairy products for immunocompromised patients using radiation treatment is not a widely accepted practice. The main reason for its limited use is that ionizing energy, through the formation of radiolytic products especially in high lipid-based foods, generates unacceptable off-odors and flavors via oxidation, polymerization, decarboxylation, and dehydration reactions even at low doses.[22,41] In particular, polyunsaturated fatty acids are prone to oxidation by free radicals produced during treatment. In addition, oxidation of casein and the production of methyl radicals has been shown to result in the generation of "wet dog" off-flavors.[42] These chemical reactions, to some extent, can be reduced if the products are initially frozen and/or treated in an environment with limited water, light, and oxygen. Yet, despite these shortcomings, low-dose radiation for the specific purpose of extending dairy product shelf life does hold promise. In such applications, the treatment should be considered as supplemental or complementary with use of other preservation techniques including refrigeration and/or preservatives such as sorbic acid.

Bongirwar and Kumta[43] reported that Cheddar cheese developed off-flavors when irradiated at 0.5 kGy; however, none was detected when the dose was reduced to 0.2 kGy. A dose greater than 1.5 kGy, when applied to Turkish Kashar cheese, not

only resulted in off-flavor development but also contributed to color deterioration.[44] By decreasing the dose to 1.2 kGy the sensory problems were eliminated and the mold-free shelf life was extended 12 to 15 days when stored at room temperature. In contrast, non-irradiated cheese became moldy within 3 to 5 days. When combined with refrigeration storage, radiation increased the shelf-life period of the cheese five-fold. With Gouda cheese, however, no taste difference was reported between irradiated (3.3 kGy) and non-irradiated samples.[45] Similar taste findings were reported in a study that evaluated the acceptability of irradiation sterilized dairy foods for immunosuppressed patients.[46] In this study, a Gouda-based process cheese was initially frozen to –78°C and then gamma irradiated at 40 kGy. Although mozzarella cheese was similarly treated, the result of sensory evaluation was far less favorable. Interestingly, both cheeses maintained their characteristic mouth-feel properties despite being frozen. In addition, the relatively high treatment dose resulted in only slight color changes. It should be pointed out that while higher doses are required for sterilization purposes, the product once treated has an indefinite shelf life from a microbiological standpoint, provided of course that sterility is maintained. For Camembert cheese, flavor changes described as burnt or musty first became noticeable when treated with 0.30 kGy.[47] In order to stabilize the cheese by preventing additional growth of *Penicillium roqueforti*, a minimal dose of 2.0 kGy was recommended. Results from a subsequent study, however, reported that full fat Camembert cheese suffered no off-flavor development up to a dose of 3 kGy;[48,49] and that treatment at 2.5 kGy was sufficient to eliminate initial populations of 10^3 to 10^4 colony forming units (cfu)/g of the pathogen *Listeria monocytogenes*.[50] In contrast, flavor changes were quite noticeable when radiation treatment was applied to cottage cheese, the minimal threshold dose being 0.75 kGy.[47] At this dosage the cheese was described as having a slight bitter, cooked, or foreign taste. However, in order to reduce spoilage by psychrotrophic bacteria by at least three logs, the applied dose would have to be nearly doubled.[47] This resulted in cheese with a definite burnt off-flavor. Using electron beam irradiation and doses of 0.21 and 0.52 kGy, the shelf life of vacuum packaged cheddar cheese at 10°C containing 10^1 cfu cm^{-2} *Aspergillus ochraceus* spores was extended by approximately 42 and 52 days, respectively.[51] Under similar conditions, inoculation of cheese with *Penicillium cyclopium* spores resulted in shelf-life extensions of only 3 and 5.5 days, respectively. Overall, the efficacy of the treatments was shown to be heavily reliant on the irradiation resistance of the target microorganisms and the temperature of storage. Increasing the post-irradiation storage temperature from 10 to 15°C, for example, decreased the extension in shelf life. Although these results are to be expected, based on the mesophilic nature of the target fungi, the increase in temperature may also have contributed to more efficient repair of injury by the survivors.

Other Dairy Products

Sterilization of yogurt bars, ice cream, and nonfat dry milk by gamma irradiation using a dose of 40 kGy at –78°C resulted in an overall decrease in acceptance.[47] Although the use of MAP or the inclusion of antioxidants appeared to reduce the level of off-flavors, the effects were product specific. Irradiation of fluid milk also resulted in unacceptable flavor scores.[52] Off-flavors and browning originating from

chemical reactions involving lactose were identified.[52] Irradiation preservation of yogurt was similarly investigated.[44] Left at room temperature, plain yogurt reached a population of 10^9 cfu/g by 6 days and was judged unacceptable; however, when treated with gamma irradiation using a dose of 1 kGy this population level was not reached until 18 days of incubation. Irradiation combined with refrigeration further extended the shelf life of yogurt to 29 to 30 days. In comparison, the shelf life of the refrigerated controls was only 15 days.

POULTRY

Chicken and Turkey

Radiation treatment of poultry products as low as 1 kGy is currently allowed in several countries. In the U.S., fresh or frozen poultry and further processed poultry products including mechanically separated meat can be radiated to an absorbed dose between 1.5 and 3 kGy.[53,54] If the products are intended for packaging, the film must be permeable to oxygen in order to reduce possible growth and subsequent toxin production by *Clostridium botulinum*. In the Netherlands the maximum dose approved for poultry is 3 kGy, while in Israel and South Africa treatment doses as high as 7 kGy may be used.[55] The latter dose is sufficiently high to eliminate salmonellae in fresh poultry; however, flavor problems invariably arise.[55] Threshold doses of 2.5 and 1.5 kGy have been reported for chicken and turkey, respectively, following radiation at temperatures of 5 to 10°C in plastic bags.[9,56,57] For this reason, doses higher than 2.5 kGy are normally reserved for application to frozen poultry products since reduced available moisture is known to render microorganisms more radiation resistant and interaction of free radicals generated during treatment with food components is largely constrained.[11,58,59]

At or below these dosages, however, there is no guarantee of producing salmonellae-free carcasses.[60] Despite this lack of assurance, a 2-kGy dosage should be sufficient to adequately reduce or even eliminate salmonellae in poultry provided that production and processing was performed under good production and manufacturing practices.[55] Indeed studies have provided data to suggest that treatment at this dose not only eliminates salmonellae from chicken wings and mechanically deboned chicken meat[61] but also reduces the initial microflora on whole carcasses by 99%.[62,63] At a dose higher than 2.5 kGy, over 99.9% of the initial aerobic plate count can be destroyed.[64] Radiation treatment of poultry is currently not allowed in Canada or Great Britain. An expanded list of countries that currently allow radiation treatment for poultry and poultry products is given by Farkas.[9]

Under normal processing conditions freshly eviscerated and chilled whole chicken carcasses will have a shelf life that is dependent primarily on both the microbial load, principally *Pseudomonas* levels, and the storage temperature which typically ranges from 2 to 4°C. Although the numbers and types of psychrophilic microorganisms found on carcasses immediately after processing varies, they are usually less than 10^4 cfu/cm^2 and may be as low as 10^2 cfu/cm^2.[47,65] When the population of spoilage microorganisms on polyester tray or polyethylene bag-packaged carcasses reaches levels of about 10^7 to 10^8 cfu/cm^2, unpleasant off-odors,

TABLE 3.1
Extension of Shelf Life in Poultry

Product	Dose (kGy)	Storage Temp (°C)	Shelf Life/Evaluators	Ref.
Carcass	2.5	1	11-16 d/odor	91
Leg meat	3.6	2	c. 2 weeks/cooked taste	91
Breast	3.8	1	c. 3 weeks/cooked taste	91
Carcass	1.5	5	6–7.5 d[a]	59
Carcass	2.5	5	15 d[a]	59
Carcass	8.0	2	40 d[a]	59
Breast	2.5	2	22 d/odor, flavor, tenderness	69
Carcass	5.0	5	c. 16–20 d/microbiology, visual, odor	90
Carcass	7.0	5	c. 19–24 d/microbiology, visual, odor	90
Breast[b]	2.9	2	c. 8 weeks/microbiology, odor, flavor	82

[a] Evaluation parameters not indicated.

[b] Sous-vide prepared.

From Andrews, L.S. et al., Food preservation, *Rev. Env. Contaminant Toxicol.,* 154, 13, 1998. With permission.

discoloration, and slime develop. At this point the carcasses have reached an incipient spoilage level and are deemed unacceptable.[47] Both pigmented and non-pigmented pseudomonads, which represent a small portion on the initial microflora are responsible for ultimate spoilage especially at temperatures below 5°C.[66] The off-odors, which are normally detected prior to slime formation, primarily consist of volatiles including sulfides, esters, and ketones.[67] Maintained at temperatures of 4, 1, and 0°C, freshly eviscerated carcasses have a shelf life of 6, 8, and 10 days, respectively.[59,63] In contrast to cold storage, which functions primarily by inhibiting or retarding microbial growth, irradiation treatment can be targeted to eliminate and/or reduce the presence of specific spoilage microorganisms. In particular, *Pseudomonas* and members of the *Enterobacteriaceae* are easily eliminated or drastically reduced not only from poultry but also from other muscle-based foods including beef, pork, and fish.[36,68,69] The net result is an enhanced extension in shelf life (Table 3.1) especially if proper post-irradiation storage conditions such as temperature are maintained.[47,59,63,70] In addition, many if not all non-sporeforming pathogens including *Salmonella*,[25,36,59,60,68,71-75] *Escherichia coli*,[77,78] and *Campylobacter*,[36,79,80] can be destroyed. Psychrotrophic pathogens including *Listeria*,[26,28,36,81-84] *Aeromonas*,[85] and *Yersina,*[36,80,86] which are of more immediate concern, especially during protracted refrigerated storage, may also be expected to be eliminated. Additional information on pathogen control in foods by radiation is provided in reviews by Farkas[8] and Monk et al.[9]

Early studies reported that spoilage of poultry generally occurred within one week of slaughter even if the carcasses were refrigerated at 2 to 4.4°C.[87] Following a 7.4-kGy dose, however, the shelf life was extended fourfold using similar storage

conditions. One of the main drawbacks when using these relatively high-dose treatments, however, is the production of flavor defects which can be minimized somewhat only if the carcasses were frozen during treatment. Using a storage temperature of about 1°C, McGill et al.[88] reported that when fresh whole carcasses were packaged in polyethylene pouches, spoilage was observed after 11 days. This coincided with a total plate count of about 10^6 cfu/cm^2. In contrast, carcasses that were radiated using pasteurization doses of about 0.93 kGy reached total plate counts of only 10^2 cfu/cm^2 after 11 days, and indications of spoilage were not detected until 20 days of storage; overall, the shelf life was extended by at least 5 days compared to the controls. Noticeable off-odors at this dose were not detected by the taste panel. Higher treatment doses were even more effective in retarding microbial growth; however, under these conditions rancid-like odors were detected by the taste panel especially after 16 days of storage. It was concluded that a proper storage temperature, regardless of treatment dose, was paramount if optimum shelf life extension was to be achieved. Additional studies using higher application doses confirmed that shelf life extension and survivor levels were dose related.[62,89,90] For example, irradiation of whole carcasses using 5 and 7 kGy extended the shelf life at 5°C to about 16 to 20 and 19 to 24 days, respectively[90] (Table 3.1). In a recent study, Abu-Tarboush[63] reported that when whole carcasses were packaged in polyethylene bags and irradiated using doses from 2.5 to 10 kGy in plastic boxes containing crushed ice, the lag phase of the total aerobic count could be extended to 6 days at 4°C. From a microbiological perspective this represented a 12-day extension in shelf life. In comparison, no lag phase was observed in the controls. Extending the lag phase for psychrotrophic microorganisms was far less favorable except at the highest dose. No objectionable odors were detected by the trained panel for either the raw or cooked product. Following cooking, which consisted of boiling samples of breast and thigh for 12 min, the panel further failed to detect noticeable changes in appearance, texture, and taste. It was recommended that a dose higher than 2.5 kGy had little benefit in regards to shelf life extension and pathogen elimination. At a dose of 2.5 kGy, Basker[91] reported that whole chicken carcasses maintained at 1°C had a shelf life, based on odor development, ranging from 11 to 16 days.

Treatment dose and storage temperature are known to have a profound influence on the nature or types of survivors; therefore, differences in spoilage patterns including off-odors can be expected. Generally following low-dose (≤2.5 kGy) treatment *Moraxella*/*Acinetobacter*, *Serratia*, yeasts, and lactic acid bacteria including *Lactobacillus* and *Micrococcus* tend to survive in varying levels.[90,92-95] In treated foods stored at refrigeration temperatures under vacuum or 100% CO_2 (anaerobic modified atmosphere packaging), however, lactic acid bacteria normally predominate.[95,96] Additional Gram positive bacteria including *Brochothrix thermosphacta* and *Microbacterium* spp. may become problematic especially during extended storage.[94] Interestingly, *Lactobacillus* spp. and the radiation resistant bacterium *Moraxella phenylpyruvica* were more easily destroyed if treated under vacuum or in 100% CO_2.[36] Although yeasts are generally more radioresistant compared to vegetative bacteria, they make up only a minor fraction of the spoilage microflora of poultry and other meat products; however, following radiation *Candida* and *Saccharomyces* have been reported to contribute to surface discoloration.[11,63]

Synergy involving chemical pretreatment of carcasses and radiation has also been examined as a means of extending shelf life. In one such study, carcasses were dipped in either fermented whey (thermophilus whey) or a 1% lactic acid solution and radiated with 2.5 kGy at 3°C.[61] Although the numbers of Gram negative bacteria, *Yersinia* and *Campylobacter* in this study, were significantly lower in radiated vs. non-irradiated samples, no differences were observed among the pretreatments, which included water dipping as a control. However, the proportion of salmonellae-positive contaminated carcasses did decrease from 67 to 20% as a result of whey pretreatment. Overall, using Gram negative plate counts of 100 million per carcass as an index of spoilage, the shelf life of the controls was extended an additional 9 days as a result of radiation treatment.

Further Processed Products

Using canned minced chicken meat Thornley[93] reported that storage life could be extended from 1 to at least 4 weeks following treatment with 2.5 kGy. From a sensory point of view, however, the product appeared pinkish and transparent and was accompanied by a distinct odor that was not attributed to microbial growth but rather to the treatment itself. However, following cooking the taste panel found it difficult to distinguish the treatment sample from the control. How the product is cooked following radiation treatment may therefore have an influence on whether or not odors are detected. For instance, in several studies experienced taste panelists could not detect radiation odors on roasted chicken which had been previously treated at 7 to 8 kGy;[98,99] however, when chicken was radiated at a lower dose of 1.25 to 5 kGy and steam-cooked, the taste panel could readily identify off odors.[89] Pinking and or the development of a red color was also reported following treatment with 1.25 to 8.0 kGy of whole eviscerated chicken and turkey during refrigerated storage.[89,98] The mechanism for its appearance is unknown but presumably is non-microbial. Irradiation of fresh or frozen tray-packed, cut-up fryer chicken shortly after processing using a dose of 5 kGy significantly delayed growth of spoilage bacteria during storage at 4.4°C for 21 days; however, objectionable off-odors were still detected 2 to 4 days post-irradiation.[100] Decreasing the dose to 1 kGy eliminated odors but did little in extending the shelf life of the product. An intermediate dose of 3 kGy was finally recommended for shelf life extension since off-odor development was minimal within the 14 days of storage; overall a 21-day shelf life was achieved. Chicken breasts irradiated using cobalt-60 at a dose of 2.5 kGy and stored at 2°C resulted in a two log reduction in aerobic bacteria counts. A level of 10^6 cfu/g, which coincided with initial signs of spoilage, was reached 19 days post-slaughter.[69] Combined with refrigeration, the gamma treatment was shown to extend the shelf life of the samples by at least twofold over the controls. At this dosage off-flavors and odors were not detected by a trained sensory panel for up to 22 days of storage; higher treatment doses resulted in lower flavor and acceptability scores. In addition, the panel reported that tenderness was significantly lower in the radiated samples. The increased firmness of the treated samples was correlated to reduced water holding capacity and increasing the treatment dose from 3.5 to 4.5 kGy further decreased the water holding capacity of the samples from about 72 to 68%, respectively.

This resulted in a further loss of tenderness compared to controls. In contrast, when skinless and boneless chicken breasts were preserved using sous-vide and electron beam radiation (2.9 kGy) moisture levels were not affected.[82] Neither appearance nor tenderness of the samples was evaluated; however, based on microbiological results, the application of radiation with sous-vide preservation compared to sous-vide alone resulted in an additional 2-week extension in shelf life at 2°C. Doses from 1.1 to 2.9 kGy contributed to off-odors which were detected immediately upon opening the package. Although the intensity of the off-odors appeared dose related, they quickly dissipated and ostensibly originated from interactions arising between the packaging material and the treatment. From a product safety standpoint, the combination of sous-vide heating and radiation appeared more effective in reducing listeriae than either treatment alone.[82] These results are in agreement with studies which indicated that heating sensitizes pathogens including listeriae to radiation treatment.[83] Further, the sensory quality of tray-packaged chicken breast meat maintained at 1°C following radiation at 3.8 kGy was acceptable for three weeks.[91] In comparison packaged leg meat treated at 3.6 kGy and stored at 2°C maintained eating quality for only 2 weeks. Although Katta et al.[62] reported that doses from 1.5 to 2.0 kGy resulted in a significant shelf life extension of refrigerated poultry, Basker[91] indicated that the use of higher dosages might better inhibit microbiological growth particularly on leg meat. Differences in the pH between leg (6.4 to 6.7) and breast muscle (5.7 to 5.9) have been reported and are known to result in distinct microbial growth patterns.[66]

Fish

Fish and Fishery Products

Compared to other food commodities, sensory evaluation of fish and seafood in terms of quality and or freshness is mainly judged subjectively. Therefore, any intervention treatment used to extend the shelf life must not diminish the characteristic appearance, odor, texture, and taste of the product. Numerous tests have been evaluated in order to assess fish quality. These include chemical: trimethylamine, total volatile bases, measurements of oxidative rancidity; physical: electrical properties, pH, Eh, texture, water-binding capacity; and microbiological: standard plate count, psychrotrophic count, coliform count methods.[101] Overall, however, no single test has been shown to be useful when judging fish quality, although in some cases, for specific situations or for a limited number of species or products, taste and odor appear the most crucial. Low-dose radiation treatment, particularly in the range of 1 to 5 kGy, is especially effective in further extending the refrigerated shelf life of various fish and fish products by reducing at least 90 to 95% of the original spoilage microflora. Fresh fishery products processed in plants adhering to good manufacturing practices would be ideal for treatment. Conversely, stale fish or fish undergoing visible spoilage would receive minimal benefit from such treatment. Radiation preservation when applied to fish has also been demonstrated to be an effective treatment against specific pathogenic bacteria.[102] By eliminating and/or reducing pathogens, radiation treatment can provide consumers with an increased margin of safety especially for shellfish and raw fish products including sushi, which are routinely consumed

raw and or with minimal heating. The greater variability in chemical composition, particularly fat content and color of the various species of fish and shellfish, however, makes radiation treatment much more discerning.

Some of the earliest research performed on fish preservation, which included mackerel and haddock fillets, utilized high-voltage cathode rays at dosages sufficient to result in sterilization.[103,104] Although the storage life of the products was extended, based on bacteriological and chemical analysis, off-flavors were invariably reported. Generally the occurrence of irradiated induced flavors and odors intensified as the dosage was increased and therefore appeared dose-related. Subsequent research focused on identifying specific spoilage bacteria which were involved in the production of off odors during growth on non-irradiated refrigerated fish.[105,106] Using low-dose (2 kGy) radiation, Maclean and Welander[106] demonstrated that many odor-generating bacteria could be eliminated. One spoilage bacterium, *Micrococcus*, however, appeared to be very resistant to this type of treatment.[106,107] In an effort to decrease the dose required to extend the shelf life of various fish, particularly high fat containing types such as mackerel and herring, the addition of an antibiotic, oxytetracycline, was investigated.[105] For low-fat fish such as sole fillets, the combination of beta radiation treatment using the lowest (0.118 kGy) or intermediate dose levels with the antibiotic (5 ppm) had a similar effect on spoilage retardation as did the radiation treatment alone using the highest dose (2.8 kGy).[105] With medium and high fat containing fish, spoilage at 5.5°C was complicated by the onset of rancidity, ostensibly non-microbial in origin. It was suggested that in order to realize a significant extension in the refrigerated shelf life of high-fat containing types including salmon, inclusion of an antioxidant should be considered. In addition, when higher doses were utilized (3.0 kGy) with salmon, color bleaching was observed. Increasing the dose to 10 kGy resulted in a brown coloration which was also reported by Groninger et al.[108] However, low-fat white-flesh fish such as halibut was much less affected both in regards to color loss and textural changes. Nevertheless, flavor differences in both types of fish were detected when compared to the non-irradiated controls.

Anticipating the possible commercialization of radiation for the preservation of fish, Miyauchi[109] screened raw fillets of Pacific cod for sensory changes. Dosages from 2.3 to 7 kGy resulted in samples having a slight radiation odor and flavor which was described as burnt or scorched. Increasing the dose to 9.3 kGy resulted in samples that were borderline of acceptability. Based on scores provided by a taste panel, the control sample had a storage life from 1 to 2 weeks. Treated samples exposed to a maximum dose of 7 kGy were acceptable following more than 3 but less than 6 weeks of refrigerated storage. Overall, the optimum dose for radiation appeared to be 4.6 kGy or lower. When maintained at refrigeration temperatures, the shelf life could be extended threefold over the controls. Total plate counts (Table 3.2) and concurrent chemical analysis, which included total volatile base, acid number, and trimethylamine concentration, were not reliable indicators of acceptability. In part this may be due to the fact that only a certain portion of the microbial survivors remained active during spoilage or were specifically involved in producing off-odors. In contrast when yellow perch fillets were radiated and maintained at approximately 1°C, microbiological and chemical analysis closely correlated with taste panel scores.[110] Following exposure to 3 and 6 kGy, the storage

TABLE 3.2
Time Course Aerobic Plate Counts on Fish and Seafood Following Radiation

Product	Storage Temp (°C)	Storage Time	Treatment dose (kGy) Aerobic plate count (Log_{10} cfu/g)		
			0	**2**	**4**
Smoked salmon	2–3	0 (months)	5.46	3.54	2.99
		1	6.79	3.95	3.38
		2	7.87	5.23	4.04
		3	—[a]	6.94	5.25
		4	—	—	6.82
			0	**3**	**6**
Perch fillets	3	0–1 (days)	4.98	3.07	2.0
		5	8.23	—	—
		8	7.90	3.77	—
		12	8.79	5.04	2.77
			0	**2.5**	**5**
Crab	3.3	0 (weeks)	5.00	3.92	2.23
		1	6.67	6.14	3.62
		3	7.62	6.68	5.60
		6	8.25	7.07	6.11
		9	8.37	7.74	6.71
			0	**5.0**	**5**
Shrimp	3.3	0 (weeks)	3.65	—	—
		1	5.00	1.47	—
		3	7.43	3.46	—
		6	8.25	4.20	2.50
		9	8.41	—	—

[a] Not determined

Adapted from Hammad et al.,[123] Scholz et al.,[128] and Emerson et al.[110]

life was extended approximately four- and fivefold, respectively, at 5 to 6°C; however, shelf life was reduced by approximately 50%. By decreasing the storage temperature for hake from 4 to –7°C it was also possible to decrease the dose from 4 to 2 kGy and yet still maintain acceptable product.[111] Ampola et al.[112] similarly reported that when ocean perch, pollock, and cod fillets were radurized, the shelf life increased approximately twofold especially when the storage temperature was reduced from 5.5°C to 1°C. This finding demonstrated that radiation in itself is incapable of extending shelf life unless accompanied by strict temperature control which must be maintained during all phases of treatment, storage, distribution, and

retailing. The researchers also explored the possibility of vacuum packaging prior to radiation treatment and reported that lower doses using this regime could be used to achieve similar extensions in the shelf life for some species of fin fish. Ostensibly, radiation of fishery products under vacuum compared to air-packaging resulted in survivors with either higher diminished or altered microbial activity. A subsequent study on the benefits of packaging fish prior to radiation treatment was performed by Vengopal et al.[113] Eviscerated Indian mackerel immersed in crushed ice was radiated (1.5 kGy) and stored at 0°C. A sensory panel judged the product acceptable up to 20 days. Similarly treated fish which was packaged in polyethylene pouches and placed in crushed ice exhibited a shelf life of 25 days. It was concluded that for practical purposes, packaging of mackerel prior to treatment was not necessary as long as the fish was exposed to melting ice made from potable water. Overall, the use of a 1 to 2 kGy treatment for most species of fish including Bombay duck, cod, haddock, mullet, hake, and catfish did appear to impart off-odors and tastes especially if treatment was performed at temperatures of 1 to 4°C. The outcome of such treatments have resulted in shelf life extensions from 5 to 7 to about 14 to 20 days.[114-120] and were attributed to the destruction of specific spoilage microorganisms, particularly psychrotrophs such as *Acinetobacter*, *Moraxella*, and *Pseudomonas*.[119]

Since *Pseudomonas* is a strict aerobe, the use of MAP containing CO_2 would be expected to halt its growth. If used in concert with radiation, the combination treatment could result in a further extension of shelf life via increased efficacy or synergy and or a reduction in the radiation dose. Indeed such findings have been reported, albeit with mixed conclusions. In one study using cod fillets a combination treatment involving MAP (60:40%; CO_2:O_2) and a 1-kGy treatment resulted in a total shelf life of 24 days.[121] In comparison, the control and samples treated only with radiation had a total sensory shelf life of 10 and 19 days, respectively. Vacuum packaged and radiated fillets, however, had a total shelf life of 21 days indicating that the MAP treatment was of marginal benefit when used in combination with radiation. In all cases, sample assessment was based on raw odor and cooked flavor and texture. Increasing CO_2 levels to 80% also failed to have a significant impact on the shelf life extension of catfish fillets when used in conjunction with radiation at 0.5 to 1.0 kGy.[117] Steaming or cooking [121] and the use of chemical preservatives such as sorbates and benzoates[120,121] to control growth of surviving yeasts during product storage have also been assessed. Based on aerobic plate counts and chemical analyses including tri- and dimethylamine and hypoxanthine, it appeared that the sorbate-radiation treatment provided the longest (25 d) shelf life. In contrast, the control and air radiated sample had a shelf life of 10 and 19 days, respectively.[121]

Hammad et al.[123] reported when lightly salted (1.8%) cold-smoked (28 to 30°C) salmon was radiated at a dose of 4 kGy, despite favorable reductions in spoilage and pathogenic microorganisms aside from *Clostridium perfringenes*, a noticeable loss in color was observed. In this regard, the sensory panel reported that the normal cherry red color of the smoked fish faded to beige white. Only slightly lower scores were reported for taste and texture when compared to non-irradiated controls. Decreasing the dose to 2 kGy had no effect on color fading; however, the microbiological quality was reduced substantially. Overall, the researchers concluded that a maximum dose of 2 kGy could be used to extend the shelf life of these products

by about 3 months if maintained under refrigerated storage. In contrast, a previous study on the sensory properties of radiated smoked white fish (less fatty compared to salmon) concluded that dosages from 3 to 5 kGy had no adverse impact on flavor and texture.[124]

Although the sensory properties of dried sardine and flounder were not assessed following a 5-kGy treatment, Ito and Abu[125] reported that based on reductions in putrefactive microorganisms, such as *Pediococcus halophilus*, *Vibrio costicola*, and *Planococcus* sp. product shelf life in polyethylene pouches could be extended 2 to 4 times. This represented a storage period of approximately 2 months.

Fish mince, which serves as an intermediate raw material for the manufacture of various analog seafood products, is a further example of a processed fishery product that has been investigated using radiation.[126] Since a major portion of the processed fish mince is preserved by freezing, cryoprotectants including carbohydrates are frequently incorporated to reduce protein denaturation. The application of radiation was investigated as a means of pasteurizing the mince, thus eliminating the need for freezing and the inclusion of a cryoprotectant. Following treatment with 0.66 and 1.31 kGy a taste panel concluded that the shelf life of the radiated mince could be extended by about 12 and 18 days, respectively, when accompanied by storage at 3.3°C. Based on a cut-off of 10^6 cfu/g, a level established by the sensory panel which coincided with unacceptable product, the microbiological quality of the mince was extended by 6 and 13 days, respectively. However, a functional property of the mince, specifically the gel strength, decreased following treatment. This decrease appeared to be dose-dependent.

Shellfish

Early studies for the preservation of shrimp involved the use of chloretracycline (CTC) with radurization as a means of lowering the treatment dose.[105,127] During refrigerated storage over a period of 8 to 10 weeks, it was reported that radiation at 5 kGy with 5 ppm CTC was just as effective in extending the shelf life as treatment with 7.5 kGy.

Gamma radiation of fresh refrigerated shrimp using doses between 5 and 7.5 kGy extended freshness four- to fivefold.[127,128] However, the latter dose contributed to odor which apparently decreased during product storage. When fresh, peeled shrimp was maintained at an abusive temperature of 10 to 12°C following 1.5 and 2.5 kGy treatment, the storage life was extended by 10 to 14 and 20 days, respectively. However, at a storage temperature of 2 to 4°C, the shelf life was extended 21 and 25 days, respectively. A blanch treatment involving steaming for 4 min followed by treatment with 1.5 kGy resulted in a sensory acceptable product following 60 and 130 days storage at 10 to 12 and 2 to 4°C, respectively. Further, treatment at this dose did not result in fading of astaxanthine, the characteristic pigment found in fresh shrimp. In terms of color, taste, and odor, shrimp treated at 1.5 kGy scored similar to non-irradiated controls. Gamma radiation of fresh prawn at 1.45 kGy resulted in about a 1 log reduction in total counts and a 1.3 log reduction in proteolytic microorganisms. Increasing the dose to 2.5 kGy brought about larger reductions of 2.5 and 2.2 log, respectively.[129] During storage for 28 d at 1°C,

microbial counts on treated samples increased by 2.0 to 2.5 and 1 log, respectively; *Lactobacillus* spp. constituted a major portion of the microflora and a D_{10} value of 0.59 kGy indicated that these bacteria were of moderate resistance when compared to other spoilage bacteria. For example, *Pseudomonas* spp., which normally constitute the major portion of the spoilage microflora of refrigerated prawn,[130] have D_{10} values ranging from 0.10 to 0.23 kGy[93,131] Pathogens including *Salmonella*, *Vibrio*, *Listeria*, and *Shigella*, which can also contribute to shellfish spoilage, have similar D_{10} values.[132-135] Attempts to lower the treatment dose and consequently the production of radiation-induced odor and taste problems have been a primary objective of various investigators. In part this has been achieved by combining ionizing energy and freezing. Obviously differences among shrimp species and processing conditions must be considered. From a sensory perspective Hau et al.[136] reported that the threshold treatment dose, relative to off-flavor development, for frozen (–10°C) headless prawn, packaged in polyethylene pouches was 4.5 kGy. This value is higher compared to previously reported data; however, it was reasoned that treatment using a frozen product decreased free radical generation and mobility thereby reducing the production of off-odors and flavors.[57,137] Treatment doses used in the study ranged from 2.5 to 7.5 kGy, with the highest dose reducing the initial population to about 10^2 cfu/g. In contrast, the population in non-irradiated samples maintained at –10°C surpassed 10^6 cfu/g after 48 h of storage. At this level the product was considered borderline in regards to acceptability.

Shucked clam meats and soft shelled clams, gamma treated at doses up to 8 kGy and stored at 6°C, were periodically deep fried and evaluated by a taste panel; at 40 days of storage product acceptability was still maintained.[138] By lowering the storage temperature to 0.6 to 1.7°C, Ronsivalli et al.[139] demonstrated that the treatment dose could be reduced to 4.5 kGy, and the chowder prepared from treated clam meats was acceptable even after 15 days. Acceptability of clam meat was also reported by Conners and Steinberg[140] after treatment using 2.5 to 5.5 kGy. In contrast, a maximum dose of 2.0 kGy was reported by Novak[141] for treatment of Gulf Coast oysters. When stored in crushed ice the oysters maintained high acceptability as judged by a sensory panel even after 21 days of storage. Non-irradiated controls were off-flavored by 7 days. The extended shelf life of the treated oysters was due in part to a 99% reduction in the initial microbial population and was accompanied by decreases in methylamine and ammonia levels suggesting that chemical changes were closely correlated with microbial numbers. Overall, radiation treatment of oysters is perhaps more important and/or urgent than previously mentioned products for two main reasons: they do not freeze well in terms of texture and taste and therefore are rarely preserved using this method, and they are frequently consumed raw which can constitute a potential health hazard. In regards to the latter point, although many pathogenic bacteria associated with shellfish including oysters are killed by low dose radiation treatment thereby allowing pathogen-free fresh raw seafood in the market place, viruses including hepatitis A and rotavirus tend to be more resistant. Therefore, higher doses, usually greater than 2.0 kGy, are required.[22,102]

When examining vacuum-packaged king crab meat following radiation, a panel consistently noted sensory changes when the microbial population approached 10^8 cfu/g. In contrast, controls were judged unsatisfactory when the total bacterial

plate count approached 10^6 cfu/g.[142] Ostensibly the levels of specific spoilage microorganisms are more important compared to total or spoilage associated numbers.[143] In addition, although trimethylamine and total volatile base levels increased with storage time in both radiated and control samples, only values in excess of 0.9 and 12%, respectively, could be related to product acceptability. Following treatment at 2 kGy the shelf life was extended to 3 weeks storage at 1°C. Doubling the dose concomitantly doubled the shelf life under similar storage conditions. In a previous study,[128] a treatment using 2.5 kGy was demonstrated to extend the shelf life of crabmeat to 3 to 4 weeks when stored at about 3.5°C compared with 1 week for control samples.

Chilled saucer scallops (*Amusium balloti*) when treated with 0.5, 1.5, and 3 kGy exhibited a shelf life of 18, 23, and 42 days, respectively.[144] The initial total plate count levels, ranging from 10^5 to 10^7 cfu/g, were reduced by a 2-, 3-, and 4-log reduction following radiation at 0.5, 1.5, and 3 kGy, respectively. The initial psychrotrophic count (PC), which is perhaps a better index for potential spoilage, was about 10^6 cfu/g and was reduced by 1, 2, and 3 logs, respectively. Improvements in the shelf life of the product were partly based on the observation that during chilled storage PC levels in the treated samples did not reach 10^7 cfu/g until 4, 20, and 28 days of storage, respectively. In contrast, control samples reached this level after only 7 days. When the samples were baked and presented to a taste panel, no differences in odor or texture were noted between the treated and control samples, at least prior to 8 days of storage. In contrast, scallops (*Plactopecten magellanicus)* treated with 1.5 and 3 kGy received no practical benefit in regards to shelf life extension because of development of off-odors and flavors described as burned.[145]

Meat

Beef

In 1997 both the U.S. Food and Drug Administration (FDA) and the Department of Agriculture (USDA) approved a petition for the use of safe irradiation dosage levels with fresh and frozen beef, lamb, and pork products to control foodborne illnesses; however, packaged and processed products such as frankfurters were excluded. Approvals for irradiation include maximum doses of 4.5 and 7.0 kGy for refrigerated and frozen meat, respectively.[146]

Fresh beef has a limited shelf life even when refrigerated and as such long-distance transport especially by ship can be unsatisfactory. At temperatures of 0 to 2°C putrefactive spoilage occurs in 6 to 7 days and results principally from the growth of Gram-negative psychrotrophs. Typically spoilage occurs when the aerobic plate count reaches 10^7 cfu/cm^2 and is accompanied by the formation of surface slime, green discoloration, and off-odors.[147,148] The major microbial participants include the *Pseudomonas-Acinetobacter-Moraxella* group and members of the *Enterobacteriaceae*.[147] Although *Pseudomonas* is the most important bacterium with regards to spoilage of fresh refrigerated meat, it is, as previously mentioned, easily destroyed by relatively low doses of radiation. In this regard, Wolin et al.[149] more than 40 years ago demonstrated that beef steaks which were radiated at dosages less than 1 kGy had a shelf life at 2°C which was 4 to 5 times greater than non-irradiated controls. Furthermore, at this level of application irradiation-induced sensory

changes were minimal and were in agreement with the 2.5 kGy threshold dose for beef as reported by Sudarmadji and Urbain.[57] If products do not become recontaminated during storage, spoilage will result from a secondary flora consisting of radiation resistant survivors including the *Moraxella-Acinetobacter* group, *Brochothrix thermosphacta*, yeasts, and lactic acid bacteria.

In order to minimize microbial contamination, packaging prior to radiation treatment has been proposed.[22,150] For this purpose MAP using CO_2 flushing is most commonly used although vacuum packaging is also considered a form of MAP; the packaging material provides a physical barrier to entrance of recalcitrant microorganisms and the carbon dioxide inhibits the growth of aerobes including *Pseudomonas*.[151] Using low permeability films (for example, <100 ml of O_2/m^2 per 24 h atm measured at 25°C and 98% RH) slow growing, psychrotrophic Gram-positive lactic acid bacteria predominate following radiation, and souring due to acid production replaces putrefaction by *Pseudomonas*. Oxygen impermeable films also limit lipid oxidation which results in the development of product rancidity.[11] This is of particular importance since oxidative reactions occur more quickly when fat-containing meats are processed with ionizing energy. In this regard peroxide values are known to accumulate more rapidly in radiated compared to non-irradiated fat-containing meats.[150,152] The development of off-odors, which in some cases has been reported to dissipate within minutes of treatment,[153] and flavors, which are often dose-related, have been described by various researchers as being metallic and/or slightly tallowy.[152] In addition, fat sections on the surfaces of the meat may appear distinctly bleached in comparison to the lean.[152] Additional improvements to minimize flavor changes were achieved by decreasing the temperature during treatment. Chemical and biochemical reaction rates decrease concomitantly; however, overall dosages employed during radurization are often insufficient to inactivate many enzyme systems.[11] Combining freezing and packaging is ideal although not always possible or practical. As previously stated, when radiation is applied to frozen foods, reactive intermediates generated from water radiolysis are immobilized and therefore are prevented from interacting with each another and/or with the food.[11] It should be remembered, however, that freezing of foods during treatment also enhances microbiological resistance and therefore additional survivors may be expected especially if marginal doses are utilized.

Urbain and Giddings[154] reported that aerobic mesopilic bacteria levels in vacuum-packaged beefsteaks following a 2.5 kGy treatment remained below 10^4 cfu/g even after 21 days when stored at 4°C. The researchers further reported that treating steaks with phosphates prior to treatment increased overall acceptability. A subsequent report indicated that decreasing the dose to 1 kGy still resulted in an acceptable product in comparison to the non-irradiated control.[155] Niemand et al.[156] reported a doubling in the shelf life of vacuum-packaged sirloin samples when stored at 4°C following radurization using a 2-kGy dose. The extension in shelf life from 4 to approximately 10 weeks was accompanied by a 99.9% reduction in aerobic plate count and the elimination of various resident microflora including pseudomonads, *Enterobacteriaceae*, and enterococci.[13] The more radiation-resistant lactic acid bacteria, principally lactobacilli, and to a lesser extent *Leuconostoc* spp. and pediococci, survived and progressively increased with storage time and ultimately resulted in

TABLE 3.3
Growth of Microorganisms (log_{10} cfu/g) on Beef Top Round at 1°C Following Radiation Treatment at 2 kGy

		Storage time (d)				
Microorganism		**0**	**3**	**7**	**10**	**21**
Psychrotrophs:	T[a]	1.66	—[c]	—	3.11	6.16
	C[b]	3.45	4.40	6.06	6.55	—
Mesophiles:	T	<1.58	—	—	2.66	3.13
	C	2.84	3.67	6.11	4.98	—
Pseudomonads:	T	<1.58	—	—	<1.58	<1.58
	C	2.54	4.19	5.49	6.38	—

[a] Treated
[b] Control
[c] Not performed

Adapted from Rodriguez et al.[157]

product spoilage. A sensory panel determined that the radiated samples were superior in both appearance and odor throughout the storage period when compared to the controls. Decreasing the treatment temperature from 25 to 0 to 2°C was also recommended as a means of minimizing sensory changes. The shelf life of top round beef cuts packaged in polyethylene wrap was similarly extended following treatment with 2 kGy.[157] Based on psychrotrophic bacterial counts (Table 3.3) and using a cutoff value of 10^7 cfu/cm^2, which coincided with undesirable sensory changes, product shelf life at 1°C was extended an additional 17 days compared to controls. At 8 to 10 days of storage, both odor and color had deteriorated in the controls; however, treatment samples even after 4 weeks maintained an overall acceptability score of 7.5, with 10 being the highest. As shown in previous studies, a pronounced reduction in spoilage bacteria including *Pseudomonas* and *Brochothrix thermosphacta* was achieved during treatment.[156]

Ground or Comminuted Beef and Pork

Freshly ground or comminuted beef also has a relatively short shelf life, typically from 4 to 7 days, even when maintained under proper refrigeration temperature.[158] Various factors contribute to shorten the shelf life; however, a large surface-to-mass ratio and high initial numbers of spoilage bacteria in the range of 10^5 to 10^6 cfu/g, which originate from trimmings and/or resulting from process contamination, are mainly responsible. [159-161] Principally, *Pseudomonas*, *Enterobacteriaceae*, and lactic acid bacteria are isolated.[161] Although *Pseudomonas* is easily eliminated, lactic acid

bacteria, which are especially resistant during logarithmic growth, and yeasts normally survive and predominate especially if vacuum-packaging is utilized.[13,162,163]

Studies by Roberts and Weese[158] indicated that vacuum-packaged ground beef patties having an initial aerobic plate count of 10^4 cfu/g had a shelf life of 14, 21, 42, and >42 days at 4°C following radiation treatment at 1, 3, 5, and 7 kGy, respectively. In contrast, with ground beef patties having an initial aerobic plate count of 10^2 cfu/g, microbiological acceptability was maintained even after 42 days of storage at 4°C following a 1-kGy dose. Further, non-irradiated patties having an initial aerobic plate count of either 10^2 or 10^4 cfu/g had a shelf life of less than 7 days and a corresponding plate count greater than 10^7 cfu/g. Microbial levels of this magnitude are well recognized to contribute to objectionable off-odors and flavors in meats and are indicative of product deterioration.[159] The importance of using high microbiological quality meat, especially if shelf life extension is to be expected using a low-dose application, cannot be over stated. Additional studies have provided results corroborating the notable extension in shelf life of ground beef following radurization.[159,162,164-166] In the study performed by Niemand et al.,[162] it was reported that beef patties radurized at 2.5 kGy maintained acceptable odor and appearance for up to 9 days at 4°C. Factors such as storage temperature and product access to oxygen were demonstrated to have a major influence on shelf life.[162] In general reducing oxygen by vacuum-packaging and/or the use of oxygen impermeable films had positive effects on shelf life extension. Combining radiation treatment at 2.5 kGy with lactic acid was reported to result in a synergistic effect which further improved shelf life.[162] Unfortunately, inclusion of acid gave a bleached appearance to the product. Dempster[166] also reported that following radiation at 1.03 and 1.54 kGy the surface of beef burgers appeared discolored or bright red. Although internal color was not affected, irradiation odors described as "wet dog", "musty", and "burnt popcorn" were detected by the taste panel when the burgers were treated at both doses in the presence of oxygen; the odors dissipated somewhat after treatment. Vacuum packaging combined with low temperature storage following treatment was advocated to reduce the formation of free fatty acids in the burgers. Ostensibly produced by autolytic tissue enzymes, the production of free fatty acids would definitely contribute a shortened shelf life and prompted investigations into the use of antioxidants.[167] Interestingly, Cohen et al.[168] reported that fat levels and the degree of grind did not affect product acceptability following radiation. The impact of radiation on filet américan, a raw ground beef product containing a mayonnaise-like sauce has also been examined, but only from a safety perspective.[169] Current research associated with elimination of pathogens in ground meat is given by various authors.[25,169,170]

Ground pork, which is an equally perishable product, can also benefit from low dose radiation especially if processed using microbiologically sound trimmings.[172] For example, Ehioba et al.,[173,174] reported that doses as low as 1 kGy could extend shelf life, based on psychrotrophic total counts corresponding to 10^7 cfu/g, from 8 to 11.5 days if the meat was vacuum packaged and stored at 5°C. Following similar treatment and storage conditions, however, a simulated poor quality grind containing approximately 10^5 cfu/g exhibited a shelf life extension from 4.5 to only 6 days.

After 9 days of storage, Gram-positive bacteria, mainly *Lactobacillus*, predominated in both the good and poor quality treated meats. Radiation did not result in any change in product pH or thiobarbituric acid value (indication of lipid oxidation). Although the microbial spoilage pattern was similar for both the treated and control samples, the rate of spoilage for the former was much reduced.[172] At a dose of 1.91 kGy or higher, survivors were absent even after 35 days of storage at 2°C. Raw ground pork and beef, which is commonly used in fermented sausage, was radiated at 0.2 and 0.5 kGy.[175] Although total plate counts were only reduced by 1.3 and 2.2 logs, respectively, the real benefit arose from the reduction of coliforms and staphylococci. It was also suggested that a decrease in the levels of competing microorganisms would produce a more uniform product allowing better process control and the possibility of reducing starter culture inocula.

Pork

Approval for the irradiation of pork by the U.S. FDA was granted initially in 1985.[54,176] The USDA Food Safety and Inspection Service (FSIS) granted similar approval;[177] however, since the initial purpose was for elimination of parasites, namely, *Trichinella spiralis*, only absorbed doses between 0.3 and 1 kGy were sanctioned.

Despite the relatively low approval dose, Mattison et al.[178] reported that boneless vacuum-packaged pork loins treated with 1 kGy contained significantly lower levels of both mesophiles and psychrotrophs when compared to non-irradiated controls. Even after 21 days of storage at 4°C neither bacterial group reached spoilage levels of 10^6 cfu/cm^2. From a sensory perspective, treated cooked samples were indistinguishable from the controls even after 14 days of storage, indicating that perhaps any off-odors generated as a result of treatment slowly dissipated. In any event, the lack of detectable odors is in agreement with a reported threshold dose for pork of 1.75 kGy.[57] By increasing the dose to 3 kGy, Lebepe et al.[19] reported that vacuum-packaged boneless pork loins stored at 2 to 4°C exhibited a shelf life of approximately 90 to 91 days. The shelf life was based on microbiological spoilage levels of 10^7 and 10^8 cfu/cm^2 for mesophiles or psychrotrophs and lactobacilli, respectively. In contrast, non-irradiated loins spoiled after 42 days. Although a sensory evaluation was not performed, treated samples were invariably darker compared to the controls; pH and thiobarbituric acid values did not differ significantly from the control. Treated loins following storage at 2 to 4°C for 41 days were further demonstrated to have a retail shelf life of 10 days at 5 to 7°C, based on psychrotrophic counts, when cut into pork chops and packaged using an oxygen permeable film.

Using a combination of MAP and radiation, Grant and Patterson[180] reported that a mixture of 25% CO_2 and 75% N_2 improved the sensory and microbiological quality of radiated pork. The presence of oxygen, however, was shown to dramatically affect the sensory (based on color and odor) shelf life of this product.[181] Following radiation of loins in packages containing 20% O_2 at 1 kGy, they were immediately rejected by a sensory panel on the basis of strong off-odors and discoloration. In comparison, the sensory shelf life of loins packaged in barrier bags without any O_2 and radiated at 1 kGy was 26 days at 5°C. Non-irradiated controls with 20% O_2 had a sensory shelf life of only 4 days.

Other Meats

Radiation treatment of lamb, which serves as a principal meat source in various countries including India and Greece, was reported by Paul et al.[182] The study concluded that radiation with 1 and 2.5 kGy extended the shelf life of minced lamb an additional 1 and 3 weeks, respectively, over the control when stored at 0 to 3°C. Chunk lamb, which was similarly treated, exhibited an enhanced shelf life of 2 and 4 weeks, respectively, over the control. Both the chunk and mince control samples appeared greenish and slimy within a week and contained an aerobic plate count of 10^7 and 10^8 cfu/g, respectively. In contrast, samples that were initially radiated did not exhibit these characteristics during spoilage, primarily due to the elimination of *Pseudomonas*. Irradiation odors, which were not detected in any of the treated samples by a sensory panel, have previously been reported to occur with dosages only greater than 6.25 kGy.[57] Interestingly, venison was reported to have a similar threshold dose.[57]

In order to mimic conditions likely to occur during transportation and retail display, especially in rural India, Naik et al.[183] maintained freshly packaged buffalo meat at ambient temperatures of 28 to 30°C following slaughter. Under these conditions, a shelf life of approximately 18 h was realized. Following irradiation at 2.5 kGy the shelf life, based on bacteriological, sensory, and chemical analyses, was extended to 42 h.

Consumed to a lesser extent but still economically important, the shelf life of refrigerated red or edible offal including tripe (first and second bovine stomach) and lamb liver was also demonstrated to improve after radiation.[150]

Vacuum-packaged, sliced corned beef normally has a shelf life from 2 to 3 weeks when maintained at 5°C. Spoilage normally coincides with bacterial counts of approximately 10^8 cfu/g.[147] In an experiment performed by Wills et al.[184] (Table 3.4) it was demonstrated that a dose of 2 kGy was sufficient to double the shelf life of the product at 5°C, based on taste panel ratings of flavor, aroma, and bacterial plate

TABLE 3.4
Shelf Life Extension of Vacuum-Packaged Sliced Corned Beef Maintained at 5°C Following Radiation Treatment

Radiation Dose (kGy)	Log_{10} Units Reduction in Initial Population	Time for Population to Reach 10^8 cfu/g (weeks)[a]	Major Spoilage Microorganisms
0	—	2	Lactic acid bacteria
1	1	3	Lactic acid bacteria
2	2–2.5	4–5	*Brochothrix thermosphacta*
4	>4	>7	Gram-negative bacteria

[a] Point of visible spoilage.

Adapted from Wills et al.[184]

counts. Although a slight radiation odor was detected in samples treated with a 2 kGy dose, it was suggested that other products including smoked ham, which has a more robust aroma, would be more suitable. Irradiation in regards to microbial safety in processed meats including bacon and ham is given by Anellis et al.[185,186] and Thayer et al.[187] while sensory evaluation of radiated treated frankfurters as impacted by phosphates and nitrites is given by Terrell et al.[188]

Fruits and Vegetables

Fruits

During the past 30 years there have been many studies on the application of irradiation for improving shelf life of fresh fruits. These include tropical fruits such as bananas, mangos, and papaya; subtropical fruits such as citrus and grapes; and temperate fruits such as pome fruits, stone fruits, and berries.

Gamma irradiation at low dose levels has been shown to improve shelf life of bananas, mangos, and papaya by delaying the ripening process and these dose levels have been found to be effective as a quarantine treatment against fruit flies and mango stone weevil infestations.[189]

Cumming reported recently that low-dose radiation (0.2 kGy) delayed ripening of green bananas for up to 10 to 12 days.[190] There was only minimal changes in pulp texture and vitamin C losses were lower than the controls. Earlier work by Ferguson et al.[191] showed retardation of yellowing at a dose of 0.2 kGy and an increased preference for the flavor of the irradiated bananas 7 days after treatment. Thomas reported on shelf life extension of 10 to 12 days for some varieties.[189] Several studies have shown that gamma radiation at low levels extends the shelf life of mango fruit by slowing down rates of ripening and senescence. Irradiated fruit remain edible for longer periods before they pass into the senescence phase, thus improving the overall market life of the fruit.[189] Mangos and papaya are irradiated mainly to delay ripening and for disinfestation.[192] The irradiated fruit can be detected by GC-MS analysis of the TCB (2-tetracyclobutanone) peaks present in lipid extracts from the fruit. This has proved to be a better method of analysis for irradiated fruit than ESR spectroscopy. Willemot et al.[193] recently reviewed the applications of gamma radiation to the preservation of mangos, papaya, and strawberries, while others[194] studied the effects of irradiation and a combination of hot water dip-treatment plus irradiation on the storage, sensory, nutritional physical characteristics, and biochemical changes in mangos. They reported that irradiation alone reduces respiration and color development and increases fruit softness, but did not significantly affect sensorial quality. They also reported that the combined treatment of hot water and irradiation had a synergistic effect on increased shelf life of mangos up to 32 days, without affecting nutritional quality.[195-199] Furthermore, they found that the combination treatment was more effective in post-harvest decay control than irradiation alone. Paul[200] reported that irradiated Hawaiian papaya softened more uniformly than the controls, and for fruit irradiated with 30% of the skin yellowed softening was at a slower rate. Irradiation[201] at the 15 to 30% yellow stage resulted in no significant change in skin and pulp color, or pectin methyl esterase activity.

Immediately after irradiation, pectin in the 10 to 30% yellow papaya showed depolymerization and demethoxylation. However, irradiation at doses from 0.5 to 1.0 kGy of fruit at the 25 to 30% yellow ripeness stage had less depolymerization of pectin and a firmer texture when ripe than the controls. Firmness of the irradiated fruit lasted 2 days longer than the controls. Earlier work in the U.S. showed a consumer preference for irradiated papaya.[202] The International Atomic Energy Agency has recently published an excellent book on the use of irradiation as a quarantine method for treatment of fruit such as mangos, papaya, and grapefruit in several countries.[203] Thomas[204] has reviewed possible applications of irradiation for the preservation of subtropical fruits, citrus, grapes, and avocados. Miller and MacDonald[205,206] have reported that the treatment of Florida grapefruit with irradiation at 0.3 kGy delayed ripening and increased fruit firmness without damaging fruit quality. Mitchell et al.[207] reported that low dose irradiation of several Australian fruits, such as mango, lychee, lemon, mandarin, nectarine, nectarine, peach, and persimmon, produced small changes in soluble solids, pH, pulp color, vitamin C, organic acids, and sugars for some of the fruits. However, the effects of storage were greater than the effects of radiation. Similar effects were found in bananas, plantain, and nectarines by Cummings.[190] Irradiation at doses from 50 to 250 Gy has been used to reduce post-harvest losses in pineapple due to senescence and fungal diseases.[208] Jobin et al.[209] reported on the effects of radiation combined with hot water on the physical, chemical, and organoleptic properties of tangerines. They found that although radiation caused a loss of firmness of the skin, the appearance, texture, flavor, pH, color, and soluble solids content of the pulp was not affected by up to 14 days of storage. Earlier O'Mahony[210] reported on the low post-harvest doses (0.6 to 0.8 kGy) treatment of controlled atmosphere navel oranges and compared them to the controls for taste, flavor, odor, firmness, peel and flesh color, peel blemishes, and ease of peeling. The greatest differences were found in the degree of blemishing together with smaller differences in flavor, odor, color, texture, and ease of peeling. Abbas[211] reported that low dose (up to 50 Gy) irradiation could be used to delay the ripening of jujube fruits with no significant loss in nutritional value. Irradiation alone or irradiation in combination with hot water dip has been used to reduce post-harvest fungal rot in table grapes.[212] Organoleptic quality, fruit firmness, and soluble solids content were not affected, but decreases in acidity and ascorbic acid were reported. McLaughlin et al.[213] reported that gamma irradiation of lychee at 75 or 300 kGy may be used for disinfestation purposes with no adverse effects on fruit quality.

Thomas[214] has reviewed the applications of irradiation in controlling post-harvest losses due to fungal decay, senescence and physiological storage disorders in temperate fruits such as apples, pears, peaches, nectarines, plums, and berries. Unfortunately for most fruits the irradiation dosage required to inhibit or destroy storage rots and catabolic enzymes is more than enough to cause serious injury to the plant tissue (Table 3.5).[215]

The application of gamma irradiation for improving the storage of climacteric fruits like apples and pears has been extensively studied with respect to the control of physiological disorders and reduction of decay caused by fungal pathogens. As the results appear to be contradictory, later workers have turned to UV radiation.

TABLE 3.5
Relationship Between Dosage (kGy) Causing Quality Damage and Inhibiting Storage Rots

Commodity	Max. Estimated Tolerable Dose	Max. Estimated Dose Required for Control
Apricots	0.5	2.0
Boysenberries	1.0	2.0
Lemons/limes	0.25	1.5–2.0
Nectarines	1.0	2.0
Oranges	2.0	2.0
Peaches	1.0	2.0
Raspberries	1.0	2.0
Strawberries	2.0	2.0
Table grapes	0.25–0.5	10.0

Lu et al.[216] reported that treatment with UV radiation reduced storage rots in peaches and apples. Flesh firmness of peaches and acidity of both fruits increased with dose, while the percentage of soluble solids and pH decreased. Later work[217] confirmed that both UV and gamma rays reduced storage rot and delayed ripening. However, UV treated peaches contained less sugar, total phenolics, anthocyanins, and had a lower weight loss than gamma irradiated peaches. Tsang[218] has reported that irradiation of peaches at 1.0 kGy improved ripening and red color development, with minor changes in aroma, flavor, and texture. This dose was found to inactivate a population of brown rot by 2 D but also significantly decrease ascorbic acid. Lester[219] reported the successful irradiation of muskmelons at a dose of 1.0 kGy, prior to storage to slow down the progression of senescence. Pirouzmand[220] reported the use of irradiation for the deinfestation of larvae in apples at a dose of 1.2 kGy. This decreased emergence of adult codling moths where there was minimal change in texture of the fruit. Use of irradiation for insect disinfestation is more promising than shelf-life extension, especially where fumigants formerly used have been banned, like EDB.[215] Another promising use of irradiation is for the quarantine treatment of imported produce. The process has been approved for insect disinfestation in the U.S. since 1998 for doses up to 1 kGy. Fruits such as apples, cherries, dates, guavas, mangos, nectarines, papayas, peaches, raspberries, strawberries, and tomatoes suffer little phytotoxic damage at this dose. Comparisons of the effects of irradiation and methyl bromide fumigation[221] have been carried out on Egyptian semi-dried date fruits used for the manufacture of date paste. Irradiation at 3.0 kGy was more effective for preventing insect infestation of the dates and inhibiting the growth of fungi and thus preventing aflatoxin production.

Yu,[222,223] who reported that electron beam radiation can be used to extend the shelf life of strawberries, found that fruit firmness and red color decreased, but an off-flavor increased as the radiation dose increased from 0 to 2 kGy. However, irradiation at these levels did suppress fungal growth leading to an extension of shelf life by 2 to 4 days. Recent work by Cumming[224] using gamma irradiation also

resulted in a similar extension of shelf life for fresh strawberries. Marcotte[225] and Pszczola[226] reported earlier on a successful test market introduction of irradiated strawberries in the U.S. and Brecht et al.[227] has reported on the successful use of irradiation in conjunction with modified atmosphere storage. Miller et al.[228-230] report on the use of low dose electron beam irradiation to treat two blueberry varieties and gamma irradiation for another two. In the electron beam treated blueberries, firmness, flavor, and texture decreased as irradiation dose increased from 0.25 to 1.0 kGy, but skin color, decay, soluble solids content, and pH remained unchanged. In the case of gamma irradiation, there was minimal changes in these quality factors at the dosage levels required for commercial applications for quarantine disinfestation (i.e., 0.5 to 1.0 kGy). Some recent work by Cumming[224] using gamma irradiation suggests blueberries will tolerate doses up to 4.0 kGy, which significantly decreases mold growth by approximately 50% after 2 weeks of storage.

Gamma irradiation also has been successfully used on cherries as a replacement for methyl bromide fumigation.[231] The cherries were irradiated at doses from 0.1 to 1.0 kGy, then stored for 14 to 21 days at 10°C before quality was determined. No variation of fruit or stem color, soluble solids content, acidity, or sensory quality was noted. However, there was some loss of firmness in the irradiated fruit treated at doses above 0.4 kGy. Later work by Warner[232,233] confirmed that irradiation was preferrable to fumigation for preservation of quality in cherries.

Vegetables

Irradiation at low doses (50 to 150 Gy) has been commercially used in many countries to suppress sprouting in many products such as potatoes, yams, turnips, beets, Jerusalem artichoke, onions, carrots, garlic, sweet potatoes, and ginger.[215] The use of ionizing radiation at these doses to inhibit sprouting of white potatoes was approved in the U.S. in 1964.[234] The applications of ionizing radiation for sprout inhibition and its effect on quality of potatoes has been reviewed by Matsuyama and Umeda.[235] A minimum dose of 20 to 30 Gy for onions and 30 Gy for potato tubers is required for sprout inhibition and smaller doses may actually stimulate sprouting. Doses greater than 150 Gy may cause detrimental effects in both tubers and bulbs. At sprout inhibiting doses, approximately 15% of the initial ascorbic acid was lost; however, after prolonged storage the differences between irradiated and unirradiated potatoes were not significant. There were some changes noted in the free amino acids of potatoes irradiated at 150 Gy, but these changes disappeared during storage. Earlier in an excellent review, Thomas[236] reported that a dose of 0.1 kGy inhibited sprouting irreversibly regardless of variety and storage temperature. Best results were obtained when good quality tubers harvested with minimal injuries and cured to heal bruises were irradiated. However, irradiation of potatoes, even at antisprouting doses, induces or enhances three types of discoloration: black spot, vascular browning, and darkening after cooking.

Although most of the work reported on irradiation of potatoes was at low doses for antisprouting, Cumming[237] has reported on experiments conducted at higher doses up to 10 kGy. He found that at a dose of 2 kGy, polyphenol oxidase activity is reduced, resulting in less darkening on slicing. There was some softening of texture

but minimal change in starch degradation at this level. Leszczynski et al.[238] studied the effect of irradiation at 150 Gy on potato quality and chip production. They found that the irradiated samples were lower in starch and higher in sugar levels along with some flesh darkening. All stored samples showed complete inhibition of sprouting, whereas in the controls sprouting and related changes in composition increased with duration and temperature of storage. Quality of chips depended on storage temperature and only slightly on irradiation. Diehl[239] reported that irradiation of potato slices followed by refrigerated storage instead of using freezing and frozen storage would save the industry an estimated 300 million kwh of energy. He further reported that in Germany consumer testing of potatoes irradiated at 120 Gy showed a preference for the treated samples over the controls, particularly after 3 to 5 months storage. Cumming[240] found similar results for yams irradiated at doses from 0.5 to 5 kGy, with the optimum dose for color preservation occurring at 2 kGy. A 100-fold reduction in surface microbes occurred at this dose level. Adesuyi and Mackenzie[241] reported excellent sensory results and reduced rotting from irradiation of Nigerian yams at 75 to 125 Gy for antisprouting purposes. Later work on sweet potatoes showed significant increases in sugar concentrations even at doses of 0.1 kGy.[242,243] The sweeter irradiated samples were not preferred to the controls, in part due to the darkened appearance of the treated samples, especially after baking.

Diehl[244] and other workers[245] have reported that low dose irradiation (50 Gy to 5 kGy) prolonged the storage life of onions for 9 months, mainly due to inhibition of sprouting, but did not prevent rotting. Later work[246,247] has also shown that neither gamma irradiation or long-term storage affected DM content or acidity, although the intensity of flavor was affected and carbohydrate content decreased significantly after 180 days storage, while ascorbic acid content remained unchanged. The irradiated bulbs were judged to be of superior appearance and firmness after 180 days storage and showed no difference in odor. Similar results have been reported for garlic irradiated at doses from 50 to 150 Gy with up to 80% reductions in spoilage of the cloves after 9 months storage.[248]

Yusof[249] reported that low dose irradiation of 25 to 80 Gy inhibited sprouting in ginger but did not extend the shelf life. For cauliflower and broccoli, low dose irradiation accelerated respiration which led to increased spoilage on storage even under CO_2.[228,250,251]

Irradiation can be used to control *Listeria monocytogenes* in pre-cut bell peppers and carrots.[254] Microbiological shelf life of bell peppers was doubled after irradiation with 1 kGy and when coupled with GMP (Good Manufacturing Practices) practically eliminated this pathogen from peppers. For carrot cubes, irradiation drastically reduced the microbial load which improved the microbiological shelf life, whereas irradiation of peppers at this dose caused an initial loss of 12% of ascorbic acid followed by a further reduction of 10% during storage, this being comparable to that occurring in the controls. After 10 days of storage, the β-carotene content of the irradiated samples of both products was slightly higher than that of the untreated samples. Previously it has been reported that doses greater than 1 kGy caused excessive softening of the tissue.[253] Cathalin and McNulty[254] have reported losses in texture in carrots, apples, and potatoes ranging from 5 to 30% at doses ranging from 0.3 to 1.0 kGy. Carrots were the most resistant to irradiation induced textural

changes. Irradiation also delayed senescence and reduced microbial spoilage of grated carrots, which is of potential interest for the maintenance of shelf life of minimally processed vegetables[255] and it has been reported[256] that irradiation was superior to conventional methods for preservation of "ready to eat" shredded carrots. Irradiation prevented losses of sugar, β-carotene, and orange color, inhibited aerobic mesophilic and lactic microflora, gave improved sensory scores, and has been reported to aid the preservation of fermented carrots.[257] Irradiation has been found to be useful in the decontamination of several dehydrated vegetables, carrots, celery, parsley, parsnips, and leeks.[258] A 5-kGy dose reduced initial microbial load by a factor of 5 to 10, lending support to the usefulness of irradiation as a part of GMP in the food industry. Cunha[259] found that the treatment of dried beans with gamma radiation increased the hydration rate while decreasing cooking time and hardness of the seeds, without affecting sensory properties. Delincee[260] reported that irradiation of legumes at doses up to 1 kGy for the purpose of insect disinfestation will not result in any adverse effects on cooking time, sensory quality, vitamin B content, or protein quality of white beans, peas, and legumes. Onyenekwe and Ogbadu[261] reported successful elimination of the fungal population of dried red chilli peppers with a dose of 7.5 kGy. Even radappertization at doses of 10 kGy did not produce significant changes in capsaicin and carbohydrate, although there was a small increase in oleoresin and lipid. Lescano et al.[262] reported that the shelf life of asparagus spears was doubled when a dose of 2 kGy was used and there was no significant differences in weight loss between control and irradiated samples. Although shear force and acidity values were slightly higher in the irradiated samples, mesophilic bacteria, yeast, and molds were reduced 3 orders of magnitude on treatment with 2 kGy. Irradiation at high doses has been reported to reduce aflatoxin contamination in corn and peanuts (83% reduction at 20 kGy).[263]

Mushrooms are the most promising product for using irradiation to delay maturation.[264] A dose of approximately 1 kGy applied soon after picking at the closed button stage prolonged shelf life at 10°C from 1 day to 5 to 6 days. In an earlier study by Kovacs,[265] a panel of judges could not detect any changes in odor, flavor, or texture of the irradiated mushrooms either immediately after treatment or during storage. Minnaar and McGill[266] reported on the optimization of parameters for the heat irradiation processing of mushrooms. Differences in texture, color, and viscosity of the cream sauce were found between different replicates but not between different temperature combinations (110, 115, and 120°C). The general appearance and color of the products processed at 120°C were superior to those processed at 110°C. Use of 120°C as the processing temperature resulted in the best product where a shorter processing time is required to achieve commercial sterility (i.e., 28 min as compared to 50 and 99 min at 115 and 110°C, respectively). The second phase of this study investigated the heat and irradiation resistance of *Clostridium sporogenes* spores in sliced mushrooms in cream and brine.[267] Gamma D_{10} values for *Clostridium* spores in cream sauce ranged from 3.1 to 4.0, whereas in brine the values ranged from 4.2 to 4.8. F_0 values for heating processes for mushroom in cream sauce and brine were determined and the combined D_{10} and F_0 values were used to propose possible combination treatments. They found no significant differences in texture measurements and sensory characteristics between treatments of heat-irradiation and irradiation-heat

processing. Sensitivity of *Clostridium sporogenes* spores were similar in both processing methods. Next the authors studied the consumer acceptability and preference for these combination processes.[268] The two most promising heat irradiation treatments were (1) F_0 value of 2 at 2.5 kGy and (2) F_0 value of 1 at 4.5 kGy. Ranked preference testing was performed to determine consumer preference. Treatment (1) performed better for appearance and color when compared to canned products, but treatment (2) was significantly favored over treatment (1) in all characteristics except texture. Earlier work by this group established the principle of using heat irradiation combination processing for producing high quality shelf-stable low acid food products.[269] Mushrooms in brine treated by heating 1 min at 120°C, followed by irradiation at 4.5 kGy or heating 2 min and irradiated at 2.5 kGy, were significantly different in all sensory properties than for either heat processing or irradiation treatment alone. Earlier work by Beaulieu et al.[270] and Lescano[271] showed that irradiation at doses of 2 to 3 kGy was effective for extending the shelf life of mushrooms. Irradiated fresh mushrooms had about a threefold extension of shelf life compared to the controls, with less browning and a delay in cap opening.

Barkai[272] reported on the use of heat-irradiation treatments to control fungal development in red tomatoes. Under natural infection conditions, hot water dip followed by irradiation at 0.5 kGy synergistically reduced fungal decay from 90 to 100%. El-Assi[273] studied the radiation induced textural changes in tomatoes, finding that green tomatoes were affected more than pink fruit and that the effects were dose dependent. Fruits irradiated green softened during storage and polygalacturonase activity decreased to about 10% of that of the controls. In the irradiated pink fruit PG activity decreased less but softening still occurred.

CONCLUSIONS

Irradiation processing has been extensively researched and is now being used for many food commodities. It has been successfully used to reduce pathogenic bacteria, eliminate parasites, decrease postharvest sprouting, and extend shelf life of fresh perishable food. Acceptance of the idea of irradiated food products in North America has been slower than in some other countries. The main problems for industry are no clear definition of the need for irradiation, large capital investment required ($3 to 4 million plant), transportation logistics, and consumer concerns. Contrary to consumer misinformation, all irradiated fruits and vegetables studied thus far are safe for human consumption and suffer no reduction in nutritional quality for doses under 2.0 kGy.[274]

For fruits and vegetables, cost and quality problems preclude the use of irradiation for extension of shelf life except for the few cases noted. Limiting factors in widespread adoption of the process are (1) cost as fruit and vegetables are low value crops; (2) much of the produce is grown on small acreages distributed over wide areas; (3) the produce is shipped to small local packing houses for shipment to large distribution centers (no single large facility handles all produce from the region); (4) the consumer is unwilling to pay the increased cost of irradiated products; (5) many other cheaper chemical methods are available. The most likely use of irradiation for these products is for insect control.

For meat, poultry, and seafood, irradiation is considered to be a viable alternative for improving their safety. There are two radiation processes used, radurization for shelf life improvement in refrigerated storage and radicidation to prevent food poisoning by destruction of pathogenic organisms. Successful radurization of meat has been achieved,[275] but there is little interest in the process in industrialized countries where deep freezing is available for extended storage of meat. Radicidation of meat and poultry to prevent foodborne infections is of potential interest in all countries. The major organisms, *Salmonella* and *Campylobacter*, are easily destroyed by radiation so health officials have advocated irradiation of meat and poultry as a means to eliminate these and other pathogens. The situation in seafood is very similar, with radicidation being used to eliminate harmful pathogens such as *Salmonella, Vibrio parahaemolytica*, and *Shigella* from shellfish. Seafood is an ideal candidate for irradiation in that the expensive plant required for the process can be built at a central seaport facility.

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4 Packaging Considerations

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INTRODUCTION

Shelf life is a complex concept that is dependent on the nature of the food product under consideration, the preservation technologies applied, and the environmental conditions to which the food product is exposed. In the case of packaged foods, the packaging often plays a key role in maintaining the quality and shelf life of foods by serving as an integral part of the preservation system employed. Ensuring high quality products is essential for consumer satisfaction, and quality may depend strongly on packaging performance.

Shelf life can be defined generally as the period of time following harvesting, production, or manufacturing, over which a food maintains the required quality. At the end of its shelf life, the product will be judged to be unacceptable for sale or

* Sadly, Marvin Tung passed away during the production of this book.

consumption based on sensory, nutritional, microbiological, or other quality criteria. Changing the composition and form of the food product, the environment to which it is exposed, or the packaging system can lead to alterations in shelf life.[1]

Functionally, packaging is designed to contain and protect foods, while providing added value to consumers. In today's retail market, products must often sell themselves, and packaging is therefore an important vehicle for point-of-purchase communication with consumers. New developments in packaging materials and processing technologies have created the potential for new food products that have more limited shelf life expectations than their traditional shelf stable or frozen counterparts, but still meet consumer needs. Other new products can provide an extended shelf life over traditional fresh foods.[2] The appeal of minimally processed, refrigerated foods such as modified atmosphere packaged fresh pasta is attributed to the "fresh-like" quality of the product, yet with the convenience of an extended shelf life.

With few exceptions, food quality decreases with time of storage, irrespective of the preservation methods used and the control of storage conditions, even for foods held in a frozen state.[3] Undesirable quality changes that take place during storage may affect texture, flavor, color, overall appearance, and the nutritive value and safety of food products. However, the rate and magnitude of many of these quality changes can be minimized with suitable packaging systems designed to aid control of the major influencing extrinsic deteriorative factors, namely, moisture, oxygen, light, temperature, and aroma transfer.

To ensure that consumers will find food product quality acceptable at the time of purchase, and also to ensure safety, food processors and manufacturers routinely determine a shelf life for each product they produce. Procedures for shelf life testing of foods are fairly well established and have been well described by Speigel,[4] and in relation to specific food products by Man and Jones.[5] Shelf life studies have been based historically on an assessment of either physical, chemical, and/or sensory changes in product quality, often accelerated by storage at elevated temperatures. An important packaging consideration in shelf life studies is the changes that occur in the barrier properties of plastic packaging materials due to elevated temperatures and relative humidities. Changes in barrier characteristics, such as an increased permeability to oxygen, could have a substantial influence on the shelf life of some packaged products.

This chapter will discuss briefly the basic categories of packaging materials, the major environmental factors that impact on food quality changes and how they affect package properties. Finally, various approaches to shelf life testing and prediction will be addressed in relation to packaging.

PACKAGING AND SHELF LIFE STABILITY

Successful food packaging applications are highly dependent on the nature of food materials and their preservation requirements, and on understanding how the package and the food behave during and after manufacturing processes. Early studies on the influence of packaging materials and food product properties on shelf life identified moisture sorption behavior of foods to be a key factor that determined whether degradation took place by microbial, oxidative, enzymic, or non-enzymic processes.[6-8]

For example, a particular food may be relatively stable within a certain range of water activity corresponding to water contents according to its moisture sorption isotherm. If stored at the same temperature in an environment where the ambient humidity would cause a gain or loss of water, the food product could reach a critical water content level at which the rate and extent of deterioration results in excessive loss of quality. This condition has been defined as the endpoint of shelf life. Resistance offered by the packaging material to water vapor transmission serves to slow the rate at which water is gained or lost from the product, which in turn influences the nature and rate of degradative processes, and thereby controls the shelf life.

Physical and barrier properties of a packaging system can also have an important influence on the shelf life of the packaged food product. The environment to which the product is exposed during distribution and storage can in turn influence shelf life directly by promoting deteriorative reactions in foods, or by causing changes in the performance of the packaging material itself that permit deteriorative reactions to proceed. A discussion of these environmental influences is necessary to understand the critical role that packaging can play in shelf life stability. Additional influences such as the physical, chemical, and biochemical characteristics of the product are discussed in more detail elsewhere in this book.

Packaging Materials

An important function of a package is to act as a barrier between a product and the external environment, but the requirement for protection will depend largely on product characteristics. In order to enhance shelf life, control of local environmental conditions should be met by the packaging layer in closest contact with the food. Another function of packaging is to protect the product during transit, and this often requires the use of secondary or tertiary layers of packaging with different physical properties. A brief review of the various properties of general packaging material categories follows. More specific and in-depth information on these materials and their manufacture can be found in other sources.[1,9]

Glass

Glass is a traditional packaging material that has retained its high quality image for selected packaging applications. Glass containers permit excellent clarity so that the product is visible, and are resealable, recyclable, and sometimes reusable. Glass packages are valued for being relatively inert and providing a total barrier to water vapor and gas transmission, except at the container closure. However, compared to other forms of food packaging, glass is heavy, brittle, and has low thermal shock resistance. Light transmission can be a significant disadvantage for light sensitive foods packaged in clear containers, but can be reduced through the use of colored glass. For example, light in the wavelength region of ultraviolet to 500 nm can catalyze oxidative reactions in beer; however, amber glass is used commonly for single-serve beer packaging because it effectively absorbs most light in this spectral region.[10] It is also important to note that most packaged food is protected from light by secondary packaging, such as corrugated boxes, during distribution and storage.

Metal

The metals used most commonly in the manufacture of food packaging containers are steel (tin or chromium plated) and aluminum. These metals have a wide range of mechanical and forming properties, with good strength and low toxicity. Metal containers are excellent barriers to water vapor, gases, and light, and can tolerate wide extremes of temperature, such as those found under retort processing conditions. To prevent interactions between products and their containers that could lead to internal corrosion or staining, internal protective coatings are used frequently in metal containers; exterior coatings and decorations may also serve to protect the packaging material against the environment. Disadvantages of metal containers include the limited shapes available, their considerable weight, and their lack of suitability for in-container microwave heating of products. Although not generally reusable, metal containers are nevertheless readily recyclable where collection programs are in place.

Aluminum foil in the form of thin-rolled sheets of aluminum has additional applications in laminated flexible packaging and in formed or semi-rigid containers. However, thinner foils are considered to be somewhat permeable to gases and water vapor due to the presence of minute pin holes. In other applications, metallized plastic films are manufactured by condensing aluminum vapor on the surface of films to improve barrier properties.

Paper and Board

Packaging papers tend to be coarse papers made from unbleached kraft softwood pulps, and include grease-resistant, glassine papers, and vegetable parchments used for wrapping foods where resistance to oil, grease, water, and odors is required. These papers provide structure, light weight, and printability; however, they are vulnerable to water absorption and puncturing. Paperboard provides a further degree of strength in packaging applications, such as folding cartons which can also be laminated or coated to impart specific barrier properties. For example, the laminated multi-layer brick-shaped structures widely used in aseptic packaging applications consist of an internal layer of paperboard for rigidity and protection of an aluminum foil barrier layer, while outer and inner plastic layers provide additional barrier properties, heat sealability, and food contact surfaces. Transport packaging is generally made from corrugated paperboard which can be designed to meet the strength and limited barrier requirements of secondary packaging.

Plastics

Plastic films and containers have many advantages over glass, metal, and paper in terms of versatility of package shapes, sizes and structural properties, light weight, toughness, low material cost, microwavability, and generally require less energy for manufacturing and transportation. The most limiting factor for the use of plastics in food and beverage packaging is permeability to water vapor, gases, and light. For example, gas molecules can dissolve and diffuse through plastic materials, and can thereby enter or leave a closed package. However, knowledge of the gas permeation

TABLE 4.1
Oxygen and Water Vapor Transmission Rates of Some Biaxially Oriented Polypropylene (OPP) Films

OPP type	OTR[a] (cm^3/m^2 day atm)	WVTR[b] (g/m^2 day)
Clear, 30 µm	<1500	<5.5
Acrylic coated 2 sides, 30 µm	650	4.0
Metallized, 30 µm	<120	<0.8
PVDC coated 2 sides, 28 µm	16	5.0
Metallized/acrylic coated, 21 µm	1–2	1.0–1.5

[a] 23 ± 2°C and 90% RH.
[b] 38°C and 90% RH.
OTR = oxygen transmision rate; WVTR = water vapor transmission rate.
Adapted from Reilly and Man.[11]

process can be used to design packages for specific products, such as fresh lettuce mix, where the permeation of carbon dioxide out of a package would be considered desirable.

Plastic films and containers vary greatly in the degree of protection they can provide as a food packaging material. For many flexible packaging applications, mono-layer materials have been largely replaced by multi-layer laminates, coated films, and metallized film structures with greatly improved barrier properties against light and oxygen. Manufacturing techniques such as coextrusion, lamination, and metallization can be employed to produce multi-layer structures with barrier properties designed to meet specific product shelf life requirements. For example, metallized biaxially oriented polypropylene (OPP) film is commonly used for packaging potato chips which require a good barrier to oxygen and light (to prevent oxidative rancidity), and to water vapor (to prevent moisture uptake and to maintain crispness). While film thickness plays an important role in water vapor and oxygen transmission rates of OPP films, metallization and/or coatings with additional barrier materials such as polyvinylidene chloride (PVDC) and acrylic polymers can greatly enhance the barrier properties of films, as shown in Table 4.1 for OPP.

Environmental Factors

The climatic environment (e.g., moisture, oxygen, light, temperature, aromas) as well as the physical distribution and storage environment (e.g., shock, vibration, compressive loading) are major extrinsic factors involved in the numerous deteriorative reactions and physical damage that can lead to losses in food quality and decreased shelf life. Details of specific deteriorative reactions and their mechanisms have been described by Fennema and Tannenbaum.[12]

The chemical deterioration of packaged foods is largely due to transfers of oxygen, water vapor, aromas, and contaminants between the internal environment

of the package and the external environment during storage and distribution conditions; for example, the mass transfer of moisture from a humid environment into a dried product.[1] Minimizing or preventing these mass transfers, and thereby achieving a desirable shelf life, depends on package integrity (including seals and closures) and requires the use of packaging materials that provide appropriate barrier properties and physical protection.

Mass Transfer

The mass transfer of water vapor, oxygen and other gases, volatile aroma compounds, and other molecules occurs through permeation, sorption, and migration in packaging systems. *Permeation* refers to a transfer of molecules through the package, either from the product to the environment, or from the environment to the product. *Sorption* refers to the penetration and dispersal of molecules from the product into, but not through, the packaging material. The sorption of flavor compounds inherent to a food product by the packaging material is commonly referred to as "scalping". In contrast, *migration* specifically refers to the transfer of low molecular weight packaging material components into the food product as a result of contact and/or interaction between the food and the package.

Gases and vapors permeate through polymeric materials by two mechanisms: capillary flow and activated diffusion.[13] Capillary flow involves the permeation of gases and vapors through pinholes, cracks, and microscopic pores in the packaging materials. Activated diffusion is a solubility-diffusion process whereby gases and vapors dissolve in the polymer at one surface, diffuse through the polymer, and then evaporate at the other surface of the polymer.

Activated diffusion is the major permeation mechanism for multi-layered or high barrier polymeric materials. Mass transfer basically follows a three-step process: (1) adsorption, (2) diffusion, and (3) desorption. Adsorption and desorption are determined by the solubility of the permeant gas or vapor molecules in the polymer. Diffusion is the transport of mass as a result of random molecular motion in the presence of concentration or partial pressure gradients.

The basic terms used to characterize permeation are: P, the permeability coefficient, which is a measure of the steady-state transfer rate of the permeant; D, the diffusion coefficient, which is a measure of how rapidly permeant molecules pass through the barrier material in the direction of lower concentration or partial pressure; and S, the solubility coefficient, which refers to the amount of permeant molecules dissolved in the material at equilibrium conditions. The simplest relationship among these terms is given by:

$$P = D \cdot S$$

In general, the flavor scalping by a polymer is related to S, while the loss of permeant from a total package is determined by P. Measurement of these coefficients has been well described by other authors.[1,14] Demorest and Mayer[15] discussed the advantages of newer testing techniques that have been developed to rapidly and reliably measure P, D, and S for various polymer/permeant pairs used in typical food packaging applications.

Moisture

One of the most common shelf life stability concerns in relation to packaging and mass transfer arises from the exchange of water vapor between the food and the surrounding atmosphere. Moisture transfer can result in a number of undesirable changes depending on the specific product and whether moisture is taken up or lost. These changes could include: physical processes such as caking of powders and loss of crispness resulting from moisture sorption, or hardening resulting from desiccation; microbial spoilage resulting from increased availability of water due to moisture sorption; and chemical processes which can be enzymic (e.g., proteolytic hydrolysis of fish products) or non-enzymic (e.g., Maillard browning reactions in dry products).[16,17]

Foods packaged in non-hermetically sealed containers are subject to moisture gain or loss depending on a number of factors:[18] the storage relative humidity; the sorption properties of the product; the water activity gradients relative to the storage atmosphere; and the water vapor permeability of packaging materials. The barrier performance of the packaging system employed can influence the relative importance of each of these factors.

The relative humidity (RH) of a storage environment is generally of little concern for food products packed in water vapor impermeable packaging such as glass and metal. However, it should be noted that processing and environmental conditions may promote the external corrosion of metal packaging materials, especially tinplate. The presence of oxygen in retorts, corrosive vapors and chemicals in the storage atmosphere, and contact with soluble chlorides, sulfates, and nitrates or other salts should be minimized to avoid failure of package integrity through corrosion.[1] Storage RH can have a significantly greater impact on the mass transfer of moisture for paper and plastic-based packaging, primarily due to the permeability of these materials. Paper-based packaging materials are hygroscopic and considered relatively permeable to water vapor, but coating with paraffin wax or polyethylene, and laminating between surface layers of various plastics can provide additional barrier properties and suitability for specific applications in moist environments. Plastic-based packaging materials provide a broad range of resistance to the permeation of water vapor, which is dependent on the chemistry of the polymer(s). The polarity of a polymer influences its resistance to gases and water vapor permeation. Highly polar polymers such as those containing hydroxyl groups (polyvinyl alcohol, cellulose) are the poorest water barriers, whereas non-polar hydrocarbon polymers such as polyethylene (PE) and polypropylene (PP) are excellent water barriers. The hydrocarbon content which is responsible for low water vapor sensitivity, however, is also responsible for relatively high gas permeabilities of many plastics. Selection of a polymeric material that can provide suitably balanced protection against moisture and/or oxygen transmission will depend on the requirements of each food product. Hydrophilic polymers exposed to moisture can also be affected significantly in terms of reduced oxygen barrier properties as discussed in the next section.

The concept of water activity in terms of deteriorative reactions and shelf life stability was addressed in Chapter 1 of this book. Water activity (a_w), defined as the ratio of the vapor pressure of water in equilibrium with the food in comparison with the vapor pressure of pure water at the same temperature, is essentially a measurement

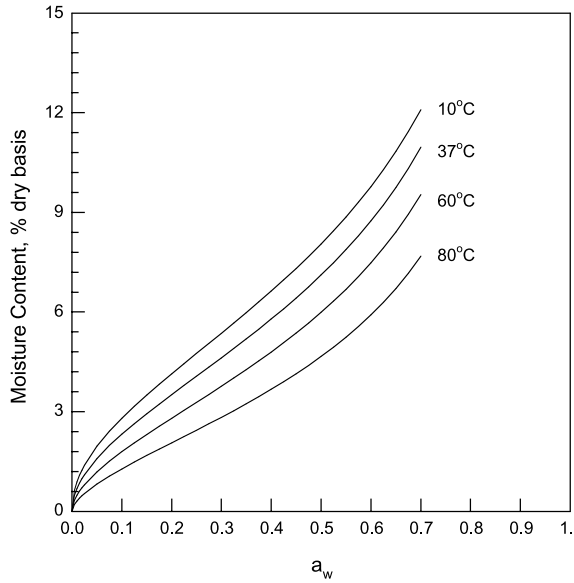

FIGURE 4.1 Representative moisture sorption isotherms for spray dried eggs. Adapted from Iglesias and Chirife.[20]

of the water affinity of that food.[19] In terms of packaging considerations, the water activity of a food product, at various moisture contents and temperatures, will determine whether this product will gain or lose moisture when exposed to an atmosphere of a given relative humidity. Water activity can be related to moisture content of a food through moisture sorption isotherms. As shown in Figure 4.1, the moisture sorption isotherm is a plot of the moisture content of a food in equilibrium with different water activities, and describes the temperature dependent water adsorption or desorption characteristics of that product. Moisture adsorbed, for example, by an inadequately protected dry food material (low a_w) exposed to a more humid environment, may initiate deteriorative reactions that lead to a reduced shelf life. The permeability of packaging materials to moisture is, therefore, a critical factor in controlling changes in the moisture content and a_w of packaged foods and, hence, their shelf life.

The water vapor transmission rate (WVTR) is the commonly used term to describe the permeability of polymer materials to water:

$$WVTR = QL/At$$

where Q is the amount of moisture passing through the barrier, L is the thickness, A is the surface area, and t is time. Previously compiled WVTR data[14] indicate that typical food packaging polymers exhibit a wide range of water vapor transmission rates (Table 4.2). Temperature and the relative humidity gradient between the two sides of the film must be stated as both affect permeability. Since WVTR values are usually specified for temperature and RH conditions, which apply to standardized

TABLE 4.2
Representative Water Vapor Transmission Rates of Various Food Packaging Polymers at 38°C and 90% Relative Humidity

Polymer	Water Vapor Transmission Rate ($g.mm.m^{-2}.day^{-1}$)
Polyvinylidene chloride (PVDC)	0.036
Polypropylene (PP), biaxially oriented	0.10
High density polyethylene (HDPE)	0.12
Polyvinyl chloride (PVC), rigid	0.36
Low density polyethylene (LDPE)	0.39
Polyethylene terephthalate (PET)	0.7
Ethylene vinyl alcohol copolymer (EVOH)	
27 mol% ethylene	2.24
44 mol% ethylene	0.56
Nylon 6, unoriented	5.9

Adapted from Brown.[14]

testing methodologies that may be different from the application conditions, permeability data from packaging handbooks should be used only to compare packaging alternatives. Estimates of changes in the moisture content of a packaged food during storage can be calculated for any packages with known WVTR properties and geometry, and for various lengths of exposure time by knowing the initial and tolerable moisture range of the product (minimum and maximum) and the ambient conditions of temperature and humidity.[1,14]

Oxygen and Other Gases

Oxygen is a critical mass transfer component in a number of deteriorative reactions that can have an effect on the shelf life of many packaged foods. Of major concern are oxidative reactions associated with food components, including lipid oxidation and the subsequent development of rancid flavors, as well as color and nutritive changes, non-enzymic browning, and vitamin C degradation. The presence of oxygen during processing and storage also permits spoilage due to growth of aerobic microorganisms and promotes various enzyme catalyzed reactions. On the other hand, the depletion of oxygen due to respiration can lead to other deteriorative effects, such as anaerobic microbial spoilage in specific products like sealed plastic packages of fresh fruits and vegetables. The shelf life of oxygen-sensitive packaged food products depends on exposure to oxygen during processing; the level of oxygen incorporated into the sealed package; and the amount of oxygen ingress into the package during storage, either through permeable packaging materials or through seals and closures.

A number of packaging innovations have been developed to control oxygen incorporation and ingress into a package based on oxygen-absorbing components or scavengers. These are successfully used mainly in Japan and Europe.[21,22] The absorbents consist of easily oxidizable substances which can be inorganic (e.g., iron

powder) or organic (e.g., ascorbic acid) and are typically contained in an oxygen-permeable sachet which is placed inside the food package. Different absorbent complexes have been designed to work most effectively at particular water activity ranges, while others can absorb both oxygen and carbon dioxide for specific use in roasted and ground coffee packages. Other packaging considerations involving oxygen ingress relate to increased oxygen permeability of some polymers exposed to moisture. Polymers such as PVDC can provide an excellent barrier to oxygen permeation under both wet and dry conditions. However, hydrophilic polymers such as ethylene vinyl alcohol copolymers (EVOH) and cellophane containing hydroxyl groups, and nylon polymers containing amide groups exhibit large increases in permeability as the polymers absorb water. EVOH has superior gas barrier properties under dry conditions; therefore, coextrusion of water-sensitive EVOH as an internal layer in structures consisting of water vapor barrier polymers such as polypropylene (PP), polyethylene (PE), or polycarbonate (PC) can help protect its oxygen barrier properties.[23] Multi-layer plastic packages containing EVOH are being used increasingly in a variety of applications, including retortable containers for thermally processed foods which may be reheated prior to consumption using a microwave oven.

Other gases such as carbon dioxide are of concern in specific packaging applications, for example, in modified atmosphere packages where maintaining a desirable gas composition within the package requires high barrier packaging materials. Permeability of packaging materials to carbon dioxide is also critical in preventing the loss of carbonation in soft drinks packaged in polyethylene terephthalate (PET), particularly in small volume containers which have a large surface-to-volume ratio. Innovative visual leak indicators based on the detection of oxygen and carbon dioxide have been reviewed recently.[24] Indicators have been developed for the detection of oxygen, for example, to confirm the effectiveness of vacuum packaging or an oxygen scavenging function, or to indicate an anaerobic state within a package. Carbon dioxide indicators have been developed to detect an increase in concentration as a sign of microbial growth.

The analysis of the transfer of oxygen and other gases through packaging materials can be carried out in a manner similar to water vapor transfer using known values for permeability of the packaging material to the particular gas, the package dimensions, and the partial pressure of the gas inside and outside the package:[14]

$$Q = \frac{PAt}{L} \Delta p$$

where Q is the total amount of permeant, P is the permeability, A is the area of permeation, t is the elapsed time, L is the thickness, and Δp is the pressure difference of the permeant across the diffusion path. This simple treatment of permeation is, however, based on a number of assumptions including that diffusion is in a steady state condition, and that both diffusion and solubility of the permeant are independent of concentration. Examples and calculations for other permeation situations, including multilayer materials, are well described elsewhere.[1,14] Table 4.3 provides representative oxygen and carbon dioxide permeabilities of some typical food packaging polymers.

TABLE 4.3
Representative Oxygen (O_2) and Carbon Dioxide (CO_2) Permeabilities of Various Food Packaging Polymers at 23 to 25°C and 75% Relative Humidity

Polymer	Permeability ($cc.mm.m^{-2}.day^{-1}.atm^{-1}$)	
	O_2	CO_2
Ethylene vinyl alcohol copolymer (EVOH)		
27 mol% ethylene	0.012[a]	0.016[b]
44 mol% ethylene	0.028[a]	0.078[b]
Polyvinylidene chloride (PVDC)	0.04	0.098
Polyethylene terephthalate (PET)	1.88	9.44
Polyvinyl chloride (PVC)	1.96	7.88
Nylon 6, unoriented	2.6	4
Polypropylene (PP), biaxially oriented	59	216
High density polyethylene (HDPE)	73	228
Low density polyethylene (LDPE)	196	984

[a] @ 20°C.
[b] @ 20°C, 65% relative humidity.

Adapted from Brown.[14]

Light

The exposure of foods to ambient lighting in food production and storage, display lighting in retail stores, and sunlight can lead to a wide variety of adverse effects which reduce shelf life, such as the development of off-flavors in dairy products or rancidity in fats and oils. The light-induced or photodegradation of foods is also a factor in the destruction of vitamins. Although the mechanisms of many of these effects have been studied extensively, research into the use of appropriate packaging to reduce or eliminate light-induced changes to improve shelf life is limited; some exceptions are cited below.

Dairy products are adversely affected by exposure to light, but the most critical effects vary depending on the specific product. The light-induced quality deterioration of milk includes the development of off-flavors and losses of vitamins A, C, and riboflavin.[25,26] The extent of the deterioration is dependent on light source, wavelength, intensity, exposure time, temperature, and therefore, also on the light barrier (or transmission) properties of the packaging material. Numerous studies, including deMan[27] and Nelson and Cathcart,[28] have been carried out to determine the most detrimental wavelengths of light and the light transmission properties of milk containers. Wavelengths in the 350 to 550 nm range were found to be the most harmful to milk quality. Paperboard cartons were shown to be opaque to wavelengths below 450 nm; however, unpigmented blow-molded polyethylene milk containers allowed 60 to 80% light transmission in the 400 to 700 nm range. Light transmission

was not entirely blocked with the use of white and yellow pigmented polyethylene. Fanelli et al.[29] found that visible and ultraviolet (UV) light screens incorporated in high density polyethylene dairy resin to protect vitamins from photodegradation provided only limited protection. Butter lipids, including cholesterol, are susceptible to oxidation where butter surfaces are illuminated by fluorescent lights or sunlight. Photooxidation can result in off-flavors, as well as cholesterol oxidation products having weak carcinogenic activity.[30] Emmons et al.[31] studied the photooxidation of butter as influenced by various wrapping materials and found that only laminates of aluminum foil and paper provided the necessary light protection required for shelf life stability. Aluminum foil was also shown by Luby et al.[32] to prevent cholesterol oxidation in butter exposed to fluorescent light, while margarine wrap, parchment, wax paper, and polyethylene films were not effective light barrier materials.

The color of red meat products, whether fresh, frozen, or cured, is one of the most important indicators that consumers use to assess quality. In frozen meats, however, discoloration by light is a significant shelf life problem because freezing does not protect against pigment photooxidation during storage. Andersen and Skibsted[33] achieved partial protection against discoloration of frozen pork patties by using polyethylene packaging which incorporated a UV-light absorber; the packaging material was also very effective in preventing light-dependent lipid oxidation in the frozen pork product. Cured meats are also very susceptible to light-induced discoloration. The pigment nitrosomyoglobin, which is responsible for the color of cured meats, dissociates rapidly upon exposure to light in the presence of oxygen.[34] Vacuum packaging using high barrier polymer films is useful to reduce discoloration; however, additional improvements in color stability have been achieved by Andersen et al.[35] with packaging systems that reduce the residual oxygen in the product below a critical limit required for photooxidation.

Temperature

The permeability of gases such as oxygen, nitrogen, and carbon dioxide through polymeric materials increases as temperature increases but the extent of these changes varies for different polymers.[1] Knowledge of the quality kinetics associated with specific food products has permitted development of mathematical models to predict shelf life from data collected at elevated storage temperatures. However, when studying foods in plastic containers or with plastic closures, it is necessary to develop new models which account for changes in permeability due to temperature for specific packages. For plastics subjected to a wide range of temperature, permeability is influenced by the glass transition temperature of the polymer, and these effects are known to vary with temperature differently for different polymers. Also, interpretation of data from multi-layer structures becomes very complex.

Another aspect of temperature in relation to shelf life is the application of thermal treatments which reduce microbial loads and retard the growth of microorganisms. When using plastic packages, processing temperatures must be kept below the glass transition temperature of the polymer in order to maintain dimensional stability of the package. Newly developed polymers and blow molding technologies have increased the maximum application temperature associated with these containers.

Clear PET and PP bottles and jars are being used for hot-fill and hold, shelf stable acid foods (pH <4.6), and pasteurized low acid foods (pH ≥ 4.6) stored below 4°C for extended periods of time. These containers have increased consumer appeal in comparison to the opaque crystalline structures developed in the past.[36]

Aroma and Flavor Transfer

Aromas and flavors are composed of volatile organic compounds. The packaging of products containing these volatiles can lead to a loss of aromas and flavors (scalping) or, conversely, the gain of off-flavors due to permeation. Flavor and aroma scalping is an increasing concern as more food products are being packaged in plastic packages.[14] Citrus juices, especially orange, have been the subject of considerable research interest with respect to flavor (limonene) scalping.[37,38]

The transport of most aroma and flavor permeants through plastic packaging materials has not been investigated as thoroughly as gas transport because of the complexity of these compounds in foods and their interaction with various polymers. In addition, the presence of co-permeant organic compounds can greatly influence permeability values compared to those values obtained for pure compounds.[22] In general, aroma and flavor compounds follow the same permeation principles in plastic materials as described previously for gases and vapors. However, determination of transmission rates is more complicated because the solubility coefficients of these organic compounds are pressure-dependent and the diffusion coefficients are concentration-dependent.[1]

Distribution and Handling

Temperature plays a key role in the rate of deteriorative reactions. Excessively high temperatures during food distribution and handling of perishable foods can accelerate deteriorative reactions and permit microbial growth. On the other hand, low temperatures can be detrimental to some fruits and vegetables that are susceptible to chilling injury, as described in Chapter 2. The development of food packaging systems that could maintain the temperature of products within a specified range would be desirable. This goal has been shown to be feasible through research on the use of phase-changing materials (such as water/ice or saturated salt solutions) that serve as a heat sink or source, by undergoing thermophysical transitions which can effectively absorb or release heat with little change in temperature.[39,40] Packaging considerations in temperature control are particularly important for refrigerated foods. Refrigerated or chilled foods include a wide range of prepared foods that require refrigeration for safety and an extended shelf life. A major challenge to the food industry is to develop strategies for maintaining a high quality temperature controlled distribution system for these perishable foods.[41] Time-temperature indicators (TTI) have the potential to be used in monitoring or predicting safety and shelf life of refrigerated foods.[42,43] These devices, either placed on food packages or on shipping containers, monitor the combination of time and temperature to which they are exposed and exhibit a visible change (e.g., color) in response to the temperature exposure history.

Product stability during distribution is also influenced by the maintenance of package integrity. Vibration and impacts during shipping and handling may cause flex cracking in plastic packages and failure in barrier materials, resulting in accelerated degradative reactions and increased exposure to off-flavor permeation.[14]

SHELF LIFE TESTING AND PREDICTION

Packaging considerations are critical to shelf life testing: namely, to understand the requirements of the product, to understand package alternatives, and then to select suitable candidate packages. An understanding of the intrinsic and extrinsic stability of a food product can have an important influence on packaging considerations for shelf life. Misdirected food product, process, and packaging development comes from not clearly recognizing and monitoring these different product stabilities during shelf life tests.[4]

Specific approaches and procedures for shelf life testing and prediction have been described thoroughly by numerous authors.[1,4,5,44] In general, the key approach to shelf life testing is to first identify the major failure modes or mechanisms for a product. Shelf life prediction becomes very complex when a food system has several modes of deterioration, which may each have its own temperature sensitivity. However, shelf life at any given temperature will be determined by that mechanism which proceeds most rapidly and thus causes the shortest life.[45] Traditional approaches to shelf life testing generally involve storage tests where products are maintained under a set of controlled (specific temperature and relative humidity) or uncontrolled (i.e., warehouse) conditions. However, an overestimation of shelf life is likely when only some of the conditions a product may encounter during distribution are taken into account. Environmental conditions can also be accelerated by a known factor so that the product deteriorates at a faster than normal rate. However, accelerated shelf life conditions may also influence the barrier properties of packaging materials and thereby introduce additional degradation reactions that do not occur under normal storage conditions.

FUTURE DIRECTIONS

Efforts to improve food shelf life stability may be attempted through the introduction of new food preservation technologies, including new packaging materials and forms, but the predominant concern must always be that the resulting products do not compromise consumer safety.[46] As illustrated in Figure 4.2, successful manufacturing and packaging of high quality food products should be considered from a systems approach, where knowledge of the properties of each component and interactions among them is used to understand and predict the performance of the overall system.

A food package will be designed to provide containment, protection, convenience, and communication. However, using the systems approach, the performance of this package must also be evaluated in terms of its compatibility with the processing conditions applied for food quality preservation, which must be maintained

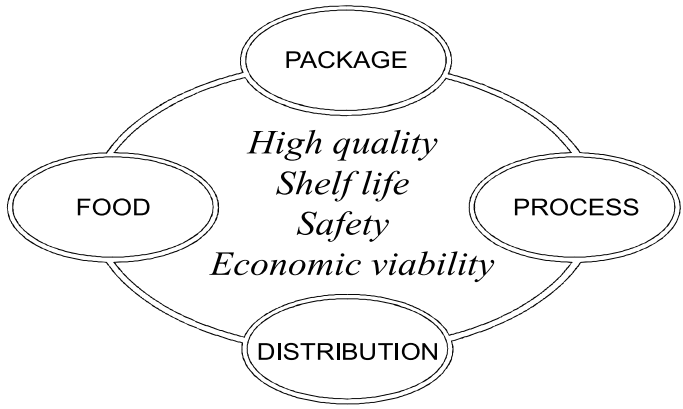

FIGURE 4.2 A systems approach to achieving shelf life stability by considering the components of an integrated system, and the central objectives of food preservation.

throughout distribution and storage. Additional factors influencing overall packaging performance include environmental responsibility, as well as the economic viability of these packaging choices. Some food products have been classified according to the degree of protection required from oxygen, other gases and volatile compounds, and moisture gain or loss, assuming a 1-year shelf life at 25°C.[1] These data are invaluable in assessing the major packaging requirements for a food item in order to predict whether a given package can provide the desired shelf life. Research to obtain additional data of this nature for a much broader variety of food products is essential for improved shelf life prediction.

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Section II

Chemical Factors

5 Controlled and Modified Atmosphere Storage

G. Mazza and D. S. Jayas

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INTRODUCTION

Controlled atmosphere (CA) and modified atmosphere (MA) storage are technologies for extending the shelf life of foods, especially fruits and vegetables; and for eliminating pests in stored grains and oilseeds. The most important application of CA and MA is for long-term storage of apples, but the shelf life of certain other fruits (pears, sweet cherries) and vegetables (cabbage) can also be extended by these methods.[1,2] In addition, there is considerable evidence that MA can extend the shelf life of meat, fish, poultry, fresh pasta, sandwiches, eggs, and bakery products.[3-6] Because grains and oilseeds are more stable than high moisture foods (e.g., fruits, vegetables, meats), CA and MA are used primarily for disinfestation rather than for increasing the shelf life.

This chapter provides an overview of the benefits and concerns of CA and MA storage, the methods for creating and maintaining MA conditions, and the effects of CA and MA on shelf life extension and quality of food products, especially fruits, vegetables, grains, and oilseeds.

CONTROLLED AND MODIFIED ATMOSPHERES FOR FRUITS AND VEGETABLES

Principle

The principle behind controlled and modified atmosphere technologies is to reduce the rate of respiration, reduce microbial growth, and retard enzymatic spoilage by changing the gaseous environment surrounding the food product. This is achieved by reducing the concentration of oxygen (O_2), which is required in respiration, or by adding an inhibitory gas such as carbon dioxide (CO_2) or carbon monoxide (CO). The balance between O_2 and CO_2 is critical, and an optimal ratio is required for each specific product. A major difference between CA and MA storage is in the degree of control of the gaseous composition of the storage atmosphere. The CA implies a higher degree of control than MA in maintaining specific levels of O_2, CO_2, and other gases. Also, in MA storage the composition of the atmosphere surrounding the product is generally created and maintained by the interaction of the commodity's respiration with the permeation of respiratory gases through the packaging material.[2,8,9] Modified atmosphere conditions can also be established and adjusted by pulling a slight vacuum and replacing the package atmosphere with a desirable gas mixture, which can be further adjusted through the use of O_2, CO_2, or ethene (C_2H_4) absorbers.[2,9]

In CA storage facilities, both temperature and gas composition of the storage atmosphere are regulated or controlled. The gas concentration ranges encountered in CA storages are 1 to 10% O_2, 0 to 30% CO_2, and the balance is nitrogen (N_2).[10-12] Air consists of approximately 78% N_2, 21% O_2, 0.03% CO_2, and traces of several other gases that have no physiological significance.

Benefits and Concerns of CA and MA Storage

The benefits and concerns of CA and MA storage have recently been reviewed by several authors.[2,5-8,13-16] The benefits can be divided into quality advantages and marketing and distribution advantages. The improvements in quality arise from the general reduction in the rates of metabolic processes, retardation of physiological aging, enzymatic spoilage, and reduction in microbial growth. In fresh fruits and vegetables stored under optimal CA or MA, practical quality advantages include:

1. Reduction in chlorophyll breakdown, with a resulting higher color stability.
2. Reduction in enzymatic browning in cut produce, whenever low levels of O_2 are used.
3. Improvement in texture caused by the action of CO_2 on enzymes acting on cellular membranes.

4. Reduction in some physiological disorders induced by C_2H_4, such as scald of apples and pears and chilling injury of citrus fruits, avocado, chili pepper, and okra.
5. Reduction in microbial activity, especially molds.

The marketing and distribution advantages of CA or MA technologies include:

1. Reduction in fresh food spoilage and quality loss through the distribution at the retail level.
2. Expanded radius of distribution systems and market area.
3. Improved branding options and product differentiation.
4. Potential for increased profitability in all fresh or chilled food operations.

In considering the above described benefits, a number of potential problems associated with CA or MA storage must be recognized. Above all is the potential health hazard associated with these technologies, especially modified atmosphere packaging or MAP.[3,17] It has been pointed out that the same principles of atmosphere modification responsible for all the benefits of CA or MA are also the main cause of controversy surrounding the potential health hazards associated with these technologies.[3,18,19] Modification of the atmosphere and, in particular, the reduction or elimination of O_2 from the package head space will in many cases disturb the equilibrium of the atmosphere in favor of anaerobic microorganisms. The aerobic bacteria that normally spoil the product, and in so doing warn consumers of any potential health hazard, may find themselves at an atmospheric disadvantage and their growth inhibited. In the absence of competing aerobic organisms, anaerobic nonproteolytic toxin producers, such as *Clostridium botulinum*, are likely to have the right conditions for optimum growth, but their presence may not be obvious to the senses. The food may appear to be acceptable long after it has become microbiologically unsafe. According to Lioutas,[15] this issue probably represents the greatest vulnerability for the CA or MA technology and could potentially inhibit any further application.

Other concerns associated with CA or MA technology include:

1. Development of off-flavors due to accumulation of ethanol, acetaldehyde, and other volatiles.
2. Increased softening in products such as cucumbers, cauliflower, celery, and onion.
3. Development of physiological disorders, such as brown stain on lettuce, internal browning and surface pitting of pome fruits, and blackheart of potatoes induced by inappropriate modified atmospheres.
4. Increased susceptibility of some products (celeriac, carrot, pepper, Chinese cabbage, and citrus fruits) to post-harvest pathogens.

Methods for Creating and Maintaining MA

The reduction of O_2 levels inside an enclosed space (storage room or polymeric film package) can be achieved biologically through respiration of the food product, or it

can be obtained by replacing the atmosphere of the storage space with the desired gas mixture. In the first case, the reduction of O_2 to levels of 2 to 3% may require up to 25 days[1,20] and depends on the characteristics of the commodity and the degree to which the storage room is airtight or the gas permeability characteristics of the packaging film.[2,21] When a non-biological or active modification system is used, O_2 can be reduced to levels of 2 to 3% within hours. It has been shown, for some commodities, that a shorter O_2 reduction or pull-down period results in better post-storage quality.[1,20,22] Rapid O_2 pull-down or rapid CA requires the shortest possible time for crop harvest, room loading, product cooling, room closure, and O_2 reduction. Rapid O_2 reduction is obtained by either N_2 flushing or catalytic combustion. In addition, O_2 absorbers may be used, especially in MA storage. There are a wide variety of O_2 absorbers, e.g., dithionite, ascorbic acid, alkali containing glucose, H_2 gas in the presence of palladium catalyst, and iron-based products.[23] Most commercially available O_2 absorbers utilize iron oxide (FeO), which becomes iron oxides (Fe_2O_3 and Fe_3O_4) and hydroxides ($Fe(OH)_2$ and $Fe(OH)_3$) after absorption of O_2.[9,23] From the reaction mechanism of the O_2 absorber(s) and volume of the storage space, it is possible to determine the amount of FeO needed to lower the concentration of O_2 to approximate desired pre-chosen values.

Modified atmosphere packages are dynamic systems where respiration and permeation occur simultaneously. In order to achieve and maintain a satisfactory atmosphere within a package, the rates of CO_2 production and O_2 consumption by the food product must be equal to diffusion rates of the respective gases through the package at a given temperature.[8,24] Factors affecting respiration of produce in the package include mass and type of food product, temperature, O_2 and CO_2 partial pressures, ethylene levels, and light. Factors affecting gas permeability through the package include type, thickness, and surface area of the packaging film, as well as temperature, relative humidity, and gradient of CO_2 and O_2 in the sealed package.[2,7,8,25]

There is a wide variation in respiration rates between commodities (Table 5.1).[26] In intact produce this is a function of genotype, anatomical part, and physiological state at harvest. Even within a species, the same anatomical tissue can have different respiration rates depending on the cultivar. Cutting of produce can also influence

TABLE 5.1
Respiration Rates of Selected Commodities

Commodities	Respiration Rate at 5°C (mL CO_2 kg^{-1} h^{-1})
Dried fruits and vegetables, nuts	<5
Apple, grape, potato (mature), onion, garlic	5–10
Apricot, carrot, cabbage, cherry, lettuce, peach, plum, pepper, tomato	10–20
Blackberry, cauliflower, lima bean, raspberry, strawberry	20–40
Brussels sprouts, green onion, snap bean	40–60
Asparagus, broccoli, mushroom, pea, spinach, sweet corn	>60

Adapted from Rizvi.[26]

TABLE 5.2
Influence of Cutting on Respiration of Broccoli and Carrots

Product	Respiration Rate (mL CO_2 kg^{-1} h^{-1}) 10°C	20°C
Broccoli		
Whole heads	59	104
Cut florets	78	147
Carrots		
Whole unpeeled	9	29
Whole peeled	12	26
Julienne	65	145

Adapted from McLachlan and Stark.[27]

the respiration rate of the tissue,[27,28] and most authors have observed an increase in respiration in cut tissues (Table 5.2).[27] The difference in respiration rate for whole and cut produce has been attributed to differences in gas exchange area and metabolic activity.[29] Fruits such as apples, pears, tomatoes, and avocado experience a marked and transient increase in respiration during ripening, known as the climacteric rise, associated with an increased production of ethylene. The rate of respiration in plant products is also influenced by changes in O_2 and CO_2 in the atmosphere (Table 5.3).[2] Exposure of fresh produce to O_2 concentrations below their tolerance limits or to CO_2 levels above their tolerance limits (Table 5.4)[2] may increase anaerobic respiration and lead to development of off-flavors.

Storage Requirements and Quality Changes for Fruits and Vegetables

Fruits

Conditions for the optimal storage of fruits and vegetables are influenced by a variety of factors including crop species, cultivar, growing conditions, maturity, quality temperature, relative humidity, packaging, and storage duration. Storage under CA and MA is influenced by the concentration of O_2, CO_2, ethylene, and other gases.[30] Table 5.5[11,31] shows CA storage conditions for the most commonly grown apple cultivars. Storage life quality and susceptibility to disease and physiological disorder can be modified considerably by production practices, weather, soil, and other factors. Delay in cooling after harvest of apples, for instance, can result in reduced storage life because of accelerated softening and ripening, and increased probability of scald development, breakdown, and decay.[1,31] Most apple cultivars benefit from storage at temperatures just above the freezing point of the fruit, a relative humidity of 90 to 95%, O_2 concentration of 1 to 2%, and CO_2 level of 1 to 2% (Table 5.6).[31]

TABLE 5.3
Respiration Rates of Selected Fruits and Vegetables at Different Temperatures and in Different Atmospheres

Commodity	Cultivar	Atmosphere	Temperature (°C)	Respiration (mL kg^{-1} h^{-1})
Apple	Granny Smith	Air	0	1.0
			10	3.6
			20	7.4
		2% O_2 + 2% CO_2	0	0.1
			10	0.7
			20	1.4
Bean, green	Blue Lake	Air	5	17.5
			10	28.3
			20	59.6
		3% O_2 + 5% CO_2	5	10.8
			10	16.5
			20	28.0
Broccoli	Green Valiant	Air	0	10.0
			5	21.0
			10	85.0
			20	213.0
		1.5% O_2 + 10% CO_2	0	7.0
			5	11.0
			10	15.0
			20	33.0
Cabbage	Decema	Air	0	1.5
			10	4.0
			20	10.0
		3% O_2	0	1.0
			10	3.0
			20	6.0
Chili pepper	Anaheim	Air	5	3.4
			10	4.8
			12.5	12.8
		2% O_2	5	2.7
			10	3.3
			12.5	6.9
		5% O_2	5	3.3
			10	3.8
			12.5	10.9
		Air + 5% CO_2	5	3.2
			10	4.5
			12.5	7.6
Mango	Tommy Atkins	Air	10	15.0
			15	31.0
			20	61.0

TABLE 5.3 (continued)
Respiration Rates of Selected Fruits and Vegetables at Different Temperatures and in Different Atmospheres

Commodity	Cultivar	Atmosphere	Temperature (°C)	Respiration (mL kg^{-1} h^{-1})
		4% O_2 + 7% CO_2	10	8.0
			15	14.0
			20	44.0
Tomato	Ace (picked mature-green)	Air	12.5	9.0
			20	18.0
		Air + 1% CO_2	12.5	8.0
			20	16.0
		Air + 3% CO_2	12.5	8.0
			20	15.0
		Air + 5% CO_2	12.5	7.0
			20	13.0
		Air + 10% CO_2	12.5	6.0

Adapted from Kader et al.[2]

TABLE 5.4
Tolerance to Low O_2 and High CO_2 of Selected Fruits and Vegetables

Commodities	Minimum O_2 Concentration Tolerance (%)	Maximum CO_2 Concentration Tolerance (%)
Some cultivars of apples and pears	1.0	2
Lettuce, cabbage, apricot	2.0	2
Pepper, tomato, artichoke	3.0	2
Avocado	3.0	5
Citrus fruits, potato, asparagus	5.0	10
Garlic, onion, broccoli	1.0	10
Brussels sprouts, cauliflower, kiwifruit, peach, nectarine, plum	2.0	5
Green beans, pineapple	2.0	10
Strawberry, sweet corn, cantaloupe	2.0	15

Adapted from Kader et al.[2]

For apples, as well as for most other fruits, it is important that whenever O_2 concentration in the store is low, CO_2 concentration must also be low (Tables 5.5 and 5-6).[11-31] This is to prevent physiological alterations and to secure better organoleptic characteristics of the fruits. In pears stored under CA approximately 30% of the volatiles were detected, as compared to storage in air; and in apples stored

TABLE 5.5
Controlled Atmosphere Storage Conditions for Apple Cultivars

Cultivar	Temperature[a] (C)	Relative Humidity (%)	O_2 (%)	CO_2 (%)	Approximate Storage Life (Months)
Belle de Boskoop	3	92	2	1	5–6
Cox's Orange Pippin	3.5	92	2	1	5–6
Delicious	–0.5	90	2	1	6–7
Elstar	3	90	3	<1	5–6
Empire	0	90	2.5	3	4–6
Gala	0.5	90	2	2	5–6
Gloster	0.5	90	3	3	4–6
Golden Delicious	–0.5	92	<3	5	5–7
Granny Smith	0	90	2	1	5–6
Idared	0	90	3	5	6–8
Jonagold	0	90	3	5	5–7
Jonathan	0	88	2	2	5–6
Laxtons	2	92	3	3	5–6
McIntosh	3	90	3	5	6–7
Mutsu	0.5	92	3	2	8–9
Rome Beauty	0	90	2	2.5	8–9
Spartan	0	90	2	2.5	6–8
Stayman	3	92	2	2	6–7

[a] Stated value derived from information received from the apple growing areas throughout the world.

Adapted from Meheriuk[11] and Gorini et al.[31]

TABLE 5.6
Controlled Atmosphere Storage Conditions for Selected Fruit Species

Species	Temperature (C)	Relative Humidity (%)	O_2 (%)	CO_2 (%)	Approximate Storage Life
Avocado	7–12	90	2–3	3–10	2 months
Cherry	0	95	3–10	10–12	30 days
Chestnut	0	90	3	10	3 months
Kiwifruit	0	98	2	4–5	7 months
Nectarine	–0.5–0	95	2	5	50 days
Peach	–0.5–0	95	2	4–5	40 days
Pears					
Anjou	–0.5–0	90	1–2	0.5–2	6–7 months
Bartlet t	–1.0–0	90	2–3	4–5	4–5 months
Plum	0	95	2	5	45 days

Adapted from Gorini et al.[31]

under low O_2 conditions, the loss of volatiles is considerable.[32-40] The severity of flavor loss depends on the atmospheric composition and duration of storage. The higher the CO_2 concentration, the lower the O_2 concentration, and the longer the duration in CA storage, the greater the flavor loss. Disorders of apples likely to occur in storage include bitter pit, scald, shriveling, water core, chilling injury, coreflush or core browning, decay, and breakdown. Of these disorders, scald, coreflush, breakdown, and decay can be modified by handling and storage practices.[41] Delays in storage, high temperature, hot weather before and during harvest, immaturity, high fruit nitrogen, and extended storage periods all tend to increase scald. Controlled atmosphere and low O_2 storage reduce scald.[20,34] Coreflush, or core browning, a common storage disorder of McIntosh apples is associated with low storage temperature and senescence.[42] This disorder, which is accentuated by immaturity and excess fruit nitrogen level, generally appears in apples after 3 to 4 months of storage at –1 to 0°C and is intensified by a further 5 to 6 days at room temperature.

Apricots, peaches, cherries, raspberries, strawberries, and plums have a short storage life. In air at –1 to 0°C and 85 to 95% relative humidity, sweet cherries have a storage life of about 3 weeks, sour cherries only a few days. Mature soft fruit normally has a maximum 2-week storage life, whereas firm fruit can be stored for 2 to 4 weeks. Fresh strawberries can be held for a maximum of 10 days and raspberries can be held for a maximum of 5 to 7 days at 0°C and 85 to 95% relative humidity. Controlled atmosphere storage of soft and stone fruits can provide additional storage life. Controlled atmosphere storage of apricots using 3 to 5% CO_2 and 2 to 5% O_2 at –1 to 0°C can extend the storage life from 12 to 14 days to 18 to 20 days.[43] Similarly, storage of sweet cherries in atmospheres containing 20% CO_2 and 21% O_2 at –1 to 0°C can extend storage life for 5 to 6 weeks.[44] Recommendations for CA storage of pears, peach, nectarine, plum, avocado, cherry, chestnut, and kiwifruit are given in Table 5.6.[31] For peach, the CO_2 and O_2 concentrations given in that table may be too low. In tests with various atmospheres, Deily and Rizvi[45] found optimum gas concentrations for peach to be 10 to 15% O_2 and 15 to 25% CO_2.

Vegetables

Recommended storage temperature, gas concentration, and storage life expectancy of selected vegetables are given in Table 5.7.[10] For vegetables, such as potatoes, carrots, garlic, and horseradish, which can be successfully stored in air, controlled atmosphere storage is not an economical option. For most other vegetables, the benefits of CA are generally low and consequently the level of application is slight.[10] The highest level of appreciation of CA in vegetables is with broccoli, cabbage, lettuce, asparagus, and Brussels sprouts. In broccoli, CA may extend the storage life by 1 to 2 weeks over that normally expected in cold storage.[46,47] Optimum CA conditions (10% CO_2, 1% O_2 at 3 to 5°C) have been shown to retard chlorophyll loss, flower bud senescence, and toughening of broccoli.[1] At O_2 levels below 0.5%, however, strong off-odor and off-flavor develop.[47] A recent study by Forney et al.[48] identified ethanol, methanethiol, hydrogen sulfide, ethyl acetate, dimethyl disulfide, acetaldehyde, methyl acetate, and octane in broccoli stored under a CA containing 0.5% O_2. They considered the methanethiol primarily responsible for the objectionable

TABLE 5.7
Controlled Atmosphere Storage Conditions for Selected Vegetables

Vegetable	Temperature (C)[a]	Atmosphere[b]		
		O_2 (%)	CO_2 (%)	Level of Application
Artichokes	0	2–3	2–3	Slight
Asparagus	2	Air	10–14	High
Beans, green snap processing	7	2–3	4–7	Slight
Broccoli	0	1–2	5–10	High
Brussels sprouts	0	1–2	5–7	Slight
Cabbage	0	2–3	3–6	High
Cauliflower	0	2–3	3–4	Slight
Celeriac	0	2–4	2–3	Slight
Celery	0	2–4	3–5	Slight
Chinese cabbage	0	1–2	0	Slight
Cucumbers, fresh	12	1–4	0	Slight
Pickling	4	3–5	3–5	Slight
Leeks	0	1–6	5–10	Slight
Lettuce (crisphead)	0	1–3	0	Moderate
Cut salad	0	1–3	0	High
Lettuce (leaf)	0	1–3	0	Moderate
Cantaloupes	8	3–5	10–20	Slight
Mushrooms	0	Air	10–15	Moderate
Okra	10	Air	4–10	Slight
Onions	0	0–1	0	Slight
Onions (bunching)	0	2–3	0–5	Slight
Parsley	0	8–10	8–10	Slight
Pepper (bell)	12	2–5	0	Slight
Pepper (chili)	12	3–5	0–5	Slight
Processing	5	3–5	10–20	Moderate
Radish (topped)	0	1–2	2–3	Slight
Spinach	0	7–10	5–10	Slight
Sweet corn	0	2–4	5–10	Slight
Tomatoes	12	3–5	2–3	Slight
Witloof chicory	0	3–4	4–5	Slight

[a] A relative humidity of 90 to 95% is usually recommended.

[b] Specific CA recommendations depend on cultivar, temperature, and duration of storage.

Adapted from Salveit.[10]

odor. Packaging of broccoli in polymeric films has been shown to extend shelf life and improve retention of color and nutrients, especially ascorbic acid.[49,50]

Winter cabbage, held at 0°C and 90 to 95% relative humidity has a storage life of 3 to 7 months. In CA (5% CO_2; 2.5% O_2 at 0°C) cabbage can be stored for up to 10 months. This treatment is effective in preserving green color, maintaining succulence, and greatly retarding senescence.[1,51] Cauliflower has a relatively short

shelf life of only 4 weeks under optimal conditions of 0°C and 100% relative humidity.[52] Storage life of this produce, however, can be extended with the use of CA. Romo-Parada et al.[53] reported that under a CA of 3% O_2 and 2.5 to 5% CO_2 cauliflower was still commercially acceptable after 52 days of storage. Storage under higher CO_2 levels, however, causes yellowing, softening, and microbial breakdown Lettuce is also a short storage crop, and when its temperature is reduced rapidly to 0°C and held at this level while the humidity is kept very high, head lettuce can be stored for 2 to 3 weeks.[1] Under CA of 2.5% CO_2 and 2.5% O_2 at 2°C head lettuce can be stored for up to 75 days.[54] Studies of CA storage of minimally processed lettuce have shown that CA (3% O_2, 10% CO_2) can prolong the shelf life of shredded iceberg lettuce by limiting plant and microbial enzyme activity, without appreciably affecting microbial development.[55]

CONTROLLED ATMOSPHERE STORAGE OF CEREALS AND OILSEEDS

Principle

In CA storage, an environment that is lethal to stored-grain pests is created by changing the proportions of CO_2, O_2, and N_2 in the atmosphere surrounding the bulk grain in storage structures. Controlled atmosphere storage is different from an air-tight storage where gas ratios change naturally, although both are carried out in more or less gas-tight storage structures. In air-tight storage, the depletion of O_2 and the accumulation of CO_2 occur due to the metabolic processes of the insects, microflora, and the stored grain. Because the effectiveness of the air-tight storage is largely dependent upon the build-up of an infestation in the grain, it is not considered a satisfactory method. Controlled atmospheres are attained by introducing CO_2 or N_2 from external sources, possibly prior to the build-up of infestation, thereby preventing damage to the stored grain. Air-tight storage and CA storage are collectively referred to as modified atmospheres (MA) and typical compositions of various controlled atmospheres in stored grain are given in Table 5.8.[56]

Advantages and Limitations

The main advantage of CA to disinfest grain is its potential to replace pesticides used in the grain industry. The CA-treated grain does not have any chemical residues which can cause considerable health concerns. In addition to providing an effective control of pests, CA storage prevents mold growth, preserves grain quality, and maintains a high level of germination in the stored grain.[57] However, as with any other method of pest control, CA storage has limitations. The major limitation appears to be the high initial cost of air-tight storage structures, and the cost of sealing existing structures to the desired air-tightness.[58,59] There is also the cost of the generation and transportation of the gas. The interaction of CA gases with the storage structure can cause some practical problems. The introduction of CO_2 or N_2 into airtight structures has the potential to increase the internal pressure on bin walls, and steps need to be taken to permit pressure equilibration. The only chemical

TABLE 5.8
Composition of Typical CAs Easily Created in Sealed Grain Storages

Type of CA	Atmosphere Source	Composition (%)			
		O_2	CO_2	N_2	Ar
Low O_2	Nitrogen from liquid or other source (<0.1% O_2)	0.5		99.4	
Low O_2	Propane burner	0.5	13.4	85.1	1.0
Low O_2	Producer gas burner	0.5	20.8	78.2	0.5
Low O_2	CO_2 from liquid or other source (<0.1% O_2)	0.5	97.5	2.0	
Hermetic storage	Metabolism within the storage	2.0	18.0	81.0	1.0
High-CO_2	CO_2 from liquid or other source (>98% pure)	4.2	80.0	15.6	0.2
High-CO_2	CO_2 from liquid or other source (>98% pure)	8.4	60.0	31.2	0.4
High-CO_2	CO_2 from liquid or other source (>98% pure)	12.6	40.0	46.9	0.5

From Banks, H.J. and Fields, P.G., Physical methods for insect control in stored-grain ecosystems, in *Stored Grain Ecosystems,* Jayas, D.S., White, N.D.G., and Muir, W.E., Eds., Marcel Dekker, Inc., New York, 1995, 353. With permission.

reaction observed with CA involves CO_2 in concrete silos.[60] Carbon dioxide is bound by concrete through carbonation, which can result in reduced pressures developing in well-sealed new concrete bins. Also, carbonation of concrete can gradually extend to a depth where reinforcing steel is exposed and the steel may eventually corrode and weaken the storage structure.

Effect of Controlled Atmospheres on Mortality of Insects

The effectiveness of CA in eliminating insects is dependent on several abiotic (e.g., gaseous composition, relative humidity, temperature, exposure time, and total gas pressure) and biotic (e.g., insect species, life stage, and infestation level and distribution) factors. Interaction of all these factors must be understood to create an environment that is lethal to all the pests in the stored grain. Researchers around the world have been conducting empirical studies to define atmospheric conditions for eliminating several pest species from grain.

Harein and Press[61] studied the mortality of adults and larvae of the beetle *Tribolium castaneum* (Herbst), and larvae of the moth *Plodia interpunctella* (Hubner), when exposed to binary and ternary combinations of the atmospheric gases (CO_2, N_2, and O_2) at various temperatures (15.6, 26.7, and 37.8°C). In general, the mortality of insects increased with a decrease in O_2 concentration, an increase in CO_2 concentration, an increase in length of exposure (from 7 to 14 day), and an increase in temperature (15.6 to 37.8°C). Atmospheres containing >36% CO_2 were lethal to test insects even when O_2 concentration was as high as 15%. In the absence of CO_2, however, the O_2 concentration must be reduced to <1% to obtain lethal gaseous atmospheres. *P. interpunctella* larvae were more susceptible than *T. castaneum* adults or larvae under all test environments. Lindgren and Vincent[62] determined the mortality of all life stages of the weevil *Sitophilus granarius* (L.) and *S. oryzae*

(L.) in various atmospheres of CO_2, N_2, or He, and in various combinations of CO_2-air and CO_2-O_2. Adults of both species when exposed to either of the three gases had an increase in mortality with an increase in temperature from 16 to 27°C. It was noted that 100% CO_2 was less toxic to both species than any combination of CO_2 (40 to 90%) with either air or O_2. Adults of *S. granarius* were the most susceptible to atmospheres of CO_2, N_2, or He, followed by larvae, pupae, and eggs. Likewise, adults of *S. oryzae* had the highest susceptibility followed by larvae, eggs, and pupae under similar gas conditions. Jay and Pearman[63] tested the susceptibility of the adults of *T. castaneum*, *T. confusum* (duVal), and a malathion-resistant strain of *T. castaneum* under several gas compositions. In most cases an increase in CO_2 or N_2 combined with a decrease in O_2 resulted in an increase in insect mortality. Three days of exposure to <1% O_2 coupled with >95% CO_2 or >99% N_2 resulted in 100% mortality of all insects. Both inter- and intra-specific differences in mortality were observed under altered gas conditions when other variables like temperature, relative humidity, and insect age were kept relatively constant. In a similar study, Aliniazee[64] investigated the effect of several binary gas mixtures containing CO_2, N_2, or He with O_2 (2 to 20%) on the mortality of *T. castaneum* and *T. confusum*. The adults of both species were killed when exposed to <2% O_2 in combination with N_2 or He for about 96 h. Carbon dioxide (80 to 98%) alone appeared to be responsible for mortality of insects when used in combination with O_2 concentration ranging from 20 to 2% as all combinations produced similar results. The data obtained by exposing mature and immature stages of both species to 100% CO_2 indicated that adults were the most susceptible stage followed by larvae, eggs, and pupae. The insect mortalities increased with an increase in temperature (15.6 to 26.7°C), and a decrease in relative humidity. Under airtight conditions, the adults of *T. castaneum* were able to use the available O_2 more efficiently (O_2 concentration reduced from 20.9 to 1.7% in 7 d) than those of *T. confusum* (O_2 concentration reduced from 20.9 to 1.6% in 5 d) indicating differential respiration rates of these species.

The adults of five stored-grain insects (*S. granarius*, *T. castaneum*, *Oryzaephilus surinamensis* (Linnaeus), *Cryptolestes ferrugineus* (Stephens), and *Rhyzopertha dominica* (F.)) were exposed to atmospheres similar to those obtained by the combustion of hydrocarbon fuels (10 to 30% CO_2, 0.5 to 2.6% O_2, and balance N_2) at 20°C and 70% relative humidity.[65] The gas mixtures containing 1.0 to 1.6% O_2 and >10% CO_2 were lethal to all species after 7 days of exposure. *C. ferrugineus* was relatively more tolerant than any other species when O_2 concentration was 2.0 to 2.6%. *S. granarius* survived the longest at an O_2 concentration of 0.5%. Jay[59] treated immature stages (1 to 5 week old) of *S. oryzae* and *R. dominica* with an atmosphere containing high concentration of CO_2 (>50%) and low O_2 (<12%) at high relative humidity (>50%), and at two different temperatures (2 and 16°C). After an exposure for 2 weeks to 60% CO_2, 100% reduction in emergence was obtained at both temperatures. *S. oryzae* was relatively more susceptible than *R. dominica* at low temperature. He also studied the mortality of *Trogoderma glabrum* (Herbst) and *T. variabile* (Baillon) larvae when exposed to 61 and 99% CO_2 at elevated temperatures (33 and 38°C). Complete mortality was obtained only with 99% CO_2 at 38°C. The mortality ranged from 15 to 32% at 61% CO_2 at both temperatures.

Similar results were obtained with pupae of these two species. The exposure of larvae, pupae, and adults of *O. surinamensis* to 77% CO_2 at a relative humidity of 50% and temperatures ranging from 16 to 32°C showed that the pupae were more tolerant than either adults or larvae. The exposure time to obtain 100% mortality decreased with an increase in temperature. In another study, various developmental stages of *Trogoderma granarium* Everts were subjected to controlled atmospheres containing 60% CO_2 in air at 20 or 30°C and a relative humidity of 60%.[66] Although the eggs, pupae, and adults all died within 6 days of exposure, some larvae survived even after 16 days. Further exposure of larvae to 45, 60, and 75% CO_2 in air indicated that an exposure of >15 days to 75% CO_2 at 30°C is required for 100% mortality of larvae of this species.

The importance of relative humidity in the control of stored-product insects with controlled atmospheres was studied by Jay et al.[67] The adults of *T. castaneum*, *T. confusum*, and *O. surinamensis* were exposed to binary mixtures of O_2 and N_2 (<1% O_2: >99% N_2), and ternary mixtures of O_2, N_2, and CO_2 (9.8% O_2: 30.5% N_2: 59% CO_2 or 13.1% O_2: 49.3% N_2: 37.6% CO_2) at four different relative humidities (7 to 9, 30 to 33, 53 to 60, or 68 to 73%). The mortality of insects increased significantly with a decrease in relative humidity under all gas compositions. Navarro and Calderon[68] studied the effect of relative humidity (20 to 22, 54 to 55, and 95 to 96%) on the mortality and weight loss of 0- to 24-h-old pupae of *Ephestia cautella* (Wlk.) under various concentrations of CO_2 (0, 21, 51, 88%) at 26°C. The pupal mortality was high at low relative humidity (<55%) at all CO_2 concentrations. At high relative humidity (95%), however, complete mortality was obtained only when the CO_2 concentration was 88%. After 6 days of exposure, the pupal weight loss was high (45 to 55%) at low relative humidity (<55%) but minimal (<16%) at high relative humidity (95%). Therefore, the pupal mortality may be attributed to the loss of water at the low relative humidity. Spratt[69] investigated the productivity of *T. castaneum* and *T. confusum* in an atmosphere containing 5 to 20% O_2 with or without 10% CO_2. The relative humidity (70%) and temperature (30°C) were kept nearly constant. The egg production was about 60% higher in the presence of CO_2 at O_2 concentrations of 5.0, 7.5, and 10.0%. No difference in egg production, however, was observed in the presence or absence of CO_2 at 21% O_2. Egg hatch was seriously impaired at 5% O_2 with or without CO_2. No significant adult mortality was observed in any of the gas conditions and in all cases the normal fecundity was regained when normal gas atmosphere was restored.

White et al.[70] observed the effects of CO_2 concentrations and temperature on adult survival and reproduction of beetle *C. ferrugineus*, and determined that fumigation of grain for 1 week at 20°C would require 94% CO_2 and <1% O_2. White et al.[71] exposed all life stages of *C. ferrugineus* to different concentrations of CO_2 at 25 ± 3°C or at temperatures declining from 21 to 7°C. Insects were controlled in 4 to 6 weeks at 25 ± 3°C when CO_2 levels were approximately 20% and O_2 levels were between 5 and 10%. At temperatures declining from 21 to 7°C, 99.6% of *C. ferrugineus* populations were killed in 12 weeks, when CO_2 levels gradually fell from 20 to 9% and O_2 levels rose from 16 to 19.5%. Further tests indicated that four CO_2 purges between 15 and 50% on weeks 0, 1, 2, and 4 in bins with 6-day half-lives for CO_2 loss virtually eliminated insect and mite pests in 42 days at 12 to

15°C.[72] Rameshbabu et al.[73] exposed adults and eggs of *C. ferrugineus* to various levels of gas composition, relative humidity, and temperature. Mortalities of both adults and eggs increased with increased CO_2, temperature and exposure time and decreased with increased relative humidity and O_2. Maximum mortality in 96 h for adults (99%) and eggs (85%) was obtained at high CO_2 (90%), low O_2 (<1%), the highest temperature studied (20°C), and the lowest relative humidity studied (60%). Shunmugam et al.[74] determined the mortality of adults, pupae, larvae, and eggs of *C. ferrugineus* at CO_2 concentrations of 30, 40, and 60% at 30°C. The O_2 concentration was maintained constant at 10% and the relative humidity of the gas mixtures was maintained at 75%. For a 60% CO_2 concentration in the atmosphere, 100% mortality of all life stages of beetles was obtained in 3 days. For 30 and 40% CO_2 concentration in the atmosphere, all the adults were killed in 8 days and all the pupae, larvae, and eggs were killed in 4 days.

Jay et al.[75] studied the mortality of malathion-resistant *T. castaneum* adults under CA containing about 35% CO_2 and 14% O_2. A concrete silo (9.1 m in diameter and 34.4 m high), reasonably sealed and fitted with inlets for gas introduction and distribution, gas sampling, and insect introduction, and filled with 2203 m^3 of inshell Spanish peanuts, was used for the experiment. The mortality of insects in cages, placed 0.5 to 1.5 m below the surface, was 93.3% after 96 h of exposure to the altered atmosphere as compared to only 9.2% mortality in the normal atmosphere. Steel tower silos containing shelled peanuts were treated with 63% CO_2 or 99% N_2 to study the mortality of *T. castaneum* and *E. cautella*.[59] The average temperature was 27°C and the relative humidity 66%. The larvae of both species were more tolerant to 63% CO_2 than any other life stage. The larvae of *E. cautella* were more tolerant to 99% N_2 than pupae, but the reverse was true for *T. castaneum*. In another study, Jay and Pearman[76] treated a silo containing 958.1 m^3 of shelled corn at 11 to 16% moisture content with CO_2 for the control of potential natural infestation by stored-product insects. During the test the average composition of the atmosphere was 61% CO_2, 8% O_2, and the rest N_2 and other gases. The temperature ranged from 13.9 to 25.0°C and the relative humidity from 51 to 68%. The samples taken before treatment and held for 60 days contained an average of 144.4 live and 59.8 dead insects, and the samples taken after 96 h of treatment and held for 60 days contained an average of only 0.1 live and 1.1 dead insects. The majority of the insects in the untreated corn were *Sitophilus* spp. and *Sitotroga cerealella* (Olivier), but over 98% of those found in treated corn were *Sitophilus* spp.

Shejbal et al.[77] studied the mortality of *S. granarius*, *T. castaneum*, and *T. confusum* in hermetic silos treated with gaseous N_2 containing 0.1 to 1.0% O_2. The relative humidity ranged from 70 to 100% and the temperature was about 22°C during the experiment. Nearly 9 days of exposure to <0.9% O_2 was required to achieve complete mortality of adult insects. *S. granarius* was significantly more tolerant than either of the *Tribolium* spp. In a similar study, a concrete bin (2500-t capacity) filled with wheat was purged with gases generated from an exothermic gas generator to determine the mortality of *S. granarius*.[78] The atmosphere generated contained <0.5% O_2, 11 to 12% CO_2, and 86 to 87% N_2 and other gases. The temperature varied from 10 to 12°C during the experiment. Complete mortality of adult insects was obtained after 21 days of exposure. The immature life stages of

the insect, however, survived as indicated by the emergence of adults during an incubation period for samples after the treatment. Therefore, an extended exposure may be required for complete mortality of all life stages of *S. granarius*.

Hoey[79] reported on the use of CO_2 for the control of stored-grain insects in Australia. The gas was introduced at a slow rate at the bottom of vertical steel silos conforming to a satisfactory standard of gas tightness. After the initial purge at a rate of 1 t CO_2 per 1000 t of grain, the gas was recirculated for at least 10 days. The treated silos were generally insect-free for more than 4 months. Annis[80] studied the effectiveness of CO_2 fumigation of bag stacks of rice sealed in flexible PVC enclosures. The gas was added until the gas leaving an exit vent at the top of stack contained >60% CO_2. The stack was then sealed. All of the insects (*S. oryzae*) placed in cages were killed. In another trial, the grain with a natural infestation of 15 live insects per kilogram of rice, mainly *R. dominica* and *T. castaneum*, was subjected to a similar gas treatment. No live insects were found in the bags opened 133 days after the treatment.

Development of Insect Resistance to CA

Bond and Buckland[81] investigated the response of *S. granarius* to selection under high CO_2 atmospheres. The exposure of adults to 42% CO_2 for seven successive generations, and to 75% CO_2 for four successive generations produced insects with 3.3- and 1.8-fold increases in their resistance to the elevated CO_2. Navarro et al.[82] reported on the development of resistance to CO_2-rich atmosphere among the adults of *S. oryzae*. Two groups of insects were exposed to 40% CO_2 in air for seven successive generations, and to 75% CO_2 in air for ten successive generations at 26°C and 100% relative humidity. Insects subjected to selection pressure were compared with those of a control for their lethal tolerance factor (LT_{95} selected generation/LT_{95} non-selected generation). The results indicated that *S. oryzae* has the genetic potential to develop resistance to CO_2-rich atmospheres. The tolerance factor at the 7th generation (under 40% CO_2) and 10th generation (under 75% CO_2) was 2.15 and 3.34, respectively. Reduction of relative humidity to 60% and augmentation of O_2 concentration to 21% at these CO_2 levels did not markedly alter the tolerance factor indicating that the tolerance in these insects was largely due to the action of CO_2. Removal of selection pressure for five generations in the case of the 40% CO_2 group and for four generations in the case of the 75% CO_2 group resulted in a significant reduction in their tolerance to the CO_2-rich atmosphere indicating that the strains obtained were not completely isogenic. Donahaye[83] exposed *T. castaneum* for 40 generations to atmospheres containing 65% CO_2, 20%O_2, and 15% N_2 at 95% RH. A resistance factor to CO_2 of 9.2 was found and the offspring of the insects removed from the selection pressure maintained their resistance to CO_2.

Quality Changes in Grain Under CA Storage

Seed Viability

Grain can be stored for relatively long periods without a loss in viability, with a decreasing scale for longevity in oats, rice, barley, wheat, triticale, rye, sorghum,

and corn.[84] The main factors that affect longevity are moisture and temperature[85] with each 1% increase in seed moisture content (MC) halving the life of seed. This rule applies when seed moisture content is between 5 and 14%. Below 5% MC the speed of aging may increase because of auto-oxidation of seed lipids and above 14% MC storage fungi kill the seed. Also, for each 5°C increase in seed temperature, the life of seed is halved (from 0 to 50°C). The presence of O_2 decreases seed germination, even at low partial pressures[86] in dry grain because of membrane damage caused by the production of free radicals[87] and accumulating chromosome damage.[88] Peanut seeds with 6.2% MC stored at 38 to 40°C had unaffected germination after 26 weeks under N_2 or CO_2 atmospheres, while viability decreased sharply in air.[89] Packaging of shelled peanuts, under vacuum or N_2 atmospheres for distribution in Senegal after 7 months at room temperature, resulted in similar or better germination than in other systems.[90] Saly et al.[91] indicated that storage of shelled peanuts at room temperature and low O_2 had no significant effect on germination. Corn stored for 360 days in Mexico in hermetic storage lost germination at the same rate as in air at 14% MC but at 15.5 and 17.6% MC viability was higher under hermetic storage than in air at the same moisture level;[92] fungi were not present on seeds in hermetic storage, but the *Aspergillus glaucus* group, *A. tamarii*, and *Penicillium* spp. were present on all seeds stored in air. Green gram seeds stored for 6 months at 12, 14, and 16% MC in hermetic storage had lower sugar values and free fatty acid values than seed stored in air.[93] Bass and Stanwood[94] stored sorghum seed for 16 years at 4, 7, and 10% MC under vacuum, air, hermetically sealed, CO_2, N_2, He, or argon atmospheres at temperatures from –12 to 32°C. Under these conditions, controlled atmosphere storage had no effect on seed germination, which was not significantly different from that of seeds stored in hermetic containers. Seed in air had less germination than all sealed treatments at temperatures above 0°C. Petruzelli[95] stored wheat at 15 to 33% MC under hermetic storage and reported that seed viability decreased more rapidly at higher moisture content, while in aerobic storage longevity was enhanced as moisture content increased from 24 to 31% (under hermetic storage, germination fell to 0% in 8 days at 32.7% MC and to 60% in 150 days at 15% MC). When moisture content increased above 31%, longevity was greater under hermetic, rather than aerobic, storage. Above 24% MC at 25°C, seed metabolism was activated and could be sustained only in the presence of O_2. At <24% MC O_2 had a negative effect on longevity. Wheat stored at 10.5% MC and various combinations of N_2, CO_2, and O_2 for 13.5 months at 27°C showed no decline in germination under N_2, CO_2, or a mixture of N_2 and CO_2. Atmospheres of <1% O_2, 9 to 9.5% CO_2, and a balance of N_2, generated by the combustion of petroleum fuels, does not affect germination of dry wheat, rice, or malting barley during 6-month storage.[96] Moor[97] conducted tests in commercial silos (950 t) of malting barley stored under N_2 (0.5% O_2) for 5 or 9 months in Australia. There was neither a negative nor a beneficial effect on germination. Storing unpolished rice in containers pressurized with N_2, CO_2, or air had negative effects on its chemical composition. At atmospheric temperature and pressure there was no difference in the effects of N_2 or CO_2 on rice quality in storage.

Atmospheres of 35% CO_2 were reported to have a negative effect on the germination of wheat, soybean, and rapeseed and little effect on corn or green pea

germination after storage for 12 months, but moisture content of the grain was not given.[98] Generally, low O_2 or high CO_2 atmospheres have no negative effects on viability of dry grain and both low O_2 (<0.5%) and high CO_2 (>50%) help preserve the germination of wet grain.[57]

Nutrient Changes in Grain

A controlled atmosphere of 97 to 98% N_2, 1 to 2% CO_2, and 1% O_2 slows hydrolytic processes in the lipids of rice grain compared to grain stored in air.[99] Pure N_2 atmospheres at 20°C stabilize protein and amino acids and the cooking properties of rice at 18.4 and 23.2% MC compared to storage in air.[100] Elevated N_2, with negligible O_2, retain higher gluten quality in stored wheat than storage in air[101] and the milling and baking properties of wheat are maintained longer than in air even at high moisture contents.[102] An anoxic environment (N_2) slows the oxidative activity and better preserves the organoleptic properties of grains and even hazelnuts.[103]

Storing dry grain under airtight conditions (15 to 25% CO_2 for several months) maintained its quality[104] but wet grain (>16% MC) was tainted with fermentation odors, was discolored, and was unsuitable for bread-making (after 2 months at 21% MC). At high moisture, there was an increase in reducing and a decrease in non-reducing sugars.[105] After storing wheat for 3 to 4 years under vacuum, CO_2, or N_2, Stopczyk[107] concluded that changes to sugars depended mainly on moisture and temperature rather than on gas concentrations. Starch viscosity decreased more in air storage than in CO_2 storage. Munzing[107] indicated that flour from 20% MC wheat stored for 6 months under CO_2 led to reduced loaf volume in 6 months and that an N_2 atmosphere (negligible O_2) was preferable for long-term storage of wet wheat. Dry wheat stored for 18 years at low O_2 had no decrease in loaf volume.[108] Wet rapeseed or sunflower seed (13 to 15% MC) stored under airtight conditions produced off-odors due to microbial fermentation but the production of free fatty acids was slower than when stored in air.[109]

Effect of CA on Fungi and Mycotoxins Production

Elevated CO_2 (20 to 60%) inhibits fungi and the production of mycotoxins by fungi in stored grain[110] including T-2 toxin,[111] patulin,[112] ochratoxins,[113] penicillic acid,[114] and aflatoxin.[115] The prevention of aflatoxin production in wet corn is of considerable importance in animal feed grain.[116] Reduction of O_2 is less effective in preventing mycotoxins than the elevation of CO_2.[117]

In dry grain, 20% CO_2 inhibits microflora; in wet grain 80% CO_2 is needed. Some species of *Fusarium*, *Aspergillus*, and *Mucor* are tolerant to high CO_2 levels.[117] In wet grain, at 1 to 2% O_2 and 15 to 40% CO_2, typical microflora were the yeasts *Hansenula* and *Candida* (60 to 80% RH) followed by anaerobic fermentation caused by lactic acid bacteria and yeasts (>90% RH). Filamentous fungi gradually disappear during storage.[105] Fungi do not grow at <1% O_2.[118] Yeasts can survive at <0.5% O_2. Richard-Molard et al.[119] found that corn stored at 21% MC in hermetic storage produced a CO_2 atmosphere with 0.5% O_2 in 4 to 5 days and there was a slight

alcoholic fermentation. After 12 months grain quality was still acceptable, although lactic bacteria had noticeable metabolic activity after 6 months. An absence of O_2 will inhibit but not quickly kill fungi.[120]

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6 Antioxidants and Shelf Life of Foods

N. A. M. Eskin and R. Przybylski

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INTRODUCTION

The least stable macro-components in foods are the lipids. Depending on the degree of unsaturation, lipids are highly susceptible to oxidation resulting in the development of rancidity. When this occurs, the food becomes unacceptable and is rejected by the consumer. In addition to the development of oxidized off-flavors, many of the oxidized products of rancidity are now considered to be unhealthy. To enhance

the shelf life of foods and prevent the occurrence of rancidity, the presence of antioxidants is required. These may be indigenous to the food itself or are added to the food product during processing. Because of health concerns surrounding synthetic antioxidants, there has been an increasing interest in natural antioxidants, not only as agents for enhancing the shelf life of foods but also as therapeutic agents. This chapter will discuss the process of rancidity and the type and role that antioxidants play in minimizing these reactions to prolong the shelf-life of foods.

LIPID OXIDATION

Free Radical Oxidation

Many comprehensive reviews have been written on the mechanism of lipid oxidation.[1,2] This process can be initiated by light, temperature, metals, metalloproteins, pigments and air pollutants as well as microorganisms. Lipid oxidation generates free radicals which are catalyzers of this process. The primary substrates for these reactions are polyunsaturated fatty acids and oxygen (Figure 6.1). The free radical

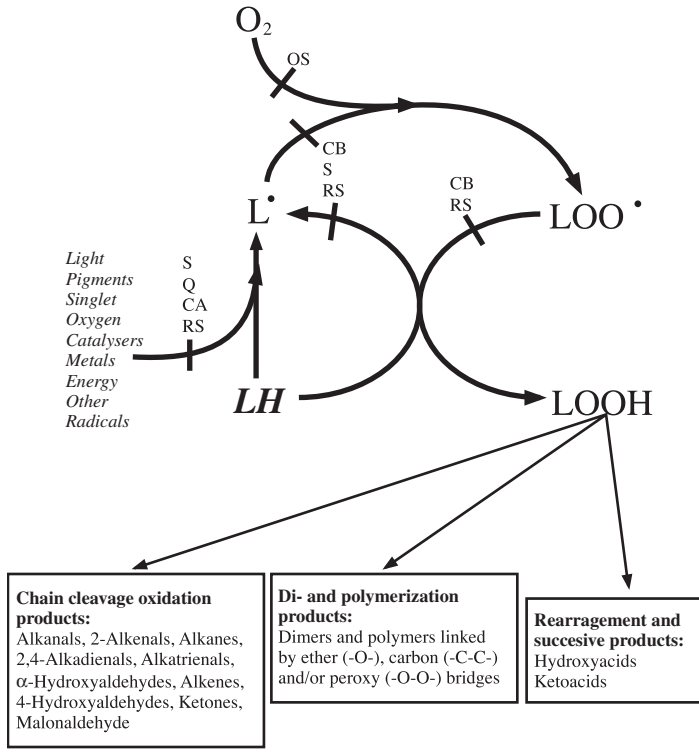


FIGURE 6.1 Mechanism of unsaturated fatty acids oxidation. LH = lipid component; L = lipid radical; LOO = lipid peroxy radical; LOOH = lipid hydroperoxide. Antioxidants: OS = oxygen scavengers; CB = chain reaction breakers; S = synergists; Q = quenchers; CA = chelating agents; RS = radicals scavengers/blockers.

mechanism of lipid oxidation is a three-stage process: (1) initiation, (2) propagation, and (3) termination.[3] During the initiation stage, lipid radicals are formed directly from both unsaturated fatty acids in the presence of light, heat, other radicals, and catalyzers including metals. At the propagation stage, lipid radicals react with oxygen to form peroxy radicals (LOO·), which in turn abstract a hydrogen atom from another lipid molecule to form hydroperoxides (LOOH) and another lipid radical (Figure 6.1). These generated radicals cause this process to become autocatalytic. During the termination phase, free radicals interact with each other to form nonradical products. Any components that prevent or interfere with the propagation of oxidation by deactivating free radicals in the system play a key role in the termination mechanism. Chain breaking antioxidants, such as phenolic compounds, react with lipid radicals by donating a hydrogen atom to the lipid radicals, thereby stopping propagation by forming inactive components.[4] Examples of phenolic antioxidants include tocopherols, butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate.

The LOOH formed are unstable and decompose into a wide range of volatile and non-volatile products (Figure 6.1). These volatile and non-volatile products are themselves unstable and undergo further oxidation and/or decomposition to a range of oxidized products responsible for the off-flavors associated with rancid oils.[5]

The susceptibility of fatty acids to oxidation depends on their ability to donate a hydrogen atom. In Table 6.1 dissociation energies for hydrogen bonds are presented. Unsaturated fatty acids with more than one double bond are particularly susceptible to oxidation due to the presence of methylene interrupted bond configurations, where a methylene carbon atom is located between two double bonds. The relative oxidation rates for oleic, linoleic, and linolenic acids were reported to be in the order of 1:12:25 based on the peroxides formed.[6]

Photooxidation

Photooxidation is far more detrimental to the stability of vegetable oils than free-radical oxidation. Most oils contain photosensitizers, natural pigments such as chlorophyll and its degradation products, heme and related compounds, methylene blue, fluorescein derivatives, erythrosine, and polycyclic aromatic hydrocarbons capable of transferring energy from light to chemical molecules.[7,8] The following reactions outline the process of photooxidation:

$$\text{Sensitizer}_{\text{Ground}} + h\nu \rightarrow \text{Sensitizer}_{\text{Excited}} \tag{6.1}$$

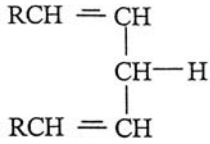
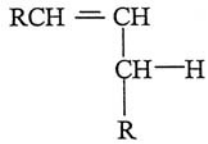
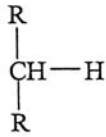
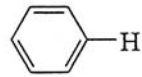
$$\text{Sensitizer}_{\text{Excited}} + \mathbf{LH} \rightarrow \text{Sensitizer}_{\text{H}} + \mathbf{L}^{\cdot} \tag{6.2}$$

$$\text{Sensitizer}_{\text{Excited}} + {}^{3}O_2 \rightarrow \text{Sensitizer}_{\text{Ground}} + \mathbf{{}^{1}O_2} \tag{6.3}$$

$$\mathbf{{}^{1}O_2 + LH \rightarrow LOO^{\cdot}} + {}^{3}O_2 \tag{6.4}$$

Energy (hv) is transferred from light to the sensitizer ($\text{Sensitizer}_{\text{Excited}}$), which may react directly with lipid (LH) forming radicals (L·), thereby initiating autoxidation (Equation 6.2). The direct formation of lipid radicals is less likely to occur

TABLE 6.1
Bond Dissociation Energies for Selected Hydrogen Bonds

Structure	Bond Energy (kJ/mol)
	317.68
	355.30
	402.53
H—H	435.56
CH_3—H	435.97
HO—H	498.67
ROO—H	367.84
CH_3S—H	383.72
	463.98

R = part of a chain attached by a single carbon–carbon bond.

due to higher energy requirement. The more damaging reaction is between the excited sensitizer and ground state oxygen to form singlet oxygen (Equation 6.3). Singlet oxygen has been shown to react with linoleic acid 1500 times faster than ground state oxygen.[9] This very reactive component is considered the most important initiator of the free-radical autoxidation of fatty acids. Exposure to light in the presence of photosensitizer and oxygen can cause the formation of singlet oxygen

and free radicals (Equations 6.2 and 6.3). This process initiates free-radical reactions in which lipid radicals are formed and autoxidation started.[10]

Sources of singlet oxygen have been discussed by Bradley.[10] Oxidation of unsaturated fatty acids by singlet oxygen can be inhibited by compounds that react faster with this initiator such as quenchers which deactivate singlet oxygen to the ground state form.[8] The most efficient natural quenchers are tocopherol and β-carotene while others include amino acids, proteins, sulfides, phenols, and metal chelators.[11]

ANTIOXIDANTS

Halliwell et al.[12] defined an antioxidant as "any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate." While antioxidants are associated primarily with inhibition of lipid peroxidation, free radicals can also damage other components, so that "oxidizable substrate" includes almost everything found in foods and in living tissues such as proteins, lipids, carbohydrates, and DNA. This chapter, however, will focus on antioxidant activity against lipid peroxidation. In general, antioxidants do not prevent oxidation, but rather extend or retard the induction period.

Mechanism of Action

Antioxidants can act at different steps in the oxidation sequence, depending on their mode of action (Figure 6.2). The primary or chain-breaking antioxidant reacts with lipid radicals to yield more stable products and such antioxidants are known as free-radical interceptors. The secondary or preventative antioxidants reduce the rate of chain initiation by a variety of mechanisms which include metal inactivators, hydroperoxide decomposers, oxygen scavengers, and synergists.

A primary antioxidant rapidly donates a hydrogen atom to a lipid radical, or is converted to other stable products.[13] Free-radical interceptors inhibit two important steps in the free-radical chain sequence of lipid oxidation. They react with peroxyl radicals (LOO*) to stop chain propagation, thus inhibiting formation of peroxides (Eq. 6.5), and with alkoxyl radicals (LO*) to decrease the decomposition of hydroperoxides to harmful degradation products (Eq. 6.6).[14]

$$LOO^* + AH \rightarrow LOOH + A^* \tag{6.5}$$

$$LO^* + AH \rightarrow LOH + A^* \tag{6.6}$$

Metal inactivators or chelating agents act as preventative antioxidants by removing or deactivating metal ions, which act as initiators as well as catalyze the decomposition of hydroperoxides. Decomposers of hydroperoxides transfer them into stable hydroxy compounds by reduction, while oxygen scavengers react with oxygen to deplete the supply of oxygen needed for autoxidation.[14] Synergism can be expected between substances with differing modes of action, so that multicomponent antioxidant systems exhibit much greater antioxidant activity beyond that expected from the additive effects of individual antioxidants.[15] Synergists generally extend the life

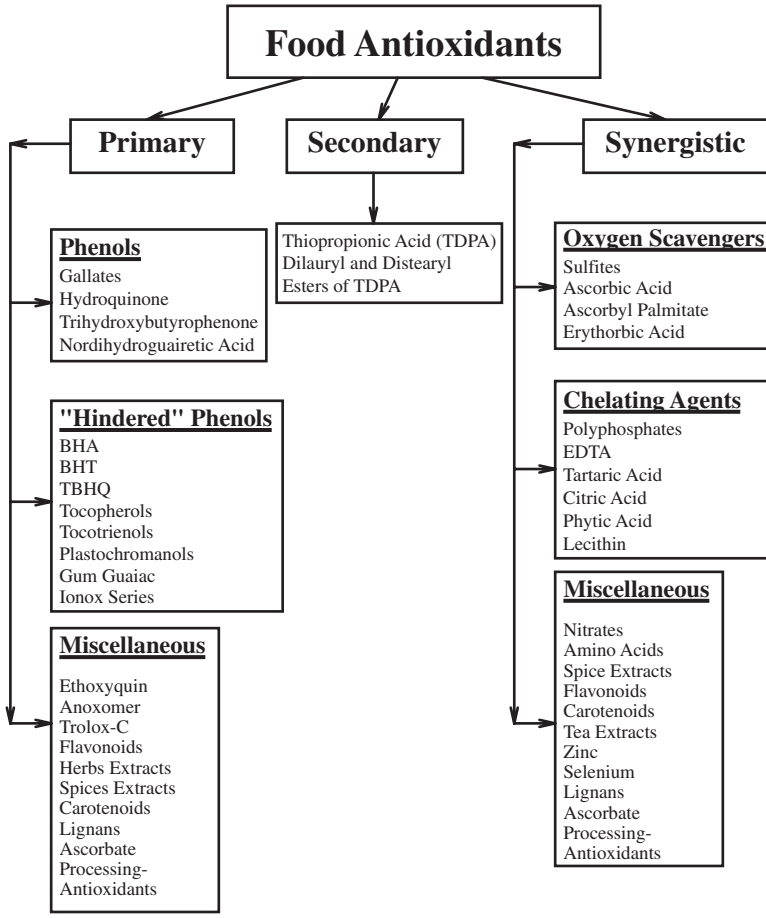


FIGURE 6.2 Types of food antioxidants.

of primary antioxidants by acting as hydrogen donors to their radicals, thereby regenerating the primary antioxidants or inactivating metal ions.[16]

Biological Antioxidants

Natural antioxidants are present in the body to protect cells from excessive oxidation and free-radical damage. These include metalloenzymes which can interfere with the production of free radicals during the initiation phase, such as superoxide dismutase which contains Mn or Cu/Zn (see Chapter 10) or catalase which contains Fe and glutathione peroxidase which contains SE. The latter is important in the decomposition of hydrogen peroxide and lipid hydroperoxides.[17]

Three essential nutrients can directly interfere with the propagation stage to scavenge free radicals. Of these, α-tocopherol is the major lipid-soluble antioxidant present in all cellular membranes. Vitamin C or ascorbic acid, the major water-soluble antioxidant, is capable of quenching free radicals and singlet oxygen, as

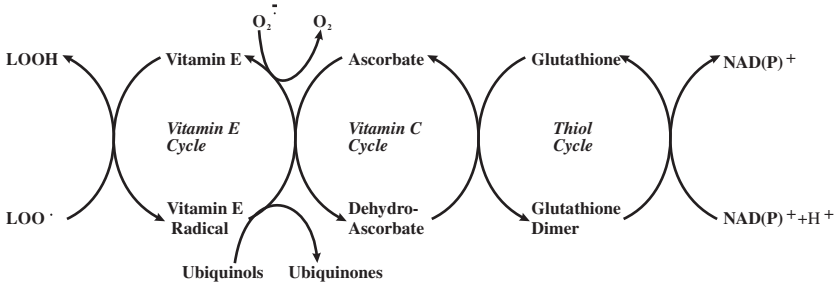


FIGURE 6.3 The vitamin E regeneration cycle. Synergistic action of water and lipid soluble components. LOOH = lipid hydroperoxide; LOO· = lipid peroxy radical. (Adapted from Tappel[18] and Packer.[19])

well as regenerating the radicals of tocopherol (Figure 6.3). Regeneration of tocopherols, an important part of other metabolic pathways, occurs in three ways: first, via the ascorbate cycle; second, through action of ubiquinols and ubiquinones; and third, by transferring oxygen radicals into ground state oxygen (Figure 6.3). β-Carotene, the main precursor of vitamin A and an efficient quencher of singlet oxygen, can also function as an antioxidant.[16,17]

Food Antioxidants

Antioxidants, as defined by the U.S. Food and Drug Administration (FDA), are substances used to preserve food by retarding deterioration by rancidity or discoloration due to oxidation.[20] They are compounds present in small quantities capable of preventing or retarding oxidation of oils and fats.[21] Antioxidants can act in cell membranes and/or food products by: (1) scavenging free radicals, which initiates oxidation; (2) inactivating metal ions; (3) removing reactive oxygen species such as oxygen radicals; (4) breaking the initiated chain of reactions; (5) quenching/scavenging singlet oxygen; (6) destroying peroxides to prevent radical formation; and (7) removing oxygen and/or decreasing local oxygen concentration/pressure.[20-22] They can be classified as primary, secondary, or synergists, depending on their particular function (Figure 6.2). A number of synthetic and natural antioxidants are used commercially to stabilize food products or pure animal fats and vegetable oils. An antioxidant acceptable for food use must meet several essential requirements: effectiveness at low concentrations, compatibility with substrate, absence of sensory influence, non-toxic, and not affecting physical properties of food products.[15]

Primary Antioxidants

This group of antioxidants donates hydrogen atoms to free lipid radicals to terminate free radical chain reactions by forming stable products. Such antioxidants include polyhydroxy phenolics as well as the hindered phenolic components listed in Figure 6.2. In addition, many natural phenolic compounds including flavonoids, eugenol, and other components from herbs and spices also form part of this group.

Secondary Antioxidants

Secondary antioxidants function by decomposing lipid peroxides into more stable end products. This group includes thiopropionic acid and it derivatives.

Synergists

Synergistic antioxidants are primarily oxygen scavengers and metal chelators. They operate by a number of mechanisms including regeneration of primary antioxidants by donating hydrogen atoms to phenoxyl radicals or by proving a more stable acid environment for these antioxidants. Ascorbic acid, sulfites, and erythorbic acid are examples of oxygen scavengers, while EDTA, citric acid, and phosphates function as metal chelators. Compounds listed under miscellaneous are capable of functioning as primary antioxidants and synergists (Figure 6.2).

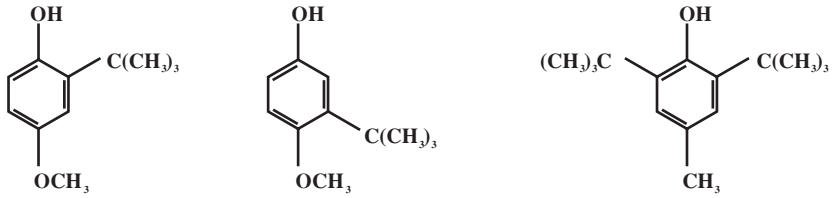
A large number of compounds found in animal and plant tissues, also available as synthetic molecules, are used in food applications. These include tocopherols and ascorbic and citric acids, often used in combination with each other or other antioxidants to take advantage of synergistic effects.[16] Although commonly used for years as a spice or flavoring agent, extracts of rosemary leaves have become the subject of increasing interest as inhibitors of lipid oxidation, and are used commercially. Rosemary extract contains many different compounds including carnosol, carnosic acid, and rosmanol thought to act synergistically as antioxidants and now available commercially.[23,24]

Synthetic

The most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ), and propyl gallate (PG), often used in combinations. Various regulations exist in different countries to control the amount or application of use of food antioxidants. In Canada, TBHQ has only recently been permitted for use in foods, even though it has been used for a number of years in other countries. Toxicological data have led to concerns regarding the potential toxicity of BHA such as the development of carcinoma in rodents if consumed routinely.[25] Some of these studies were criticized for their use of excessive concentrations and thereby overestimating the hazards. Nevertheless, there is a worldwide trend to reduce or avoid use of synthetic food antioxidants. Commercial formulations of synthetic antioxidants are shown in Table 6.2.

BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene)

The most widely used synthetic antioxidants are BHA and BHT, both monohydroxy phenols (Figure 6.4). BHA is a mixture of two isomers, 3-tertiary-butyl-4-hydroxyanisole (90%) and 2-tertiary-butyl-4-hydroxyanisole (10%). Both antioxidants are fat soluble and exhibit good carry-through effects, although BHA is slightly better.[20] BHT is particularly effective in protecting animal fats from oxidation while BHA is more effective in vegetable oils. BHA is particularly effective in protecting the



2-BHA **3-BHA**

Butylated Hydroxyanisole (BHA) **Butylated Hydroxytoluene (BHT)**

FIGURE 6.4 Structure of synthetic antioxidants.

flavor and color of essential oils.[26] It also protects effectively short-chain fatty acids, such as those found in cereal and confectionery products, from oxidation.[27] Because of their volatility, these antioxidants are not effective in frying. BHA and BHT are both used in packaging materials as they can migrate into the food. Together they act synergistically and are used as such in many food antioxidant formulations (Table 6.2).

Propyl Gallate (PG)

Produced commercially by esterification of gallic acid with propyl alcohol, PG acts synergistically with BHA and BHT (Figure 6.5). Because PG chelates metal ions such as Fe to form a blue–black complex, it is used with a chelator such as citric acid to prevent food discoloration. PG, like BHA and BHT, loses its effectiveness with heat due to evaporation and is therefore unsuitable in frying oils.

Tertiary-Butylhydroquinone (TBHQ)

Because of its excellent carry-through properties, TBHQ is considered by far the best antioxidant for use in frying oils (Figure 6.5). TBHQ does not chelate metal ions and is used in combination with citric acid. Commercially, TBHQ is used alone or with BHA and BHT at a maximum level of 0.02% or 200 ppm depending on the fat content of the food. As a diphenolic antioxidant, TBHQ reacts with the peroxy radicals forming a semiquinone resonance structure which undergoes a number of different reactions until stable products are formed.

Ethoxyquin

Ethoxyquin, chemical name 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, is used mainly as a feed antioxidant (Figure 6.5). However, it is also used to protect fish products and fish oil, poultry fats, potatoes, apples, and pears during storage. Ethoxyquin was found to effectively protect the pigments of paprika.[28] It was shown to minimize nitrosoamine formation in bacon when added at a level of 20 ppm.[29] Formulations of ethoxyquin, however, are available from only one processor, American Roland Chemical Corporation (Table 6.2).

TABLE 6.2
Active Components in Commercial Formulations of Synthetic Antioxidants (% wt)[a]

Name	BHA	BHT	PG	TBHQ	Citric Acid	Ethoxyquin	Viscosity (cP, 25°C)	Specific Gravity	Solubility[b]	Level % (FDA/USDA)
Eastman Kodak[c]										
Tenox BHA	100								Good	
Tenox BHT		100							Good	
Tenox 2	20		6		4		95	1.064	Good	0.076/0.05
Tenox 4	20	20					61	0.942	Excellent	0.05/0.05
Tenox 6	10	10	6		6		333	1.008	Excellent	0.076/0.076
Tenox 7	28		12		6		291	1.081	Very good	0.05/0.035
Tenox 8		20					49	0.925	Excellent	0.1/0.05
Tenox 20				20	10		235	1.087	Good	0.1/0.05
Tenox 21				20	1		284	0.991	Excellent	0.1/0.05
Tenox 22	20			6	4		91	1.054	Good	0.076/0.05
Tenox 25		10		10	3		190	0.938	Excellent	0.1/0.1
Tenox 26	10	10		6	6		235	0.997	Excellent	0.076/0.076
Tenox 27	28			12	6		279	1.047	Very good	0.05/0.025
Tenox S-1			20		10		244	1.127	Good	0.1/0.05

American Roland[d]									
Amerol S1			20	3		244	1.127	Good	0.1/0.05
Amerol 2	17		6	7		95	1.064	Good	0.076/0.05
Amerol 6	10	10	6	6		333	1.008	Excellent	0.076/0.076
Amerol OF[e]	5	5	15						
Amerol AP[f]					55				
Amerol P[f]					51				
Amerol ED					66				
Amerol EPC	30			10	30				
Amerol FPP			20	3					
Amerol EPC	75			4	75				

[a] Adapted from manufacturers' brochures.

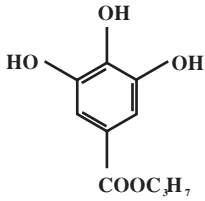
[b] Solubility in fats and oils.

[c] Carriers for formulation are application dependable and include vegetable oil, propylene glycol, or ethanol.

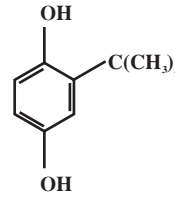
[d] American Roland Chemical Corporation.

[e] Contains 5% tocopherols and 1% ascorbyl palmitate.

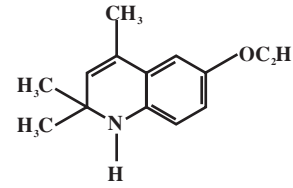
[f] Both contain 2% tocopherols.



Propyl Gallate (PG)



TBHQ



Ethoxyquin

FIGURE 6.5 Structure of propyl gallate, *tertiary*-butylhydroquinone (TBHQ) and ethoxyquin.

NATURAL ANTIOXIDANTS

Most natural antioxidants, with the exception of tocopherols, are phenolic compounds, containing *ortho*-substituted active groups, whereas synthetic antioxidants, with the exception of gallates, are *para*-substituted.[30]

The term "phenolic compound" includes a large number of secondary plant products which differ in chemical structure and reactivity, ranging from simple compounds to highly polymerized compounds. Many properties of plant products are associated with the presence of different polyphenolic compounds.[31] Phenolic compounds possess an aromatic ring bearing one or more hydroxyl groups together with a number of other constituents. At least 5000 phenolic compounds have been identified with an early reference classifying plant phenolics into 15 groupings.[32] Only some of the major groups will be considered including phenolic acids, coumarins, and flavonoid compounds such as anthocyanidins. Commercial formulations of natural antioxidants available are listed in Table 6.3.

Tocopherols and Tocotrienols

Tocopherols and tocotrienols, known as chromanols, are well recognized for their efficient protection against lipid oxidation in food and biological systems. These components are synthesized by plants and provide essential nutrients for humans and animals. Tocopherols are present in green parts of higher plants, leaves, and oil seeds[33] (Table 6.4). There are eight structurally different compounds in the tocopherol family (Figure 6.6); four known as tocopherols and four known as tocotrienols.[34,35] α-Tocopherol is present mainly in the plant cell chloroplasts, while the other three isomers are found outside of these organelles.[33] Tocopherols are present in refined vegetable oils at levels ranging from 60 to 110 mg/100 g.[36] Tocotrienols are absent or present in only very small amounts in most vegetable oils with the exception of palm.[37,38] They are found in the bran and germ parts of cereals and some seeds[39] (Table 6.4). Frega and co-workers[40] recently found tocotrienols were the only fat-soluble antioxidants in the lipid fraction of Annatto (*Bixa orellana* L.) seeds, mainly δ-tocotrienol. The basic structure of all eight of these compounds is similar (Figure 6.6), consisting of a 6-chromanol aromatic ring system containing a hydroxyl group and a 16-carbon phytol side chain.[35,41] Tocotrienols differ from

TABLE 6.3
Commercial Natural Antioxidant Formulations

	Tocopherols (%)	γ + δ Isomers (%)	α (%)	Tocotrienols (%)	Solvent
Eastman Kodak					
Tenox GT-1	50	40			Vegetable oil
Tenox GT-2	70	56			Vegetable oil
ADM					
Mixed tocopherols					
MTS-50	50		20		
MTS-70	70		20		
4-50	50	25	25		
4-80	67.2	13.5	53.7		
α-Tocopherol					
5-67	67.2		67.2		
5-87	87.3		87.3		
5-100	97		97		
α-Tocopherol acetates					
6-81	81		81		
6-100	96		96		
α-Tocopherol succinates					
Regular grade	96		96		

	Content	Solubility	Usage Rate
KALSEC			
Herbalox™	Rosemary extract	Formulations oil and water soluble	0.05–0.2%
Duralox™	Rosemary extract, spices extract, citric acid tocopherols	Formulations oil and water soluble	0.05–0.2%

tocopherols by the presence of three double bonds in the phytol side chain. Tocopherols and tocotrienols both consist of α, β, γ, and δ isomers, which differ in the number of methyl groups present in the aromatic ring (Figure 6.6). Tocopherols have three chiral centers in the phytol chain, namely, 2, 4′, 8′, making eight possible stereoisomers. Rules developed by the International Union of Nutritional Sciences[42] and the International Union of Pure and Applied Chemistry–International Union of Biochemistry[43] require tocopherols of unspecified configuration be named as methyl substituted tocols, e.g., α-tocopherol as 5,7,8-trimethyl tocol; δ-tocopherol as 8-monomethyl tocol. Natural tocopherols have the same configuration in their side chain, (+)-tocopherols or *RRR;* 2D,4′D,8′D; *d*-tocopherols. Synthetic tocopherols are usually a mixture of epimeric forms. Recommended nomenclature for these components is *SRR*-tocopherols or 2-*epi*-tocopherols. An equimolar mixture of synthetic tocopherols is composed of isomers with natural and 2-epimer configurations and these products should be named as 2-*ambo*-tocopherol (previously named as

TABLE 6.4
Sources of Tocopherols and Tocotrienols (ppm)

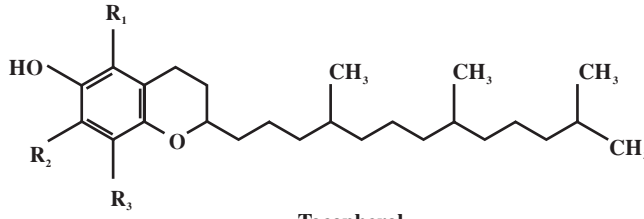
Source	αT	βT	γT	δT	P-8	αT3	βT3	γT3	δT3
Oils									
Soybean	101	—	593	264	—	—	—	—	—
Canola	313	—	287	7	74	—	—	—	—
Sunflower	1000	—	5	1	—	—	—	—	—
Flax	25	—	201	8	131	—	—	—	—
Rice	124	40	50	5	—	184	21	570	—
Palm	279	—	61	—	—	274	—	398	69
Coconut	5	—	—	6	—	7	1	19	—
Cereals									
Wheat	14	7	—	—	—	33	—	—	—
Wheat germ	239	90	—	—	—	30	100	—	—
Oat	5	1	—	—	—	11	2	—	—
Rye	16	4	—	—	—	15	8	—	—
Rice (brown)	6	1	1	—	—	4	—	10	—
Rice bran	3	15	4	2	—	1	14	22	29
Barley	2	4	—	1	—	11	3	2	—
Barley bran	11	16	36	4	—	36	25	19	11

Abbreviations: T = tocopherol isomer; T3 = tocotrienol isomer; P-8 = plastochromanol.

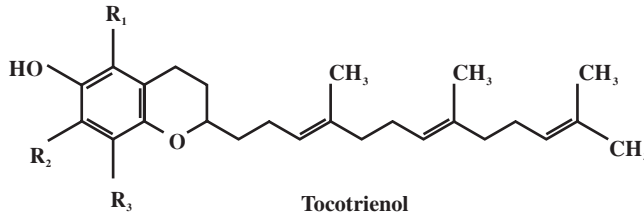
dl-tocopherol, ±-tocopherol or 2DL,4″D,8′D-tocopherol). Individual tocopherol stereoisomers have been found to have different antioxidant activities in biological systems (Figure 6.7).

Tocotrienols have one chiral center in phytol chain at the second carbon atom so that only two stereoisomers are possible, 2D and 2L. The presence of double bonds in the phytyl chain at 3′ and 7′ carbon atom, however, generate four *cis*/*trans* isomers per tocotrienol molecule. The antioxidant activities of tocotrienol isomers have not been evaluated.[45]

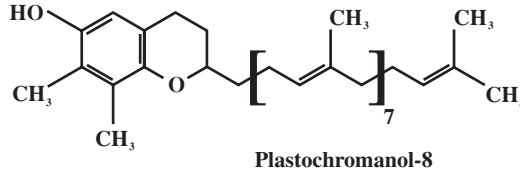
Chromanols are probably the most efficient lipid antioxidants produced by nature. The antioxidant activity of these components is related to the following: (1) phytyl chain with phenolic ring make them lipid soluble; (2) lipid radicals react with them several times faster than with other lipid radicals;[46] and (3) one tocopherol molecule can protect about 10^3 to 10^8 molecules of polyunsaturated fatty acid molecules at low peroxide values.[47] Tocopherols act as antioxidants by donating a hydrogen atom from the hydroxyl on the ring system to a free radical.[48] Unsubstituted phenols are not hydrogen donors, while the reactivity of substituted phernols is mainly attributed to two factors: (1) inductive effects of electron-releasing substitutes in the position *ortho*- and *para*- to the hydroxy group/function, and (2) stereoelectronic effects related to the orientation of substituents to the aromatic ring.[49] Electron-releasing substituents present in the *ortho*- and *para*-position increases the electron density of the active center(s) promoting release of hydrogen from hydroxyl group and improving reactivity with peroxy radicals.[50] From this mechanism, α-tocopherol,



Tocopherol



Tocotrienol



Plastochromanol-8

Isomer	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

FIGURE 6.6 Structure of plastochromanol and isomers of tocopherol and tocotrienol.

based on its structure, should be more effective as a hydrogen donor than β-, γ-, or δ-tocopherols. The oxidation reduction potentials of +0.273, +0.343, +0.348, and +0.405 volts were reported for α-, β-, γ-, and δ-tocopherols, respectively.[51] A higher redox potential indicates lower potency as a hydrogen donor. Based on these measurements, the α isomer of tocopherol is the best radical regenerator compared to the other three isomers of these antioxidants.[52] However, substitution in the *ortho* and *para* position hindered phenoxy radicals from further reactions and decreases the possibility of oxidation by atmospheric oxygen.[50,53] The antioxidant activity of tocopherols is 250 times greater than BHT mainly due to the heterocyclic ring — hydrogen donor part and chroman moiety — responsible for fat solubility.[54] Phenols with an *oxy* substituent in the *para* position to the hydroxy group produced more stable phenoxyl radicals and exhibited higher activity as antioxidants.[55] More methyl substitutes in phenolic ring improved the relative antioxidant activity of tocopherol isomers but also made these isomers more soluble in fats/oils.[56] Tocotrienols also

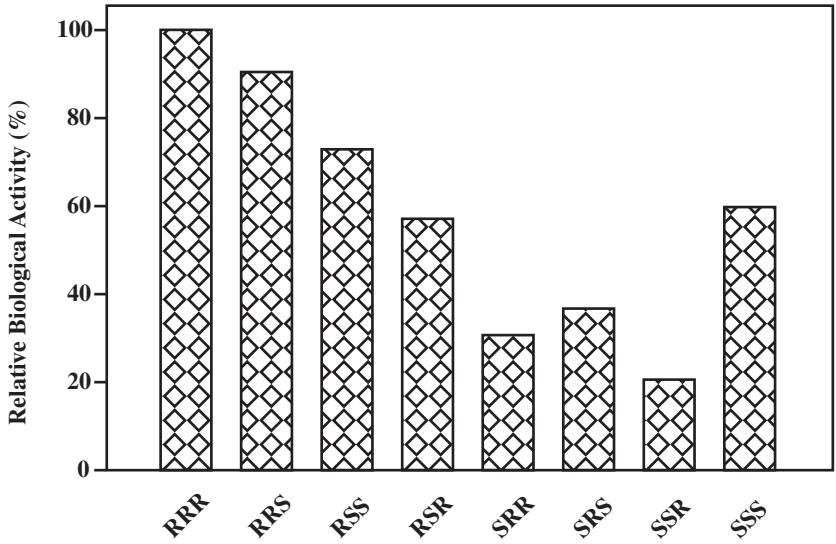


FIGURE 6.7 Biological activity of α-tocopherol acetate stereoisomers. RRR-isomers at 2.4′,8′ carbon atom. (Adapted from Pongracz.[44])

exhibit antioxidant activity as they contain a hydroxyl group in the same position found in tocopherols.[34] The mechanism of antioxidant activity for tocotrienols is expected to be similar to tocopherols.[45] The various isomeric forms of tocopherols and tocotrienols are reported to have varying degrees of antioxidant effectiveness (Figures 6.8 and 6.9). The activity of tocopherol isomers also varies with temperature[34] and concentration.[57-59] At low and mild temperatures, the order of antioxidant activity was $\alpha > \beta > \gamma > \delta$, while the reverse order $\delta > \gamma > \beta > \alpha$ was observed at elevated temperatures.[60-62] Marianova and Yanishleva[63] reported that at higher temperatures the pro-oxidative effect of tocopherols was diminished even at high concentrations. This phenomenon was explained by oxygen solubility of oxygen in oils. The effect of temperature on pro- and antioxidative properties of tocopherols is dependent on chemical composition and physical properties of the system evaluated.

In the presence of light when photosensitized initiation occurs, tocopherols can act as free radicals and singlet oxygen scavengers.[64,65] The efficiency of tocopherols as singlet oxygen quenchers appeared to correlate well with concentration.[66] Antioxidant efficiency is also affected by the medium in which measurement is conducted. Tocopherols were more effective in animal fats than in vegetable oils.[44] Lipids with small amounts of tocopherols, such as animal fats and cosmetic lipids, can be well protected by these antioxidants.[67] The polarity of medium can also play a significant role in determining the activity of these components. The pro-oxidant effect of α-tocopherol on linoleic acid was higher in aqueous media than in polar organic solvents (ethanol, acetonitrile) and nonpolar media.[68] Takahashi et al.[69] found that α-tocopherol was a more effective radical scavenger in a non-polar substrate than in a water or polar system. These findings support the Porter theory of "polar paradox", which suggests that polar and hydrophylic antioxidants are more effective

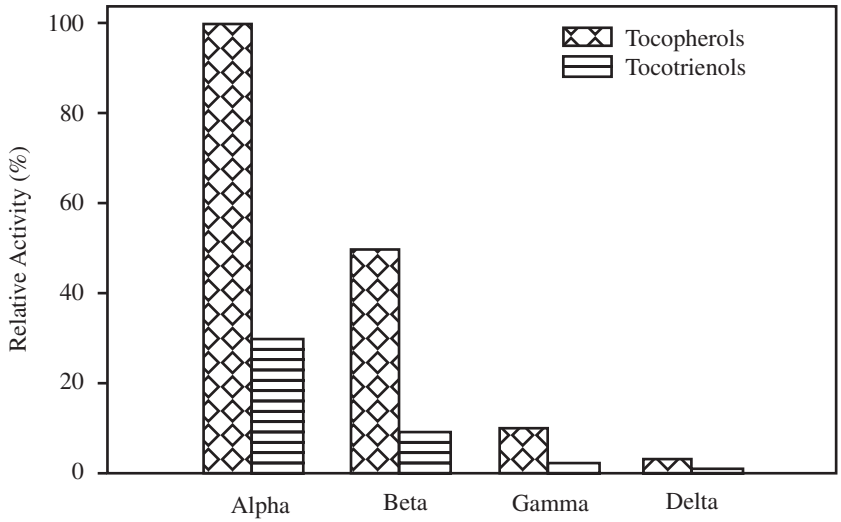


FIGURE 6.8 Biological activity of tocopherols and tocotrienols. (Adapted from Kamal-Eldin and Appelqvist.[45])

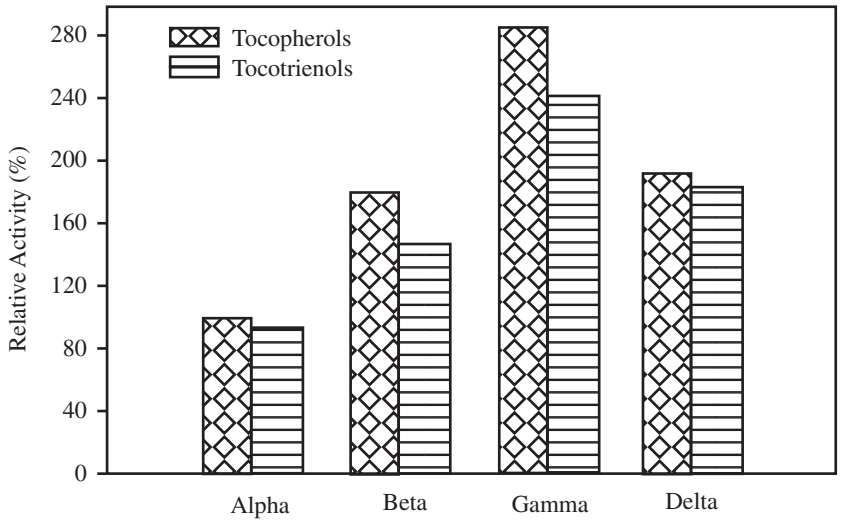
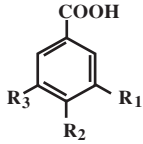
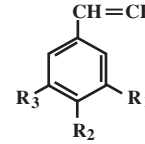


FIGURE 6.9 Antioxidant activity of tocopherols and tocotrienols. (Adapted from Kamal-Eldin and Appelqvist.[45])

in low surface-to-volume ratio (bulk oils) while nonpolar components are desirable when high surface-to-volume ratio exists, such as in an emulsion.[70] Frankel et al.[71] found that α-tocopherol and ascorbyl palmitate were more efficient antioxidants in an oil-in-water emulsion compared to bulk oil, while Trolox and ascorbic acid showed the opposite trend.



Benzoic Acid



Cinnamic Acid

Acid	Functional Group Position R_1	R_2	R_3	Acid	Functional Group Position R_1	R_2	R_3
p-Hydroxybenzoic	H	OH	H	Coumaric	H	OH	H
Protocatechuic	OH	OH	H	Caffeic	OH	OH	H
Gallic	OH	OH	OH	Ferulic	OCH_3	OH	H
Vanillic	OCH_3	OH	H	Sinapsic	OCH_3	OH	OCH_3
Syringic	OCH_3	OH	OCH_3				

FIGURE 6.10 Structure of selected phenolic acids.

Phenolic Acids and Coumarins

A range of substituted benzoic acid and cinnamic acid derivatives comprise two families of phenolic acids commonly found in plants (Figure 6.10). Both types occur in conjugated and esterified forms.[72]

Vanillic, *p*-hydroxybenzoic and syringic acids are found in lignin. Gallic acid has been found less frequently than its dimeric condensation product, ellaigic acid.[32]

Caffeic and *p*-coumaric acids are the most common cinnamic acids and usually occur as chlorogenic acid, esters of quinic and shikimic acids, or as sugar esters. The double bond in the side chain causes these acids and their derivatives to exist as *cis* and *trans* isomers.[72]

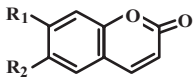
These compounds exist as a variety of sugar esters or glycosides, and are restricted to a few plant families.[72]

Flavonoid Compounds

These compounds are based on a C6-C3-C6 skeleton structure and include by far the largest and most diverse group of plant phenolics. They are classified according to substitution patterns, and the position of ring B[30] (Figure 6.11). The major subgroups are flavonols, flavones, isoflavones, catechins, proanthocyanidins, and anthocyanins. Most flavonoids occur as glycosides in which the aglycone moiety is esterified with various sugars. The point of attachment of the sugar varies for different flavonoids, and to add to the complexity, it may be acylated.[72] The pale yellow flavonols frequently occur in leaves.[72]

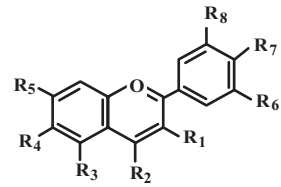
Flavones differ from flavonols by lacking the 3-hydroxyl group on the heterocyclic ring. Apigenin and luteolin are common in angiosperms, while tricin is present in grasses[32] (Figure 6.11).

Anthocyanidins all possess the basic flavylin ring structure and normally exist as glycosides, the latter referred to as anthocyanins (Figure 6.12). These are widely distributed in leaves, flowers, and fruits of higher plants, with cyanidin (red) being the most common. The substitution patterns on the B ring modify the color.[72]



Coumarins

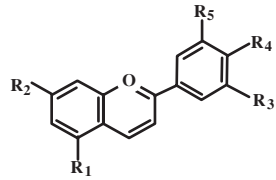
Compound	Functional Group	
	R_1	R_2
Umbelliferone	OH	**H**
Aesculetin	OH	**OH**
Scopoletin	OH	**OCH_3**



Flavonoids

Compound	Functional Group Position							
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Kaemferol	H	H	OH	H	OH	H	H	H
Quercetin	OH	H	OH	H	OH	OH	OH	H
Myricetin	H	H	OH	H	OH	OH	OH	OH
Apigenin	H	H	OH	H	OH	H	OH	H
Luteolin	H	H	OH	H	OH	OH	OH	H
Tricin	H	H	OH	H	OH	OCH_3	H	OCH_3
Isovitexin	H	H	OH	GLU	OH	H	OH	H

FIGURE 6.11 Structure of selected coumarins and flavonoids. GLU = glucose.



Compound	R_1	R_2	R_3	R_4	R_5
Pelargonidin	**OH**	**OH**	**H**	**OH**	**H**
Cyanidin	**OH**	**OH**	**H**	**OH**	**OH**
Delphinidin	**OH**	**OH**	**OH**	**OH**	**OH**
Peonidin	**OH**	**OH**	**H**	**OH**	**OCH_3**
Petunidin	**OH**	**OH**	**OCH_3**	**OH**	**OH**
Malvidin	**OH**	**OH**	**OCH_3**	**OH**	**OCH_3**

FIGURE 6.12 Chemical structure of anthocyanidins.

PHENOLIC ACIDS AS ANTIOXIDANTS

As discussed earlier, phenolic compounds have the capacity to function as antioxidants. Consequently, natural antioxidants are primarily plant phenolic compounds including flavonoids, phenolic acid derivatives, coumarins, tocopherols, and polyfunctional acids.[31] Synthetic antioxidants as well as tocopherols and ascorbic acid are commercially exploited as antioxidants and will not be discussed further as the focus of this chapter is on some of the natural phenolics previously reviewed.

Mechanism of Action

Phenolic compounds function as primary antioxidants by performing the role of free radical terminators. They interfere with lipid oxidation by rapidly donating a hydrogen atom to the lipid radicals, and the efficiency of these antioxidants (AH) increases with decreasing A-H bond strength. Phenolic antioxidants are excellent hydrogen or electron donors, and their radical intermediates are relatively stable due to resonance delocalization and general lack of suitable sites for attack by molecular oxygen.[31] The reaction of a phenol with a lipid radical forms a phenoxy radical, which is stabilized by delocalization of unpaired electrons around the aromatic ring.[13,31]

While phenol itself is not active as an antioxidant, substitution in the *ortho* and *para* positions with alkyl groups (ethyl or n-butyl) increases the electron density of the OH moiety by inductive effect enhancing its reactivity toward lipid radicals. The introduction of a second hydroxyl group at the *ortho* or *para* position of a phenol also increases its antioxidant activity.[13,31,73] The stability of the phenoxy radical is increased by bulky groups at the *ortho* positions.[13,31] However, the presence of bulky substituents in the 2 and 6 positions reduces the rate of reaction of the phenol with lipid radicals. This steric effect opposes the increased stabilization of the radical, and both effects must be considered in assessing the overall activity of an antioxidant.[13] Namicki[74] confirmed that *o*-dihydroxylation enhanced antioxidant activity of phenolic compounds while methoxylation of the hydroxyl groups drastically reduced this effect. Milic and co-workers[75] recently showed that the ability of phenolic acids to scavenge lipid alkoxy radicals depended on their structure and the number and position of the hydroxyl groups. Using an ESR spin trapping technique they showed the antioxidant effect increased in the order of gallic > caffeic > chlorogenic > vanillic > salicylic acid for a hydroperoxide-enriched sunflower oil model system (Figure 6.13).

Luzia et al.[76] showed that 5-caffeoylquinic acid, an ester of quinic acid with caffeic acid found in some vegetables, behaved as a primary antioxidant in soybean oil. Further work on the oxidative stability of soybean oil by Luzia et al.[77] suggested 5-caffeoylquinic acid acted as both a primary antioxidant and a metal chelator. The antioxidant activity was attributed to the ortho-dihydroxy grouping in the structure of 5-caffeoylquinic acid (Figure 6.14).

It is generally accepted that the position and degree of hydroxylation is of primary importance in determining the effectiveness of flavonoids as antioxidants and that hydroxylation of the B-ring is the major consideration for antioxidant activity.[31,78] Studies by Foti and co-workers[73] on flavonoids in micelles, however, did not find the number of hydroxy groups or their location on the B vs. A ring to be significant. All flavonoids with *o*-dihydroxylation of the B ring, with 3′ and 4′ dihydroxy configuration, possess antioxidant activity.[13,31,78] An additional hydroxyl group at the 5′ position further enhanced antioxidant activity, which explains why flavonols such as myricetin are more effective than quercetin (Figure 6.11).[31] A carbonyl group at position 4 and a free hydroxyl group at positions 3 and/or 5 also had an effect. In addition to the carbonyl group, a 2,3 double bond in the central ring participates in the radical stabilization, resulting in increased antioxidant activity.[73,79] The *o*-dihydroxy grouping

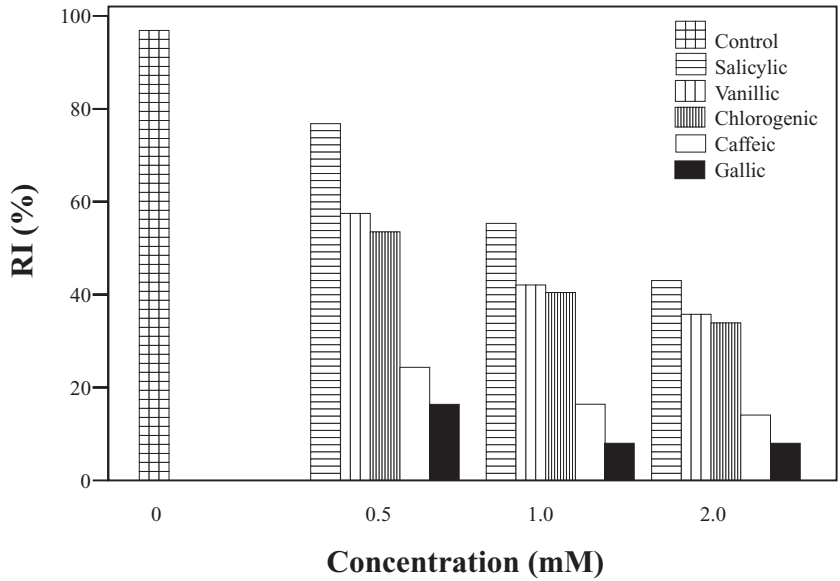


FIGURE 6.13 The influence of different amounts of phenolic on the relative intensity of the ESR signal of the spin adduct of the lipid alkoxyl radical. (Adapted from Milic et al.[75])

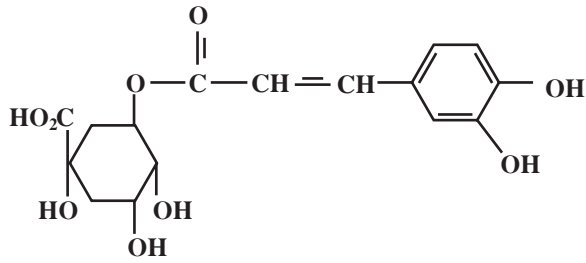


FIGURE 6.14 Structure of 5-caffeoylquinic acid.

on one ring and *p*-dihydroxy grouping on the other (such as 3,5,8,3′,4′ and 3,7,8,2′,5′-pentahydroxyflavones) produce very potent antioxidants, while *meta* 5,7 hydroxylation of the A ring seems to have little influence.[31,78]

Flavonols are also known to chelate metal ions at the 3-hydroxy, 4-keto group and/or at the 5-hydroxy, 4-keto group (when the A ring is hydroxylated at the 5 position). Chalcones, natural precursors of flavones and flavonones, are readily cyclized under acid conditions and have been shown to possess more potent antioxidant activity, more so than their corresponding flavonones. The 3,4 dihydroxychalcones are particularly effective as antioxidants. In the isoflavone, hydroxy groups at both 4′ and 5 positions are needed for significant antioxidant activity.[31]

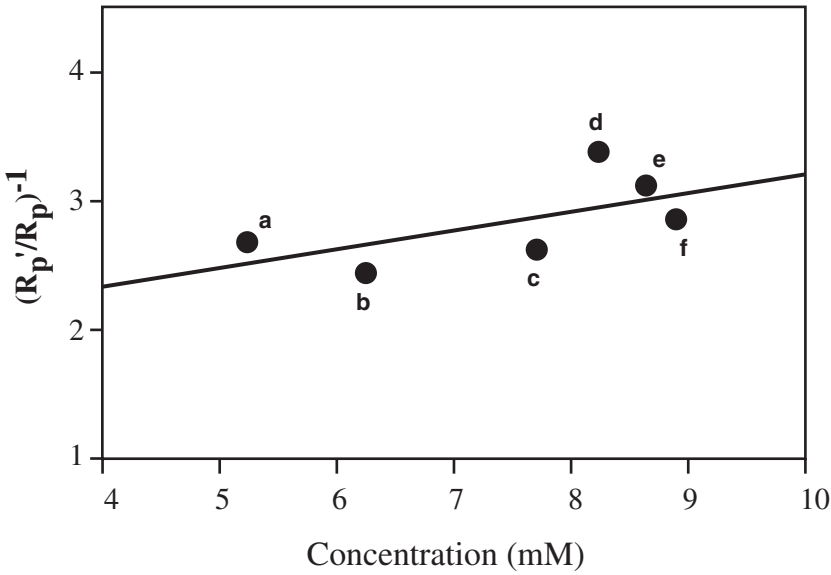


FIGURE 6.15 Relationship between the concentration of catechins corrected by the antioxidative activity of the respective catechin ([C]) and the antioxidative activity of the tea $\{(R_p'/R_p)^{-1}\}$ for (a) Matcha, (b) Gvokuro, (c) Sen-cha (H), (d) Kamairi-cha, (e) Sen-cha (L) and (f) Ban-cha. (Adapted from Kumamoto and Sonda.[81] With permission.)

Antioxidant Potential

Widespread interest in natural sources of antioxidants has generated an enormous amount of research to assess the antioxidant potential of novel sources of phenolic compounds as well as recognize the inherent activity of commonly consumed foods. This has been combined with the role of lipid peroxidation in human health through modulation with food sources of antioxidants. The average daily consumption of food phenolics has been reported to range from 25 mg to 1 g.[31,80] Using an oxygen electrode, Kumamoto and Sonda[81] evaluated the antioxidant activity of 25 different kinds of tea. They correlated antioxidant activity with the amount of the four major tea catechins (epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate). A correlation (r) of 0.6402 indicated that catechins contributed significantly to the antioxidant activity of green tea (Figure 6.15). However, the intercept at regression line was greater than 1, suggesting other compounds must also be involved such as other polyphenols as well as vitamin C.

Wanasundra and Shahidi[82] compared different flavonoids with commercial antioxidants BHA, BHT, and TBHQ on the stability of marine oils during storage at 65°C. The synthetic and natural antioxidants were added to refined, bleached, and deodorized sea blubber and menhaden oils at equivalent concentrations of 200 ppm and for α-tocopherol, 500 ppm. In the case of sea blubber oil, added rutin, kaemferol, quercetin, morin, and myrcetin proved more effective than α-tocopherol, BHA, and BHT in extending the induction period. However, myricetin proved to be the most

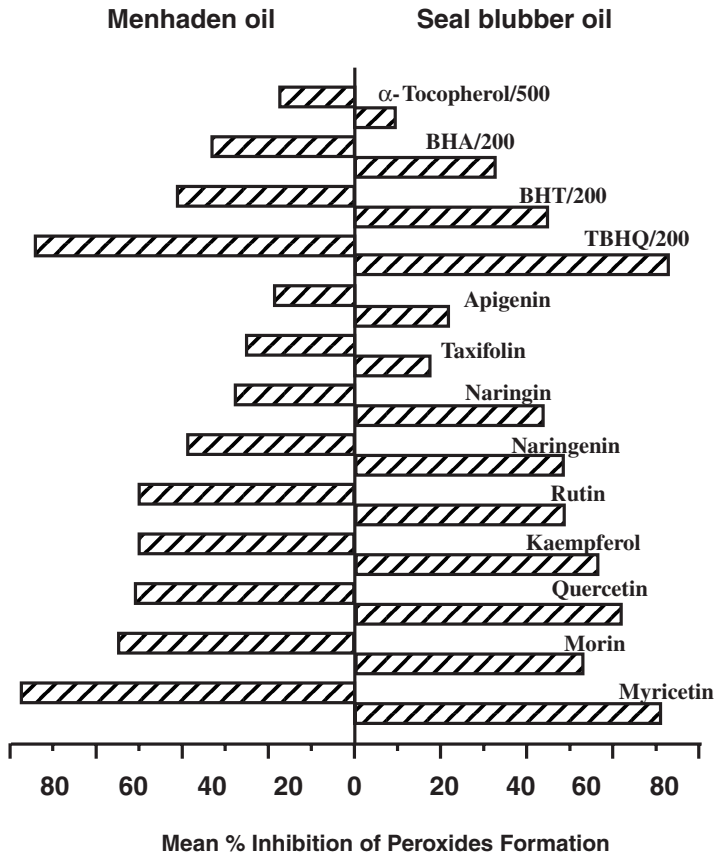


FIGURE 6.16 Effect of flavonoids and commercial antioxidants on peroxide value of refined, bleached, and deodorized seal blubber and menhaden oils during accelerated storage at 65°C without light presence.[78] With permission.

effective of all the flavonoids and was more effective than TBHQ. Flavonoids reduced the peroxide values (PV) in both sea blubber and menhaden oils, particularly the flavonols, kaemferol, morin, myricetin, quercetin, and rutin (Figure 6.16). Myricetin reduced the PV in both oils by 50%. The flavonones, naringenin and naringin, were effective to a lesser degree.

A similar pattern was observed for production of TBARS, with TBHQ the most effective antioxidant (Figure 6.17). However, myricetin, performed very similar to TBHQ throughout the storage period of both oils. Flavonols were far more effective in inhibiting TBARS formation than flavones and flavononols as shown by the following order:

myricetin > quercetin > morin > rutin > kaemferol > naringenin > naringin > apigenin > taxifolin

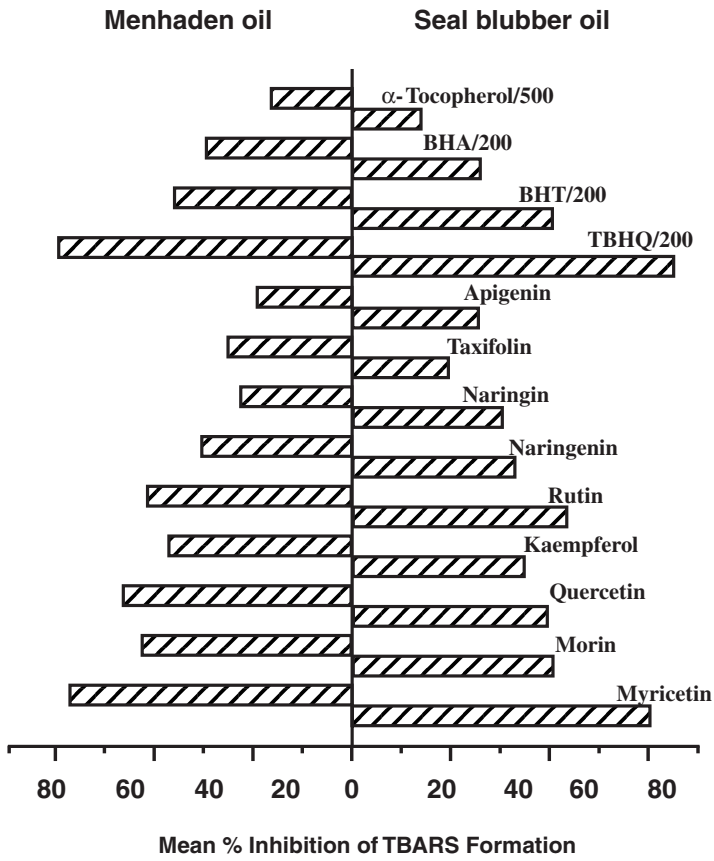


FIGURE 6.17 Effect of flavonoids and commercial antioxidants on 2-thiobarbituric acid reactive products (TBARS) of refined, bleached, and deodorized seal blubber and menhaden oils during accelerated storage at 65°C without light presence.[78] With permission.

Four catechins and rutin were isolated from buckwheat (*Fagopyrium esculentum* Moensch) by Watanabe[83] who examined their ability to scavenge peroxyl radicals formed during AMVN-initiated oxidation of methyl linoleate. The catechins identified were (–)-epicatechin, (+)-catechin-7-O-β D-glucopyranoside, (–)-epicatechin 3-O-p-hydroxybenzoate, and (–)-epicatechin 3-O-(3,4-di-O-methyl) gallate. The relative antioxidant activities of these compounds were compared to BHA and quercetin as shown in Figure 6.18. With the exception of (–)epicatechin, the other catechins exhibited antioxidant activity only slightly less effective than BHA. Subsequent studies by Przybylski et al.[84] also confirmed the presence of antioxidants and radical scavenging components in buckwheat seed extracts.

Herbs and Spices

Herbs and spices have traditionally been used to enhance the flavor of foods. Among the important members of the Libaitae famly are rosemary, sage, oregano, and thyme.

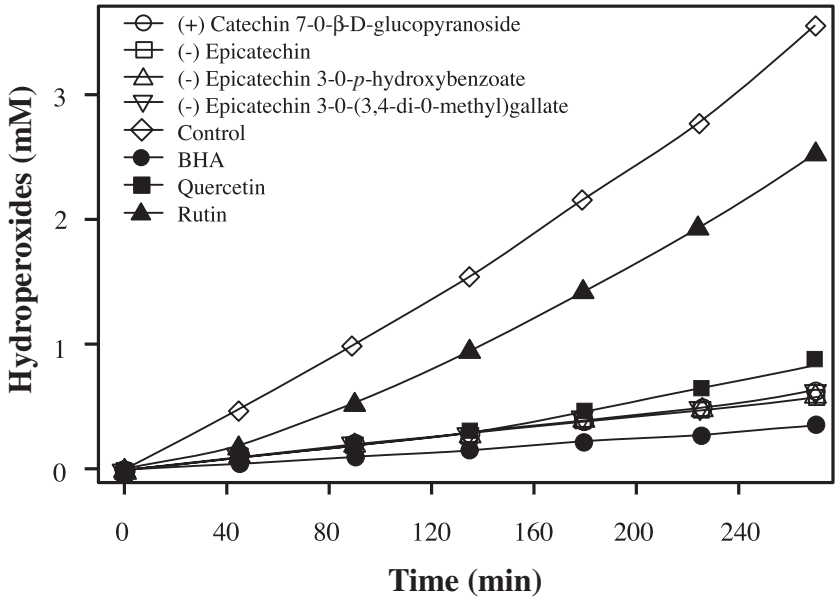
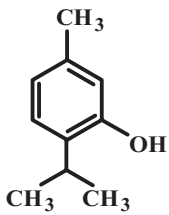


FIGURE 6.18 Antioxidative activity of catechins isolated from buckwheat.[83] With permission.

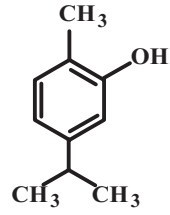
Almost half a century ago, Chipault[85] investigated the antioxidant activity of a number of these spices reporting rosemary and sage to be the most effective ones in lard, while cloves proved more effective in an oil-in-water emulsion. Subsequent research by Chipault[86] screened 17 different spices for antioxidant activity in mayonnaise-type products and reported oregano as the most beneficial. These studies pointed to the importance of identifying the type of food system used in the evaluation process. In recent years there has been a flurry of activity in identifying the antioxidant active components in herbs and spices, the majority of which appear to be phenolic compounds. Since herbs and spices have been used for centuries, their antioxidant active components are considered harmless.[87]

Aeschbach and co-workers[88] first established that the efficacy of thymol and cavracol isomers to inhibit peroxidation of liposome phospholipids was concentration dependent. These antioxidants were identified in the essential oils from plants of the oregano species.[89] Yanishleva et al.[90] examined the antioxidant activity and mechanism of action of thymol and cavracol on the autoxidation of purified triacylglycerols of lard and sunflower oil. At ambient temperature, thymol was reported to be an effective antioxidant.

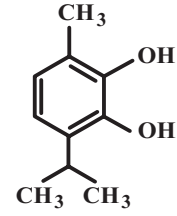
A number of studies examined the antioxidant properties of essential oil of thyme.[91,92] These properties were attributed to the phenolic components, cavracol and thymol. Schwartz and Ernst[93] studied the anitoxidant properties of a non-polar fraction isolated from thyme leaves. Besides cavracol and thymol, these researchers isolated a new phenolic compound, *p*-cymene-2,3-diol (2,3-dihydroxy-4-isoprpropyl-1-methylbenzene) (Figure 6.19). Using the Rancimat and Schaal Oven test (Figure 6.20), *p*-cymene-2,3-diol proved to be the most potent antioxidant and was



Thymol



Carvacol



p-Cymene-2,3-diol

FIGURE 6.19 Structure of natural antioxidants.

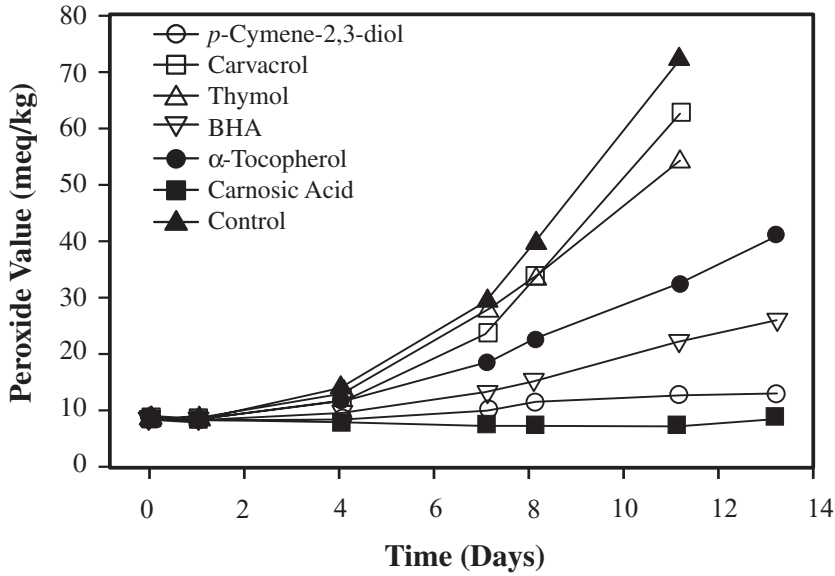


FIGURE 6.20 Antioxidative activity of *p*-cymene-2,3-diol, carvacol, thymol, BHA, α-tocopherol, and carnosic acid at a concentration of 100 µg/g in lard (Schaal Test at 60°C). Each point represents a mean value with a standard deviation by no more than 10%. (Adapted from Schwartz and Ernst.[93] With permission.

far more effective in retarding oxidation of lard compared to α-tocopherol, BHA. Wang and co-workers[94] recently isolated five flavonoid glycosides from thyme. Of these, eriodictyol-7-rutinoside and luteolin-7-0-β-glucopyranoside exhibited the strongest antioxidant properties.

Refined rosemary extract is sold commercially for its antioxidant properties particularly in foods containing animal fats and vegetable oils.[95,96] Rosemary extracts contain a large number of phenolic compounds including carnosic acid, carnosol, and rosmarinic acid, all of which are strong natural antioxidants (Figure 6.21). Frankel[97] commented that it was hard to interpret the literature on commercial

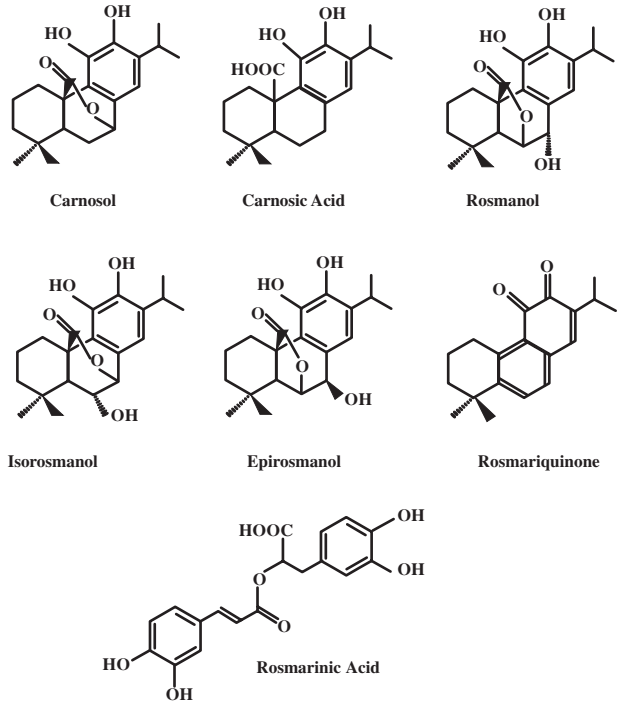


FIGURE 6.21 Structure of antioxidative components isolated from rosemary.

rosemary extracts because of the variable and questionable oxidation conditions used. Subsequent studies by Frankel and co-workers[98] evaluated the antioxidant activity of commercial rosemary extract as well as the active components, carnosol, carnosic acid, and rosmarinic acid on the oxidation of tocopherol-stripped corn oil as well as in corn oil-in-water emulsions. With the exception of carnosol, rosemary extract, carnosic and rosmarinic acids, and α-tocopherol all exhibited strong antioxidant activity. In the case of the corn oil-in-water emulsions, rosemary extracts and constituents exerted lower antioxidant activities with rosmarinic acid being the least active. Changing the pH affected the efficacy of these constituents as carnosol and carnosic acid were far more active antioxidants in emulsion systems buffered between pH 4 and 5 compared to pH 7. Richheimer et al.[99] analyzed the phenolic diterpenes in rosemary and commercial rosemary extracts by HPLC. Carnosic acid was the major diterpene with much smaller amounts of carnosol. Using the Rancimat method to monitor the stability of soybean, these researchers found carnosol to be the most potent antioxidant. Carnosol was far more effective than BHA and BHT but less than TBHQ. A recent study by Guntensperger and co-workers[100] showed the addition of refined rosemary extract, particularly after precooking, extended the shelf life of heat-sterilized meat.

The abundance of phenolic compounds in plants has resulted in a large number of publications on their presence and efficacy as antioxidants. Table 6.5 is a selection of some of the many papers published in this area in the last few years.

TABLE 6.5
Plant Phenolics as Natural Antioxidant

Sources	Phenolic Compounds	Ref.
Beverages		
Cacao	Clovamide	Sanbongi et al.[101]
Green teas	Catechin gallates	Frankel et al.[102]
Red/white wines	Flavonoids	Vinson & Hontz[103]
Red wines	Hydroxylated stilbenes	Lamikanra et al.[104]
Red wines	*cis*, *trans*-resveratrol	Goldberg et al.[105]
Red wines	Anthocyanins	Ghisella et al.[106]
Rooibus tea	Flavonoids	von Gadow et al.[107]
Rooibus tea	Phenolic acids	von Gadow et al.[108]
Tea extracts	Catechins	Kumamoto & Sonda[109]
White wines	Flavonoids, flavonols	Betes-Suara et al.[110]
Crops		
Wild rice hull	Hydroxycinnamic acids	Asamarai et al.[111]
Barley and malt	Insoluble phenolics, coumaric, ferulic acids	Mailard & Berset[112]
Buckwheat	Flavonoids, rutin	Oomah and Mazza[113]
Buckwheat	Flavonoids, rutin	Przybylski et al.[84]
Buckwheat	Catechins	Watanabe[83]
Flaxseed	Phenolic acids	Oomah et al.[114]
Herbs and spices		
Rosemary	Carnosic/methyl carnosate	Huang et al.[115]
Rosemary	Carnosol, carnosic acids	Frankel et al.[116]
Rosemary	Carnosol, carnosic acid	Hopia et al.[117]
Rosemary, thyme	Carnosic/rosmarinic acids	Pearson et al.[118]
Ginger, origanum	Carnosol, carvacrol, thymol, gingerone	
Summer savoury	Rosmarinic acid	Bertelson et al.[119]
Thyme	Carvacvol, thymol	Shetty et al.[120]
Thyme	Flavonoid glycosides	Wang et al.[121]
Fruits & vegetables		
Berries	Anthocyanins, flavonols	Henonen et al.[122]
Citrus fruit	Flavonoids	Benaverte-Garcia et al.[123]
Fruit, juices	Flavonoids	Wang et al.[124]
Grapes	Anthocyanins, flavonols	Si et al.[125]
Onion	Quercentin glucosides	Price et al.[126]
Prunes	Hydroxycinammates	Donovan et al.[127]
Tangarene	Polymethoxylated flavones	Chen et al.[128]
Tomatoes, onions, celery, lettuce	Flavonoids	Crozier et al.[129]

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7 Food Emulsifiers and Stabilizers

N. Garti

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INTRODUCTION

Lecithins, gum arabic, caseins, soy proteins, monoglycerides, and other naturally occurring molecules have been used for many years in foods because of their surface activity. Terminology such as "additives that retard precipitation of dispersed particles", "decrease creaming rates of oil droplets or foams", "prevent aggregation/desegregation of dispersed solid or liquid particles", "prevent syneresis of gelled systems", "condition or stabilize food systems", and "retard coalescence of oil droplets" is often used by food technologists to describe the role of such additives. In general, these molecules are known as emulsifiers and/or stabilizers. The use of such molecules in the food industry was based primarily on practical experience of trial-and-error. Only during the last 25 years has more scientific work been carried out to understand better the structural association and surface activity of these molecules.

Food emulsions, foams, and dispersions are very complex systems containing air, oil, and water as well as other phases such as gas cells, fat crystals, protein, dissolved salts or carbohydrates, fibers, water-soluble polysaccharides, starch granules, or gels, etc. Simple man-made food emulsions, like margarine, mayonnaise, or salad dressing, in addition to the water-oil-emulsifier, often consist of several ingredients such as minerals, spices, dyes, vitamins, etc., which complicate the system. Most foods are considerably more complex.[1] The continuous phase can be partially or completely solid, or tend to solidify with decrease in temperature (dairy products). The semi-solid or solid phase can be crystalline (ice cream) or gelatinous (desserts) and can consist of secondary dispersions of gas cells (whipped cream), additional colloidal solids, or macroscopic solid particles (meat emulsions). The dispersed phase can in itself be liquid (vegetable oil), semi-solid, solid or a combination of the two (Table 7.1). The stabilizers/emulsifiers can be complex blends of proteins (solid or soluble), hydrocolloids, and small molecules.[2] In addition, the

TABLE 7.1
Typical Food Colloids

Food		Types of Emulsion	Method of Preparation	Mechanism of Stabilization
1	Milk	O/W	Natural product	Protein membrane
2	Cream	A + O/W	Centrifugation	As (1) + particle stabilization of air
3	Ice cream	A + O/W	Homogenization	As (2) + ice network
4	Butter and margarine	W/O	Churning and invotator	Fat crystal network
5	Sauces	O/W	High-speed mixing and homogenization	By protein and polysaccharide
6	Fabricated meat products	O/W	Low-speed mixing and chopping	Gelled protein matrix
7	Bakery products	A + O/W	Mixing	Starch and protein network

Abbreviations: O = oil, A = air, W = aqueous phase.

From Darling, D. F. and Birkett, R. J., Food Colloids in Practice, in *Food Emulsions and Foams,* Dickenson, E., Ed., Royal Society of Chemistry, London, 1987, 1. With permission.

processing of food products often results in the formation of cell membrane residues, which function as food emulsifiers or stabilizers forming interfacial films consisting of lipoproteins, glycolipids, etc.

There are several reasons why food emulsions are important to the food manufacturer. A major consideration is the improvement of palatability, mouthfeel, texture, and general appearance in systems containing both oil and water. Olive oil on its own may be too greasy to taste, but is becoming widely acceptable in the emulsified oil-and-vinegar salad dressing. The two immiscible liquids are both required because some flavor ingredients are insoluble in the salad oil, whereas others are insoluble in the vinegar. Food systems and food emulsions have been reviewed in great detail by many scientists. Many excellent books on the subject are available, and many comprehensive review articles have been published.[1-11] The subject has been investigated from various angles and points of view: mechanistic and theoretical aspects of emulsion formation (adsorption, surface characteristics, film formation, rupture, and droplet formation); instability considerations (thermodynamics, kinetics, molecular, surface forces, mechanical statistics, etc.), and the role of the surfactant (chemical and structural requirements). Descriptive (macroscopic and microscopic) characterization and analysis (of components including surfactants) were extensively documented.[12-15] Systems like baked goods,[15] meat emulsions,[16] and milk and dairy products[16] were examined in view of the role that emulsifiers and stabilizers play. More practical aspects of food emulsions have been described by food technologists in terms of formulations, methods of preparation, palatability, rheology, and stability.

This chapter describes food systems and food emulsions from the food emulsifier perspective using natural and synthetic emulsifiers.

TABLE 7.2
Comparison of the Magnitudes of Various Quantities in Emulsions and Foams

Property	Value in Emulsions	Value in Foams
Particle diameter	2×10^{-7} to 10^{-5} m	10^{-4} to 3×10^{-3} m
Particle volume fraction	0.01 to 0.8	0.5 to 0.97
Density difference	10 to 100 kg m^{-3}	10^3 kg m^{-3}
Compressibility of dispersed phase	5×10^{-10} N^{-1} m^2	10^{-5} N^{-1} m^2
Interfacial tension	10^{-3} to 10^{-2} N m^{-1}	0.03 to 0.05 N m^{-1}
Laplace pressure	e.g., 10^4 N m^{-2}	e.g., 10^2 N m^{-2}
Solubility of dispersed phase in continuous phase	0(O/W), 0.15 vol% (W/O)	1.1 vol%

EMULSIONS

Immiscible phases can be redispersed by mixing or homogenizing, and one phase (oil or water) can be ruptured into droplets and dispersed into the other. With time, the system, at equilibrium, will eventually separate into two- or three-component phases, but for a restricted period of time the dispersed droplets will remain suspended. Such thermodynamically unstable systems are referred to as emulsions. The amphiphilic nature and concentration of the systems, along with the nature and concentration of the oil, will determine the kinetic stability of the emulsion. The differences between emulsions and foams are mostly due to the "easiness with which the bubbles are deformed" in comparison to the relative rigidity of the liquid droplets, and the differences in the surfactant organization and immobilization at the interface. Some of the typical characteristics of foams and emulsions are summarized in Table 7.2. An excellent overview of emulsion and foam stability was discussed by Walstra.[17,18]

Emulsion Formation

Emulsification is a set of kinetic processes that takes place during the mechanical rupture of the film in-between the two immiscible liquid layers. The emulsifier plays a significant role in these consequential kinetic steps by lowering interfacial tension between the two phases and hence facilitates emulsion formation. The main role of the emulsifier, however, is related to its ability to adsorb on the ruptured droplets and to prevent their fast reflocculation or coalescence during the emulsification process thereafter. The emulsifier controls the interfacial viscosity between droplets, helps to dissipate the energy, adsorbs on the surface of the newly formed droplets, and alters its surface charge or nature.

Emulsion Stability

The stability of emulsions has been the subject of scientific debate for many years.[19-22] Three main mechanisms of stabilization have been described involving electrostatic, steric, and mechanical stabilization of particles.

Electrostatic Stabilization-DLVO Theory

Electrostatic stabilization is based on the DLVO theory,[23,24] which describes the electrostatic repulsion forces arising from the diffused, electric double-layer present around the surface of charged particles. Opposing the repulsive forces are the Van der Waals interactions between particles, arising from the main types of attraction forces: dipole–dipole, dipole–induced dipole, and induced dipole–induced dipole. The attraction energy is proportional to the particle size, its charge, and the medium (Hamaker constant A) and is inversely proportional to the distance between two approaching particles.

The repulsive term is derived from the local accumulation of counter ions at a charged surface; the concentration of these ions being dependent on the ionic strength of the medium. The presence of a layer of counter ions around a particle is associated with an electrostatic potential which results in two like particles experiencing a repulsive force when they approach one another. Only a few food systems rely solely on electrostatic repulsion forces for their stability.

Adsorbed proteins undoubtedly carry charges, but they are also macromolecules that have the capacity for enthalpy and entropy stabilizing effects which are probably more important. Simple ionic surfactants, which are both functional and permissible in foods, are rare. One such emulsifier which makes stable oil-in-water emulsions is sodium steroyl lactylate (SSL).

Electrostatic repulsive forces must undoubtedly be involved in food colloid stability, particularly when proteins are present. In practice, however, other forces appear to predominate. Thus, dairy-cream emulsion droplets, which are stabilized by milk proteins, do not flocculate at the isoelectric point (pH 4.6) provided the temperature is kept below 10°C. This observation is exploited in the manufacture of cultured creams and related dairy products.

Steric Stabilization

When two colloidal particles approach closely, the adsorbed surfactant layers interact. With adsorbed macromolecules, the interaction can involve a reduction in configurational entropy as molecular chains overlap. Additionally, hydration of adsorbed hydrophilic components can lead to an enthalpy repulsion term when two particles are in close proximity, leading to a local osmotic gradient which tends to force the particles apart. With small-molecule emulsifiers, such as monoglycerides and other esters, the entropy term is insignificant and enthalpy forces are often too weak to provide adequate stability. Consequently, few food colloids are stabilized by small molecule non-ionic emulsifiers. Notable exceptions to this, however, are the polyoxyethylene derivatives of sorbitan fatty acid esters (Tweens), which are capable of providing stable oil-in-water emulsions at only mono-layer coverage. Use of this fact is made when stabilizing flavored oils for fruit juices and other drinks are present, where Tweens are the class of emulsifiers capable of imparting the necessary long-term emulsion stability.

For food emulsions, it is not small-molecule surfactants, but macromolecules (usually proteins) that are the universal stabilizers of oil-in-water emulsions and

foams. Emulsions in creams, ice creams, toppings, and other desserts are invariably stabilized by milk proteins. Stabilization of emulsion droplets by milk proteins has been discussed in a series of publications by Walstra and co-workers.[25,26] It is usually casein, often in aggregated form, which acts as the main stabilizer. In protein-based emulsions, the ability of droplets to coalesce can be almost infinite. Perversely, the functional requirements of practical food emulsions are not for complete stability, but rather for controlled instability. Thus, a cream must be stable during production and distribution, but must destabilize during whipping. This may be achieved by displacing protein from the interface by the addition of a second (low molecular weight) surfactant, or by controlling fat crystallization such that crystals bridge the interfacial protein membrane.[27]

Liquid–Crystalline Phases and Emulsion Stability

An important concept in the understanding of emulsions stabilized by polar lipids is the thermodynamic phase equilibria of the corresponding ternary system: oil–water–surfactant, which was first introduced by Larsen and Friberg.[4] It was demonstrated that a maximum in emulsion stability is obtained when three phases exist in equilibrium, and it was therefore proposed that the lamellar liquid–crystalline phase stabilize the emulsion by forming a film at the oil–water interface. As shown in Figure 7.1, the lamellar-crystalline phase can exhibit a hydrophobic surface toward

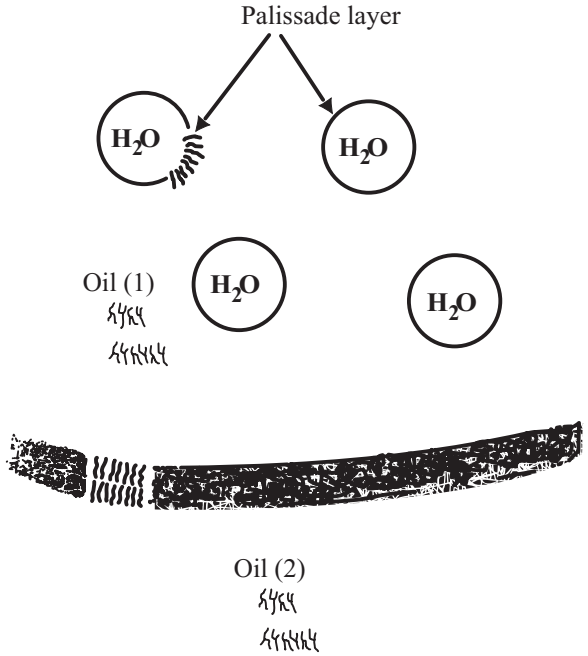

FIGURE 7.1 Schematic representation of the interfacial region of an L_2 droplet (containing oil 1; dispersed in oil 2). (From Hernquist, L., Polar Lipids in Emulsions, in *Food Emulsions and Foams,* Dickenson, E., Ed., Royal Society of Chemistry, London, 1987. With permission.)

the oil and a hydrophilic surface toward the water, and it is quite obvious that this phase possesses ideal interfacial properties needed to reduce the surface energy of oil–water interfaces.

The rheological properties of monolayers of binary surfactant systems have also been related to emulsion stability and to the structural properties of the lamellar liquid–crystalline phases formed by the surfactants in water. It was also suggested that the emulsifier molecules adsorbed at an oil–water interface will adopt the same hydrocarbon chain structure as they have in the bimolecular lipid layer of lamellar mesophases.

Particle Stabilization — Mechanical Stabilization

Stabilization of foams and emulsions by particulate material was originally described by Pickering.[28] The mechanism involves particles adsorbing at the interface, which in turn requires an appropriate balance of interfacial free energies such that particles are wetted preferentially by the continuous phase. The contact angle between the three interfaces defines the ability of the particle to stabilize or destabilize the colloid. The angle θ in Figure 7.2 should lie between 0 and 90°. Pragmatically, the most effective stabilization occurs when θ is in the range of 60 to 70°. If the angle is too close to 90°, physical pertubations at the interface can lead to destabilization.

A classical food product, in which the air phase is stabilized by particles, is whipped cream. Fat droplets adhere to air bubbles during the shipping process, forming a protective layer and preventing bubbles from coalescing. Stability of whipped cream is then described in terms of the interfacial energies between air, fat, and aqueous phases. Increasing the oil–water or air–water interfacial tension increases adhesion of droplets to air bubbles, and so enhances stability. This can be achieved in practice through the use of oil- or water-soluble emulsifiers. Mayonnaise is another example of particle stabilization, although here the size of the adsorbing particle is much smaller: egg-yolk particles (ca. 30 nm diameter) are considered the

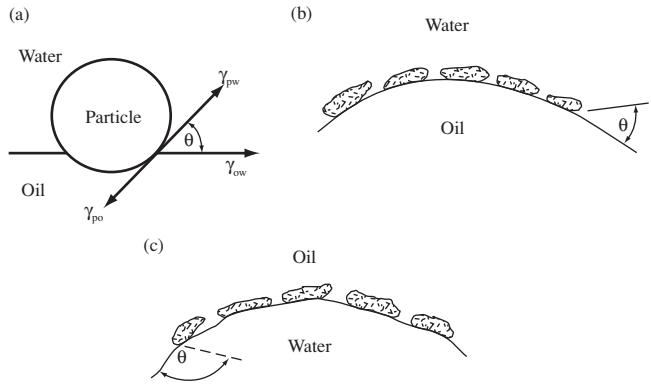

FIGURE 7.2 Illustration of emulsion stabilization by solid particles. (A) Definition of contact angle θ for a single spherical particle at the oil-water interface. In terms of g_{po}, γ_{pw} at the particle-oil, particle-water, and oil-water interfaces, respectively. (B) Oil-in-water; droplet stabilized by particles preferentially wetted by water ($\theta < 90°$). (C) Water-in-oil droplet stabilized by particles preferentially wetted by oil ($\theta > 90°$).

main stabilizing component.[29] Margarine, and possibly peanut butter, are other examples where particle stabilization mechanisms are thought to operate, but in both products the viscosity of the continuous matrix is so high that droplets are immobile and cannot be destabilized by an external shear field.

EMULSIFIERS

Considerable confusion remains in the technological usage between the terms "emulsifier" and "stabilizer". A useful distinction can be made in terms of stability. An emulsifier must confer short-term stability, since this is essential for the preparation of all emulsions. With some emulsions (e.g., cake batter or ice cream mix), a lifetime of hours or even minutes is all that may be required. Other products (e.g., cream liquors or mayonnaise) may need to remain stable for several years, and for these a stabilizer is required. Long-term stability of oil-in-water emulsions may be achieved by thickening the aqueous phase or adsorbing a film of polymer molecules on the aqueous side of the oil–water interface. Most polysaccharides act as stabilizers through their modification of the rheological properties of the aqueous dispersion medium. Proteins, on the other hand, act primarily through the properties of their interfacial films, and are therefore both emulsifiers and stabilizers in many instances. Water-in-oil emulsions such as butter and margarine are mainly stabilized through a network of fat crystals in the semi-solid continuous phase.

Hydrophile–Lipophile Balance (HLB) of Emulsifiers

Emulsifiers are designated as either hydrophilic or lipophilic according to their polar and nonpolar moieties, which affect their solubility in water or oil. The polar emulsifiers are water-soluble, and thus promote the formation of O/W emulsions. Emulsifiers that are less polar tend to be soluble in oil and promote the formation of W/O emulsions. In food emulsifiers, the lipophilic properties are generally the most important ones, but the hydrophilic–lipophilic balance (HLB) can vary considerably according to the chemical composition of the emulsifier. Griffin[30,31] introduced the concept of HLB to measure the affinity of a nonionic emulsifier to oil or water. He suggested the following equation for calculating HLB:

$$HLB = 20(1 - S/A)$$

where S = saponification value of the ester
A = acid value of the fatty acid

In certain cases it is difficult to determine the saponification number accurately, e.g., for esters of tall oil, lanolin, or rosin and for these the following equation based on composition is used:

$$HLB = (E + P)/5$$

where E = weight percentage of oxyethylene
P = weight percentage of the polyol

When the only hydrophilic group present is ethylene oxide, and also for ethylene oxide derivatives, this reduces to HLB = E/5. The W/O type emulsion will have a low HLB of 4 to 6, an O/W emulsion has an intermediate HLB of 8 to 15, and a solubilizing agent has a high HLB of 15 to 18. Several methods have been proposed to determine the HLB of nonionic surfactants[32] and tables with the HLB values are available in any surfactant science textbook. Moore and Bell[32] suggested the H/L value. Greenwald et al.[33] suggested a water titration procedure (the water numbers) of organic solution of surfactant, but the method has not been widely used. Davies[34] developed a method for calculating the HLB values of surfactants directly from their chemical formula, using empirically derived group numbers.

$$\text{HLB} = \Sigma\text{Hydrophilic group numbers} - \Sigma\text{Lipophilic group numbers}$$

They gave the values of the group numbers in a single table and the match between this method of evaluation of HLB and the empirical work is quite good. The experimental determination of the HLB of a surfactant is not an easy task.

The HLB concept is based on a simplified picture of the coalescence and formation process, and does not take into account the important role of liquid-film thinning between droplets and the importance of shear and dilution properties of the adsorbed layer of the surfactant around the droplets. Therefore, it is not surprising that the literature is full of examples of emulsifications that do not obey the HLB concept. While the validity of the HLB has been criticized, nevertheless it can be a very useful concept in the formulation of emulsions. In practice, in formulating an emulsion one must determine, with a set of known emulsifiers, the required HLB of a given oil used for the preparation of the emulsion (by defining the minimum oil separation at given conditions and constant concentration of a set of known emulsifiers). Many of the common oils have required HLB values given in tables. For any new emulsion to be prepared, the best set of emulsifiers will be determined on the basis of the required HLB and the minimum oil separation after storage. The HLB values are additive and can be easily calculated for a set of two nonionic emulsifiers.

Shinoda and co-workers[35-38] devoted many years of research to find better concepts to describe the relationship of the surfactant structure, its solubility, and the emulsification parameters. Concepts such as phase inversion (PIT), HLB numbers, and emulsion inversion point (EIP) were introduced and phase diagrams constructed. The Shinoda concepts have a sound thermodynamic basis and explain many of the phenomena attributed to the nonionic emulsifiers.

Typical examples of food emulsifiers are propylene glycol monostearate, which is strongly lipophilic with an HLB of 3.4; polyoxypropylene stearate with an HLB of 8; polyoxyethylene monostearate which is less lipophilic (more hydrophilic) with an HLB greater than 11. HLB values of the many different types of food grade emulsifiers have been given by Griffin[30,31] and Petrowski.[39]

HLB values can be very useful for selecting an appropriate emulsifier, preparing an emulsion, or for blending several emulsifiers to achieve a desired HLB value. These HLB numbers are algebraically additive calculations which work well when a given formula is composed of water and oil-type food emulsions or esters of

polyvalent alcohols and fatty acids. However, for formulations involving such food items as eggs, flour, salt, milk, starch, and sugar the selection process becomes more difficult. Therefore, others have modified Griffin's original concept to calculate HLB values of molecules of varying polarity. Finally, the use of HLB values serves as a guideline for the selection of emulsifiers, and requires trial and error and thorough experimentation when applied to food product formulations.[40,41]

Classification of Emulsifiers

Emulsifiers are classified according to three main criteria:

1. The nature of the charge on the head of the hydrophilic group (cationic, anionic, nonionic, and zwitterionic).
2. The nature of the hydrophilic group (carboxylates, sulfates, sulfonates, ethoxylates, etc.).
3. The nature of the hydrophobic tail.

Becher[41] grouped all the surfactants in one table (Table 7.3).

TABLE 7.3
Classification of Emulsifying Agents

I. ANIONIC
   A. *Carboxylic Acids*
      1. Carboxyl joined directly to hydrophobic groups.
      2. Carboxyl joined through an intermediate linkage.
   B. *Sulfuric Esters (Sulfates)*
      1. Sulfonic group directly linked to hydrophobic group.
      2. Sulfate group joined through itnermediate linkage.
   C. *Alkane Sulfonic Acids*
      1. Sulfonic group directly linked to hydrophobic group.
      2. Sulfonic group joined through intermediate linkage.
   D. *Alkyl Aromatic Sulfonic Acids*
      1. Hydrophobic group joined directly to sulfonate aromatic nucleus.
      2. Hydrophobic group joined to sulfonated aromatic nucleus through intermediate linkage.
   E. *Miscellaneous Anionic Hydrophilic Groups*
      1. Phosphates and phosphonic acids.
      2. Persulfates, thiosulfates, etc.
      3. Sulfonamides.
      4. Sulfamic acids, etc.

II. CATIONIC
   A. *Amine Salts (Primary, Secondary, and Ternary)*
      1. Amino groups joined directly to hydrophobic group.
      2. Amino group joined through an intermediate group.
   B. *Quaternary Ammonium Compounds*
      1. Nitrogen joined directly to hydrophilic group.
      2. Nitrogen joined through an intermediate group.

TABLE 7.3 (continued)
Classification of Emulsifying Agents

- C. *Other Nitrogenous Bases*
  - 1. Nonquaternary bases (e.g., guanidine, thiuronium salts, etc.).
  - 2. Quaternary bases.
- D. *Non-nitrogenous Bases*
  - 1. Phosphonium compounds.
  - 2. Sulfonium compounds, etc.

III. NONIONIC
- A. Ether Linkage to Solubilizing Groups
- B. Ester Linkage
- C. Amide Linkage
- D. Miscellaneous Linkages
- E. Multiple Linkages

IV. AMPHOTERIC (SWITTERIONIC)
- A. *Amino and Carboxy*
  - 1. Nonquaternary
  - 2. Quaternary
- B. *Amino and Sulfuric Ester*
  - 1. Nonquaternary
  - 2. Quaternary
- C. *Amino and Alkane Sulfonic Acid*
- D. *Amino and Aromatic Sulfonic Acid*
- E. *Miscellaneous Combinations of Basic and Acidic Groups*

V. WATER-INSOLUBLE EMULSIFYING AGENTS
- A. *Ionic Hydrophilic Group*

Food emulsifiers must comply with the non-toxicity, non-carcinogenic, and non-allergenic requirements and must be approved by the different health authorities in the different countries in which they are used, and within the different applications in which they are added. Only a few families of emulsifiers can be used in food, some because of health regulations and some because of practical aspects. For example, ionic emulsifiers may be of limited value in the presence of acids or bases, since they are susceptible to chemical modifications and loss of surface activity. Therefore, few ionic emulsifiers are used by the food industry. Attempts were made to group the food emulsifiers into two categories: (1) naturally occurring surfactants, including proteins (from vegetable, marine, and animal sources), chemically and enzymatically modified proteins, glycolipids, polysaccharide hydrocolloids (from various sources), and naturally occurring small molecular weight surfactants (like lecithins, monoglycerides, and saponins); and (2) synthetic surfactants. The synthetic surfactants permitted for food applications are mostly derived from fatty acids or alcohols, and are esters of the fatty chains and hydrophilic functional groups like polyol, glycol, sorbitol, sucrose, acetic, lactic, succinic, tartaric, citric, and polyethyleneglycols. It is difficult to group the synthetic emulsifiers into one group. The way we have chosen to discuss them is according to their complexity and frequency of use.

NATURAL EMULSIFIERS

Phospholipids can be obtained from various sources, two of which are oilseed lecithins and egg yolk lecithins. The phospholipid composition of soybean lecithin appears to vary according to the method of extraction. Phosphatidylcholine (PC) full content varies from 29 to 46%; phosphatidylethanolamine (PE) varies from 21 to 34%; and phosphatidylinsoitol (PI) varies from 13 to 21%. Other minor constituents are phosphatidylserine, phosphatidic acids, lyso-PC, lyso-PE, and lyso-PI.[48] In addition to soybeans, lecithin has been found in many other oilseeds including peanut, cottonseed, sesame, safflower, sunflower, and others.[49-51]

Soybean Lecithins

The soybean plant is a source for two important naturally occurring emulsifiers, lecithin and soy proteins. When soybean is processed the hulls are separated from the cotyledons (bean chips or bean meat) which are then transformed into flakes from which the oil is extracted with hexane.[49-54] The oil is rich in polar lipids. Upon hydration, using a single process followed by centrifugation, the lecithins are extracted from the oil. The oil-in-water emulsion is dried and the lecithin is isolated at various degrees of purity (Table 7.4). After the defatted flakes have been extracted, the proteinaceous matter is desolventized and toasted into different fractions of meals with various levels of proteins.

Most industrial lecithins have only 60 to 65% lecithin and 30 to 35% soybean oil as plasticizer. Many other types of lecithin are available including: clarified lecithin (carefully filtered to remove HI materials), compounded lecithin (combined with surfactants or other additives, or blended with carriers to form a product with special properties), deoiled lecithin (dry or granulated powders with a high phosphatide content), modified lecithins (chemically or enzymically, hydrogenated, hydroxylated, ethoxylated, halogenated, sulfonated, acylated, succinylated, ozonized, and phosphorylated — the most common being hydroxylated and acetylated), and fractionated lecithin (for cosmetic and pharmaceutical applications). Lecithin is considered a "GRAS" (Generally Recognized as Safe) material by FAO.

Phospholipids are found in almost all natural fats with varying compositions and amounts depending on the source of the fat. In the glycerol backbone of the phospholipid molecule, the two glycerol positions generally contain (but not necessarily) a saturated straight chain fatty acid in the one position (R in Figure 7.3) and an unsaturated straight chain fatty acid in the two position (R′ in Figure 7.3). The fatty acids are usually 16 to 18 carbons long. Phospholipids generally contain a molecule of phosphoric acid esterified at one of the glycerol positions (Figure 7.3). The phosphoric acid is usually esterified to another compound, such as choline, ethanolamine, or inositol. Thus, the nomenclature of phospholipids is similar to that used with triglycerides.

Soybean oil contains 1.5 to 3.0% phosphatides.[55] Crude lecithin has a soybean oil content of about 30%. Phosphatidylcholine (PC), one of the major active components, is present at approximately 16%. Phosphatidylethanolamine (PE) is also a major component and is found at approximately 14%. The third major phospholipid

TABLE 7.4
Specifications of Commercial and Industrial Grades of Soybean Lecithins

Analysis	Grade		
	Fluid Unbleached Lecithin	Fluid Bleached Lecithin	Fluid Double-Bleached Lecithin
Acetone insoluble, min.	62%	62%	62%
Moisture, max.	1%	1%	1%
Hexane insoluble, max.	0.3%	0.3%	0.3%
Acid value, max.	32	32	32
Color, gardner, max.[a]	18	14	12
Viscosity, centiPoise, @77°F, max.[b]	15,000	15,000	15,000
Acetone insoluble, min.	65%	65%	65%
Moisture, max.	1%	1%	1%
Hexane insoluble, max.	0.3%	0.3%	0.3%
Acid value, max.	30	30	30
Color, gardner, max.[a]	18	14	12
Penetration, max.	22 mm	22 mm	22 mm

[a] Undiluted basis.

[b] By any appropriate conventional viscometer, or by AOCS Bubble Time Method Tg 1A-64, assuming density to be unity. Fluid lecithin having a viscosity less than 7,500 centipoises may be considered a premium grade.

By Karl-Fischer Titration (AOCS Method Tb 2-64).

Using Precision cone 73525, Penetrometer 73510; sample conditioned 24 hours at 77°F. Yearbook and Trading rules, National Soybean Processors Association (1986–87).

component is a complex mixture of inositol phosphatides (PI) present at levels of approximately 12%.[50] Miscellaneous low level constituents include water, phosphatidic acid, pigments, galactosyl glycerides, various glycolipids, phosphatidylserine, carbohydrates, sterols, and tocopherols. The moisture content in commercial crude lecithin is usually 1% or less. The multifunctional properties and its "natural" status of lecithin makes it an ideal food ingredient. The major functional properties of lecithin are summarized in Table 7.5. Lecithins exhibit many beneficial properties and are used in many commercial applications, including nutrition and health. However, the main function of the phosphatides is their emulsifying ability for fats and oils.

The long-chain fatty acid moieties contribute hydrophobic properties; those properties are counterbalanced by the polar or hydrophilic character of the phosphate moiety. In an oil-in-water system, the phospholipid components concentrate at the oil/water interface. The polar, hydrophilic parts of the molecules are directed towards the aqueous phase and the nonpolar hydrophobic parts are directed towards the oil phase. Concentration of phosphatides at the oil/water interface lowers the surface tension and makes it possible for emulsions to form. Once the emulsion is formed,

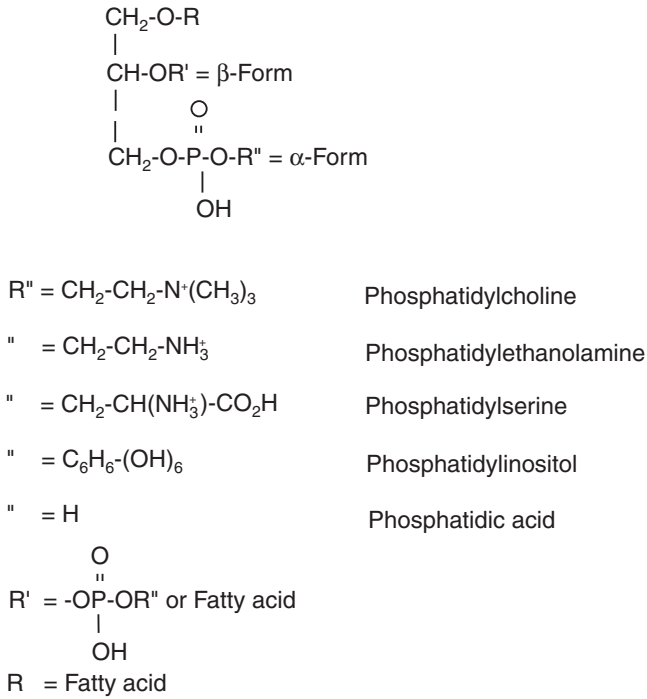

FIGURE 7.3 Several groups attached to the phosphate in phospholipids.

the phosphatide molecules at the surface of the oil or water droplets act as barriers that prevent the droplets from coalescing and then they stabilize the emulsion.[1] The following discussion deals with the major functional uses of lecithin products:

1. its functionality as emulsifier in food emulsions
2. its ability to solubilize various compounds in aqueous systems
3. its sol dispersing power
4. its foaming and defoaming activity

Emulsifier in Foam Emulsions

Examples of food emulsions include milk, butter, margarine, puddings, chocolate, bakery items, cheese, milk, replacers, and ice creams.

Crude lecithins are excellent water-in-oil (dispersed phase is water, continuous phase is oil) emulsifiers. However, modified lecithins can function to emulsify either water-in-oil or oil-in-water emulsions, depending on the type of lecithin modification and the specific parameters of a system. These system parameters can include pH, types of components, and component ratios as well as solid content. Unlike crude lecithins, hydroxylated lecithins are stable in acid systems (pH <3.5). Fractionated lecithins can be manufactured for specific types of emulsion. Since lecithin emulsifying activity is dependent on its phosphatide ratios, changing the ratio can alter

TABLE 7.5
Functional Properties of Lecithins

Food Processing Ingredients in:	
Margarine	Emulsifier, anti-spattering agent
Confections and snack foods	
Chocolate	Crystallization control, viscosity control, anti-sticking
Caramels	
Coatings	
Instant foods	
Cocoa powders	Wetting and dispersing agent, emulsifier
Instant drinks	
Instant cocoa	
Instant coffee	
Protein drinks	
Dietetic drinks	
Coffee whiteners	
Milk replacers	
Cake mixes	
Puddings	
Instant toppings	
Commercial bakery items	
Breads	Crystallization control, emulsifier, wetting agent, release agent (internal and external)
Rolls	
Donuts	
Cookies	
Cakes	
Pasta products	
Pies	
Cheese products	
Pasteurized processed cheese and cheese food	Emulsifier; release agent
Imitation cheese	
Meat and poultry processing	
Meat and poultry glazes and basting compounds	Browning agent, phosphate dispersant
Pet foods	Dietary supplement, release agent, emulsifier
Bacon	
Dairy and imitation dairy products	
Infant, milk formulas	Emulsifier, wetting and dispersing agent, anti-spattering agent, release agent
Milk and cream replacers	
Egg	
Imitation eggs	
Whipped toppings	
Ice cream	
Flavored milks	
Flavored butters (garlic, etc.)	
Basting butters	

TABLE 7.5 (continued)
Functional Properties of Lecithins

Food Processing Ingredients in:	
Miscellaneous products	
Peanut spreads	Crystallization control, emulsifier
Salad products	
Flavor and color solubilization	
Packing aid	
Polymer package interior coating	Release agent, sealant
Can interior coating	
Sausage casing coating	
Stocking net	
Processing equipment	
Frying surfaces	Internal (in product) and/or external release agent, lubricant
Extruders	
Conveyors	
Broilers	
Dryers	
Blenders	
Evaporators	

its emulsifying capabilities. When working with lecithins, one might readily conclude that lecithin products seem to emulsify on a very system-dependent basis. This is due to amphoteric (+,–) polar ends on the individual phosphatides, which makes it impossible to assign a general emulsifier rating (such as HLB) to lecithin products.

Most processed food emulsions, however, are not stabilized by emulsification alone. The particle size of the dispersed phase has to be much smaller for dynamic stability than is practically possible with foods. Emulsifier/stabilizer systems, however, are normally used to make stable food emulsions. Lecithin will break up (emulsify) the particles, while a stabilizer (water-soluble polymer, etc.) will hold the particles in a dispersed orientation giving a stable emulsion. Lysolecithins, which are more hydrophilic, show stronger oil-in-water emulsifying properties.

Solubilization Applications

Lecithins have also been used for solubilization applications. Stable microemulsions have been prepared with various fractionated lecithins and are used in direct applications as reservoirs for certain materials (flavors, pharmaceuticals, etc.) or as microreactors for enzymatic reactions.

Sol Dispersing Power

Lecithin products are still one of the best, most effective surfactants for dispersing sols. This seems to be due to lecithin's affinity for solid/liquid surface interfaces. Phosphatides seem particularly attracted to particles containing metals and metal

salts. Examples of food sols include some liquid chocolates, instant drinks, some frosting mixes, and pigmented foods.

Foaming and Defoaming Activity

Upgraded lecithins have been employed as effective foam control agents in whipped toppings, ice creams, and many types of candies. They have also been used as effective defoaming agents in foams caused by powdered proteins in water. This is an excellent example of the system specificity of lecithin products.[50] Lecithin is used in a large variety of food products such as margarine, confections, snack-food, soups, baked goods, cheese products, processed meat, poultry and fish products, dairy and dairy-type products, and dairy supplements. Commercial soybean lecithin products have been used in many food applications which are not directly related to their emulsification activities. Some of these include: (1) co-emulsification for monoglycerides to interact with amylose, (2) deoiled soy lecithin, 95% powder, as co-dispersant, (3) spray-dried combination of standard soy lecithin and milk solids (mainly lactose) as a dispersant and wetting agent, (4) spray-dried combination of modified partial glycerides and milk solids for flour treatment, assurance of uniformity and high quality bread making properties with wheat flours, (5) in instant pudding mixes, or whole-milk powders as an instantizing aid, (6) in infant formulas, to promote remixing, (7) in egg replacers as a release agent, (8) in ice cream to increase smoothness and prevent graininess, and (9) in meat products as a fat emulsifier.

Other Sources of Lecithins

Cottonseed Lecithins

Only limited quantities of cottonseed lecithins are available.[48,56,57] Most of the phospholipids are present in the non-oil materials (1 to 2%) separated from hydraulically pressed oil by alkali or water washing. Different extraction methods normally used in the oil crushing industry cause few differences in the percentage of phospholipids or fatty acids in cottonseed oil. However, limited data are available in the literature on the composition of all cottonseed phospholipids and all of the lecithin and cephalin components have not been individually separated and quantified. The published literature contains a diversity of standard research procedures for isolating the total phospholipid portion of plant materials.[54-58] They include solvent extraction of the lipids and application of various separation and purification steps for fractionation and quantifying the individual phospholipids. Additionally, these procedures include steps to concentrate the crude phospholipid extracts, to remove non-phospholipid impurities, and selectively precipitate or extract the phospholipids. These methods use solvent, solvent-solvent counter current fractionation, metal salt complexing and precipitation, column chromatography, and fractional crystallization procedures. Cottonseed lecithin contains phosphatidylcholine (23.2% of total phosphorus), phosphatidyl-ethanolamine (13.5%), phosphatidylinositiol (13.4%), phosphatidylserine (2.4%), and phosphatidic acid (8.8%). Since cottonseed oil and lecithin contain only trace amounts of fatty acids with more than two double bonds (linoleic acid), oxidative rancidity is less of a problem. Other sources of phospholipids

(e.g., soybean) contain linolenic acid in amounts that can affect flavor, color, and odor. With the potential for increasing revenues, decreasing waste disposal costs, and improving emulsification, glandless cottonseed oil and lecithin products are economically attractive.

Most cottonseed grown for commercial use has gossypol-containing glands.[48,56,57] Cottonseed products or blends containing gossypol intended for human use in the U.S. must contain no more than 0.45% free gossypol. The dark-brown color caused by gossypol in cottonseed meal, oil, and lecithin also limits its use in foods. Changes in oil extraction processes have produced oil that contains considerable amounts of free gossypol pigments in crude phospholipids causing color and toxicity problems.

Corn Lecithin

Patents for commercial preparations of corn phosphatides and for products containing lecithin (cosmetics, ointments, foaming agents, and rust inhibitors) were issued during the 1930s through the 1950s.[62-64] The growth in demand for corn sweeteners may make other products of the corn-refining industry, such as lecithin, more available and competitive. Similar compositions were noted for the major phospholipids of phosphatidylcholine, phosphatidylinositol, and phosphatidic acid. Glycolipids represent a higher proportion of polar lipids in corn than in soybean lecithin and the percentages of minor components, like stearylglycoside esters, are more than twice that of soybean. Both the glycolipids and phospholipids of corn have lower percentages of linolenic acid and are more saturated than those of soybean. Linoleic acid varies from 42 to 70% depending on the variety of corn. This genotypic effect on fatty acid composition of phospholipids introduces the possibility that lecithin with selected content of these nutritional components can be obtained by corn breeding.

Other Potential Sources of Lecithin

Other potential sources of lecithin include canola/rapeseed, sunflower seed, peanut, palm kernel, xenophytic curcurbit seed, cereal grains including wheat, barley, and rice, and olive, mango, and avocado fruit. Nonconventional sources include palash (*Butea monosperma*), papaya (*Caricapapaya*), jangli bodani (*Sterculia factida*), coriander (*Coriandrum sativum*), and carrot (*Daucus carota*) seed. Phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol have been identified as major components in all of these sources.[48,49,61,65-74] Minor components include lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidic acid, phosphatidylglycerol, glycolipids, and triglycerides. Differences were noted among minor phospholipids which could alter the functionality of lecithin derived from these seed sources. Canola lecithin has been shown to contain a greater amount of glycolipids than sunflower and soybean lecithin.

Natural Emulsifiers in Eggs

Whole liquid egg contains 11.5% fat, while liquid yolk contains 30.6%. Dried egg-white contains 92% proteins and 8% salts and sugars. The egg yolk solids contain

65 to 70% fats, which contain 65% triacylglycerols, 30% phospholipids, and 4% cholesterol.[75] Of the 30% phospholipids in egg yolk, the percentages are PC 73; PE, 15.5; PI, 0.6; lysophosphatidylcholine, 5.8; sphingomyelin, 2.5; lysophosphatidylethanolamine, 2.1; plamalogen, 0.9.[76] These lipoproteins can be separated by high speed centrifugation of egg yolk into the sediment, called granules, and the supernatant fraction, called plasma. Granules represent 19 to 23% of the yolk solids, whereas plasma represents 78% of the liquid phase. On a dry weight basis, granules contain around 34% lipids, of which 37% are phospholipids, mainly PC (82%) and PE (15%). Plasma contains a low-density lipoprotein (LDL) fraction that is comprised of 84 to 89% lipid, of which 26% is phospholipid (71 to 76% PC, 16 to 20% PE, and 8 to 9% sphingomyelin and lysophospholipid).[78]

Owing to the good emulsifying properties of egg yolk lipoproteins, oil can be dispersed in other food ingredients so that the lipoproteins can contribute to the consistency of mayonnaise and salad dressing and to the structure of cream puff shells. Whole eggs are used in rolls, sponge and layer cakes, and bread, while yolks are used in salad dressings, mayonnaise, doughnuts, sweet goods, and cakes that require more yellow color. Because of the high protein content, egg white is used in angelfood cakes, puff pastry, white pound cakes, layer cakes, cupcakes, meringue toppings, and candies, as well as in a number of premixed products.

Natural Emulsifiers in Milk

Few of these have yet to be used commercially. Besides soybean lecithins the only other natural source of lecithin used extensively in foods is that obtained from eggs or that found in milk. Milk is one of the naturally occurring emulsions found in foods. Cow's milk contains milk fat (3 to 6%), protein (3 to 4%), lactose (5%), and ash (<1%). Of the milk fat, the phospholipid fraction is generally not larger than 1% and triacylglycerols comprise 97 to 98% of the total milk fat. Other lipid soluble substances found include sterols, carotenoids, and fat-soluble vitamins which all aid emulsification.[74]

PHOSPHOLIPIDS AS LIPOSOMES FOR FOODS

Liposomes are tiny hollow bodies made up of phospholipids and filled with water. Their diameter is in the region of 200 to 500 nm. They were originally made to imitate cells, so that the transport of substances into and out of the cell could be studied. Liposomes form compartments without which the cell would be unable to function. Together with water, phospholipids form double layers in a structure that enables them to act as "cell walls", dividing the compartments from each other which has great technical benefit.[79-82] Water-soluble substances can be stored in the liposome core surrounded by the structural bilayer membrane which has two useful effects. First, encapsulated substances can be distributed in food more homogeneously, and second, encapsulated substances are immobilized because of the physical stability of the liposomes. The encapsulated substances do not easily migrate, and take longer to be released from the product. For example, a lemon-flavored cake stays fresh longer and retains the flavor.

The use of lecithin fractions as liposomes in foods is relatively new. By fractionating the complex mixture of lecithin and removing part of the phospholipids it is possible to optimize these special functions. These are discussed here.

Water Retention

This is important for maintaining the freshness of baked goods. If a "liposome cream" is made, some great portion of the water will be firmly bound in the finished bread, which will take considerable time to release thereby enhancing bread freshness.

Flavor Retention

Liposome formation can increase substantially the surface area of the product resulting in enhanced flavor. For example, the subjective freshness of a cake is greatly enhanced by the fine fat distribution brought about by the liposomes.

Protection against Inactivation

Yeast cells in frozen dough are exposed to very considerable stresses. Some of these yeast cells are inactivated by ice crystals and the processes related to the cold. In the past, attempts were made to circumvent this problem by just adding more yeast. However, by using liposomes it is possible to provide the yeast with sufficient protection against cold. The membranes of yeast have a high content of unsaturated fatty acids so that they remain fluid and not inactivated or destroyed by crystallization. When the temperature of the dough rises again, they are ready for action immediately so that baking yields improve significantly.

Liposomes can also be loaded with water-insoluble (fat-soluble) matter including vitamins and flavorings. Flavorings encapsulated in liposomes do not diffuse through the membrane until they are needed.[82] The food applications of liposomes are summarized in Table 7.6.

TABLE 7.6
Some Food Applications of Liposomes

Function	Food
Control of crystallization	Extruded snacks
Lowering of the freezing point	Convenience products
Protection against surrounding influences	Cakes and pastry goods
Separation of aqueous/fat phases	Bread and biscuits
Flavor retention	Ice cream
Water retention, control of a_w value	Cheese
Sensory impressions substitute for fat	Frozen dough portions and pizza
Enzyme functionality	
Protection of yeast cells against cold	

SYNTHETIC EMULSIFIERS

Mono- and Diglycerides of Fatty Acids

The most commonly used emulsifiers in the food industry are mono- (MG) and diglycerides (DG). These are prepared commercially by direct esterification of edible fats or fatty acids (from vegetable or animal origin) with glycerol at elevated temperatures, or by interesterification of an oil with glycerol.[4,83] During transesterification, triglycerides are heated with glycerol and a catalyst, usually sodium hydroxide, under vacuum at 200°C. As the fatty acids are hydrolyzed from the triglycerides, some re-esterify at other free hydroxyl positions to form mono- and diglycerides.[84] The yield of MG depends on the proportion of triglyceride to glycerol used. Such methods were demonstrated by reacting the oils of coconut, peanut, sesame, linseed, and sardine and direct esterification of fatty acids with glycerol.[85] Feuge and Bailey[86] demonstrated that the proportions of glycerol, mono-, di-, and triglycerides can be calculated statistically based on two assumptions: The reaction between admixtures from either method contains traces of free glycerol and free fatty acids, which can be removed by distillation. Commercial MG usually contain about 40 to 50% monester, 40% diester, and 10% triglycerides.[87]

Mono- and diglycerides of fatty acids exist in several structural modifications (Figure 7.4). In monoglycerides, if the fatty acid is attached to the middle carbon atom, the molecule is symmetrical and the monoglyceride can be termed a beta-monoglyceride. When the reaction is carried out at 20°C the relative proportions of

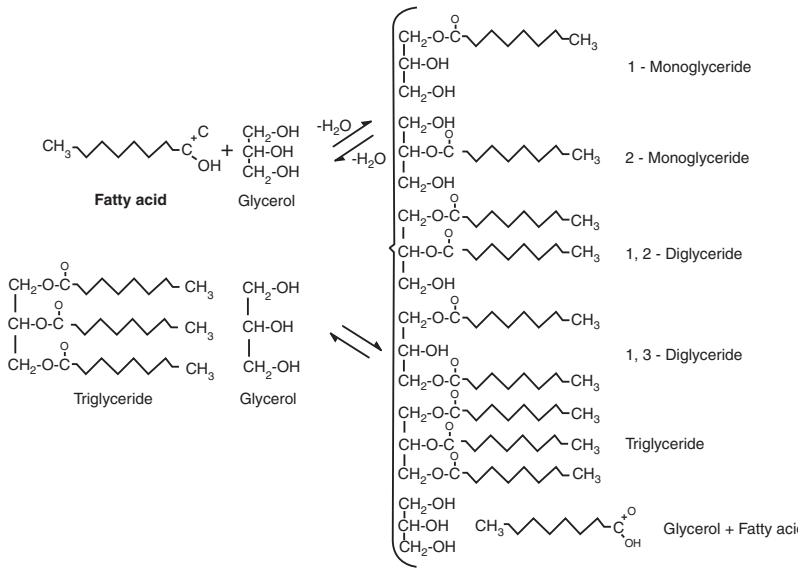

FIGURE 7.4 Preparation of monoglycerides of fatty acids by: (I) Transesterification of fats with glycerol, and (II) direct esterification from fatty acids, at elevated temperatures and in the presence of alkaline catalyst.

the two isomers are 95:5 in favor of attachment at the terminal, alpha-position, but at 200°C, the ratio changes to 82:18.[88] In diglycerides, the fatty acids can attach to any two of the three positions.

Mono- and diglycerides can be solid or liquid materials depending on the nature of fatty acids composing the molecule. Monoglycerides composed of mainly oleic acid or unsaturated fatty acids are liquid and more sensitive to oxidation and degradation while monoglycerides composed of saturated fatty acids are solid powders or waxy materials. The melting point of the product depends on the origin, structure, and composition of the fatty acids.

Since mono- and diglycerides are both hydrophilic (containing hydroxyl groups) and lipophilic (containing fatty acids), they are partially soluble in both water and fat. They are strongly adsorbed at the triglyceride/water interface and they readily form a liquid crystal phase in association with water.[89] Hence they are excellent emulsifiers. As in the case of other emulsifiers, MG are found at the O/W interface with the polar groups (hydroxyls) of the molecule in the aqueous phase and the nonpolar group (the fatty acid) in the lipid phase. In this manner, monoglycerides act to reduce the interfacial tension and to stabilize emulsions.[90] Their lipophilic character causes them to be excellent W/O emulsifiers, as in margarine.[91]

The major uses of MG and DG are in bakery products, prepared cake mixes, margarine, convenience foods, coffee whiteners, and frozen desserts. Normally they are used along with a fat system, and frequently in conjunction with other emulsifiers. MGs containing oleic acid are used as an emulsifier in ice cream. Those prepared from lard, tallow, cottonseed, soybean, and peanut oils (usually partially hydrogenated) have been used as emulsifiers in cake and icing shortenings. Fully hydrogenated MG from meat fats and vegetable oils have been used as emulsifiers in yeast-raised baked goods, cake shortenings, ice cream, confections, and many other food products.[87]

Compounds that contain esters of fatty acids and free hydroxylic or alcoholic groups are used as emulsifiers in bakery products. Of these compounds, MG and DG of saturated fatty acids and mostly those composed of stearic acids (MGS and MDGS) are most frequently used because their functions are ideally suited to those required for a shortening. To enhance MGS activity as an emulsifier, other compounds and/or modified MG are added. These include sorbitan and polyoxyethylene sorbitan esters of fatty acids, propylene glycol esterified with fatty acids, lecithin, or lactylated, acetylated, and succinylated monoglycerides.[87] MGs and MDGs and their modified forms greatly improve bakery products by functioning as dough conditioners and strengtheners, and bread and crumb softeners. They also increase the shelf life and softness, ensure good fat distribution, stabilize icing, and improve slicing, volume, aeration, and moisture retention. In baked products and other cereal-based products, the primary functions of emulsifiers are emulsification of fatty acid components in bread, complexation of starch (amylose), strengthening of protein (gluten/gluten interaction), and aeration improvement by reducing the surface tension of the aqueous phase in the batter. Emulsification is often the secondary function.[92-95] Complexation with GMS is essential for improving the shelf life of bread. Starch present in the wheat granule will swell once water is added and as the mixture is stirred amylose is released. After baking, once cooled and stored, the amylose

TABLE 7.7
Amylose Complexing Index (ACI) and Iodine Affinity of Various Food Emulsifiers

Material	Iodine Affinity	Amylose Complexing Index
DMG (distilled monoglyceride)	0	92
Soya oil	11	28
Mono-di-glycerides	11	34
Lecithin	12.8	16
Sorbitan monostearate	12.6	18
Stearoyl-2-lactylate	1.8	88
SSL	3.2	79
CSL	5.4	65
Amylose without emulsifier	15.3	0

tends to recrystallize and lose some of its water. This phenomenon, known as retrogradation of starch, leads to the staling of bread. Addition of GMS to dough slows down retrogradation as the complex interferes with the recrystallization process. The excellent fitness of MG into the helical structure of amylose makes this emulsifier ideal as an anti-staling agent. Other emulsifiers have lower complexing ability and are less efficient (Table 7.7).

In addition to bakery products, MG and DG also play an important role in many other products.[96] These include imitation dairy products, frozen desserts, pasta foods, cereals, snacks, processed potatoes, chewing gum, peanut butter, jellies, puddings, cheese spreads, syrups, candies, margarine, diet margarine, shortening, salad dressings, mayonnaise, sour cream, ice cream, whipping cream, caramel, butter cream, cake mix, and cream fillings.

The use of emulsifiers enhances the structural properties of ice cream. The emulsifiers, generally containing MG and DG, help to disperse fat globules throughout the ice cream mix and prevent them from clumping together and churning out as butter granules during the freeze-mixing operations. Emulsifiers further help to improve whipping properties to reach the desired overrun, i.e., the increase in volume caused by whipping air into the mix during the freezing process.[84] The use of emulsifiers in ice cream makes the product drier and stiffer when drawn from the freezer, which allows packaging without the product melting. Other related products that gain from these emulsifiers are sandwiches, factory-filled cones and individual servings, tarts, eclairs, and cake rolls.[98,99]

Monoglyceride Derivatives

While monoglycerides are used quite extensively as emulsifiers in food products, many compounds were reacted with MG to form new emulsifiers with different functions. Some of these compounds include ethylene oxide, succinic anhydride, tartaric acid, citric acid, lactic acid, and acetic acid (Figure 7.5).[4,12,83]

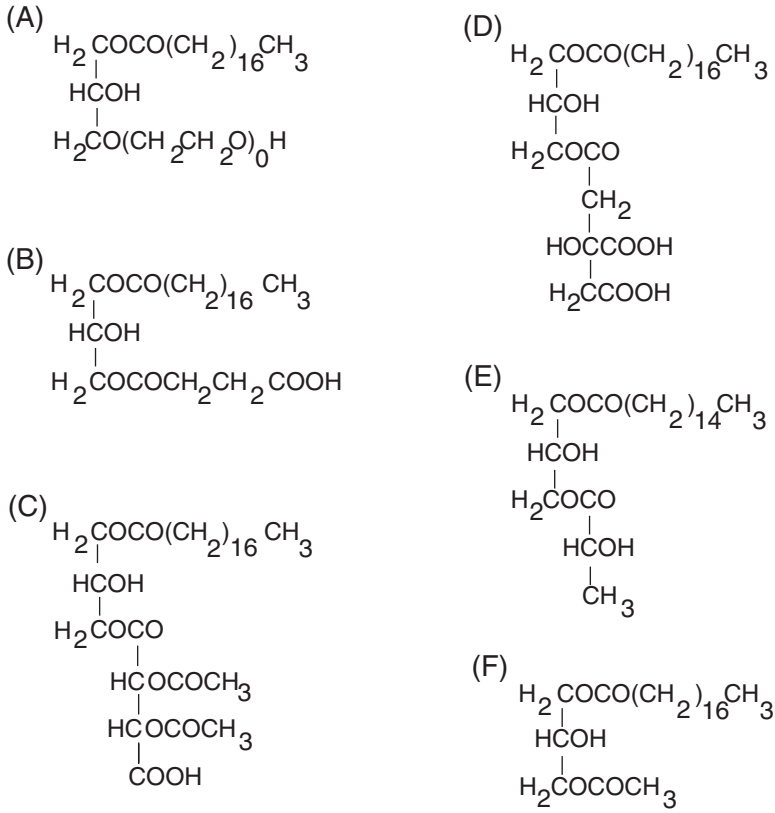

FIGURE 7.5 Monoglyceride derivatives. (A) Ethylene oxide of monostearate, (B) succinic ester of monostearate, (C) diacetyl tartaric acid of monostearate, (D) citric acid ester of monostearate, (E) lactic acid ester of monopalmitate, and (F) acetic acid ester of monostearates.

Ethoxylated Monoglycerides

These are made by treating MG with ethylene oxide. They are very hydrophilic compounds and are widely used as dough conditioners in breads. They are also used as emulsifiers in cakes and cake mixes, whipped vegetable oil toppings and topping mixes, icings and icing mixes, frozen desserts, and edible vegetable fat-water emulsions intended for use as substitutes for milk or cream in beverage coffee.

Succinylated Monoglycerides

These are mixtures of semi- and neutral succinic acid esters of MG and DG prepared by the succinylation of a product obtained by the glyceroysis of edible fats and oils, or by the direct esterification of glycerol with edible fat-forming fatty acids. They are used as emulsifiers in liquid and plastic shortenings at a level not exceeding 3%(w/w) of the shortening, and as dough conditioners in bread baking.

TABLE 7.8
Specifications and Characteristics of Some Commercial DATAE Products

Type	Type I[a]	Type II[b]	Type III[c]	Type IV	Type V	Type VI
Saponification value	480–510	380–425	480–510	325–355	380–410	490–520
Acid value	85–110	62–76	85–110	47–57	60–75	80–100
Iodine value	Max. 2	Max. 3	Max. 2	Max. 2	Max. 2	Approx. 40
Dropping point (approx.)	47°C	52°C	47°C	60°C	55°C	—
Form	Fine powder	Fine powder	Fine powder	Fine powder	Powder	Semi-liquid
Color	Ivory	Ivory	Ivory	Ivory	Ivory	Ivory

[a] Contains 20% anti-caking agent (calcium carbonate).
[b] Contains 10% anti-caking agent (tricalcium orthophosate).
[c] Contains 15% anti-caking agent (5% tricalcium orthophosphate and 10% calcium carbonate).

Diacetyl Tartaric Ester of MG (DATAE)

DATAE is more hydrophilic than MG itself and, therefore, is an excellent emulsifier. Owing to the added carboxylic group, it has the ability to bind gluten in wheat dough and, thus, improve the ability of gluten to hold gas bubbles. In the mixing process, after addition of water, the gluten proteins (gliadin and glutenin) swell to form a viscoelastic gas retaining structure. The stability and machinability of the dough depends on the number and size of gas bubbles formed within the protein structure and the elasticity and strength of the gluten network. Additional gas retention yields an increased bread volume.

The ability of an amphiphilic molecule to interact with protein is an important characteristic of the food emulsifier, since it contributes to the volume of any baked product. The molecular mechanism of such interactions is not clear. Some investigators claim that the hydrophilic head, via its carboxylic group, is interacting with the free amino groups of the proteins and serves as a cross-linker to other proteins. Other investigators have proposed that the emulsifier interacts with the hydrophobic sites of the protein. In this event, the emulsifier forms additional compartments wherein the gas released from the yeast can be entrapped.

Diacetyl tartaric acid ester of monoglycerides has been found to be one of the most effective emulsifiers as a volume improver and is widely used in baked products. The preparation of DATAE is quite difficult requiring the presence of acetic anhydride with further esterification with monoglycerides or diglycerides.[4,12,83,101,102] The specifications of some of the products that are available are shown in Table 7.8.

Monoglyceride Citrates

Monoglyceride citrates are produced by reacting MG with citric acid. They are used as emulsifiers in sausages as well as anti-spattering agents in margarine. Monoglyceride

citrate has also been used as a synergist and solubilizer for antioxidants in oils and fats.[103,104]

Monoglycerides

Several lactylated esters are used as emulsifiers in foods. One form is the lactic acid derivative of monoglycerides (MGL), while the others are sodium or calcium salts of lactic acid esters of fatty acids. MGL is used to improve aeration and foam stability in whipped toppings as well as in cake mix shortenings.[104] Glycerol lactylpalmitate, formed by reacting MG with lactic acid, is a common emulsifier used in cake, shortenings, and cake mixes,[105,106] and as an agglomerating agent for toppings.[97] Patents on methods for preparing mixed glycerol esters of fatty acids and lactic acid have been granted.[107-110]

Monoglyceride Acetate (GMA)

This MG derivative is produced by re-esterification of MG or DG with triacetin or by acetylation with acetic anhydride.[111-116] The final result is a glycerol backbone containing either one or two acetic groups and one long-chain fatty acid. These modified glycerides when fully hydrogenated have the unique property of being (and remaining) highly flexible and nongreasy plastic solids at and below room temperature.[117-120] They are bendable and can be stretched appreciably, i.e., over 800% before breaking. The flexibility of solidified acetostearin products is attributed to the shape and arrangement of their crystals. GMA can exist in several crystal forms or polymorphs but are unique in that the lower melting form is stable for all practical purposes (Table 7.9). Another property of GMA is that pure products, such as 1,2-diaceto-3-stearin, prepared by reacting acetic anhydride with molecular distilled 1-monostearin, will form practically transparent films. Melting points and other properties can be tailored to a considerable extent by changing the composition of

TABLE 7.9
Some Specifications of Typical Commercial Monoglyceride Acetates (GMA)

Properties	Type I	Type II	Type III
OH	ca. 50	ca. 70	ca. 90
Total acid (%)	11–14	15–18	20–23
Free glycerol (%)	<1	<1	<1
Bound glycerol (%)	22–23	21–22	20–21
1-Monoglyceride (%)	<20	<10	<5
Melting point (°C)	40–46	36–40	32–36
Acid value (AV)	<2	<2	<2
Ester value (EV)	260–280	300–320	320–340
Iodine value (IV)	<2	<2	<2
Ash (at 800°C) (%)	<0.05	<0.05	<0.05

the long-chain fatty acid groups and the degree of acetylation. By these changes, GMA has been made with a melting range of –24 to 54°C.

For food uses, GMA may be classified as nongreasy, plastic fats of relatively sharp melting points for use in protective coating materials; low-melting fats with extraordinary resistance to oxidative rancidity; low melting point oils with good resistance to oxidative rancidity, and either liquid or plastic at below freezing point temperatures. Over the past two decades GMA has found a number of uses in foods. Solid GMA products are used as coatings for fruits, nuts, frozen baked goods, meat, and meat products where thin films form oxygen and moisture barriers. Liquid GMA products are used as release agents in candy production and as food lubricants in nuts, raisins, and related products. Spraying the inside of ice cream sugar cones with liquid GMA has been reported to extend shelf life. GMA products belong to the group of emulsifiers that maintains their stability in the flexible alpha-crystal form. These types of compounds improve agglomeration and thus the whippability and foam stabilization of various food emulsions, such as those found in whipped toppings and other dessert products. Other uses include plasticizers for chewing gum, releasing agents, and defoamers.[121-123]

Propylene Glycol Esters

Propylene glycol mono- and diesters are prepared by direct esterification of propylene glycol with fatty acids. Their lipophilic quality makes them W/O emulsifiers. For example, propylene glycol monostearate (PGMS) is a strongly lipophilic emulsifier, having a low HLB.[124] Propylene glycol fatty acid esters are listed among the emulsifiers that may be added to fats at the manufacturer's level and have been approved by the FDA for use in foods.[125] PGMS and propylene glycol monopalmitate are most often used in cakes, cake mixes, whipped toppings, and bread.[126] Other uses are in combination with distilled monoglycerides to obtain excellent batter behavior resulting in increased cake volume and uniform structure and whipped toppings (due to their aerating and foam stabilizing properties).

Stearoyl Lactylates (SL)

Stearoyl lactylates (SL) are made by the reaction of lactic acid, fatty acids, and a suitable sodium or calcium source, at elevated temperatures and controlled conditions under vacuum and nitrogen. The products are known as sodium stearoyl-2-lactylate (SSL) and calcium stearoyl-2-lactylate (CSL), respectively. Figure 7.6 illustrates the process and the internal product composition of the reaction mixture. SSL and CSL have been approved for use as dough conditioners in yeast-raised baked products. These products combine two sets of properties: the ability to form a complex with amylose improving bread shelf life and interacting strongly with gluten to increase loaf volume (Figure 7.7). Other uses include whipped toppings, coffee whiteners, cake icings, and starch puddings.[124,125,127,128]

As emulsifying agents SL are used primarily in the baking industry as dough conditioning agents.[105,128-130] More specifically, stearoyl-2-lactylate is used in non-yeast leavened bakery products and prepared mixes, whereas stearoyl monoglyceridyl

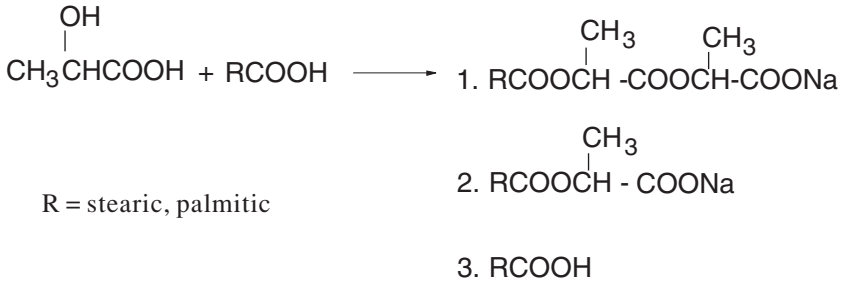

FIGURE 7.6 The reaction pathway and the product composition of SSL.

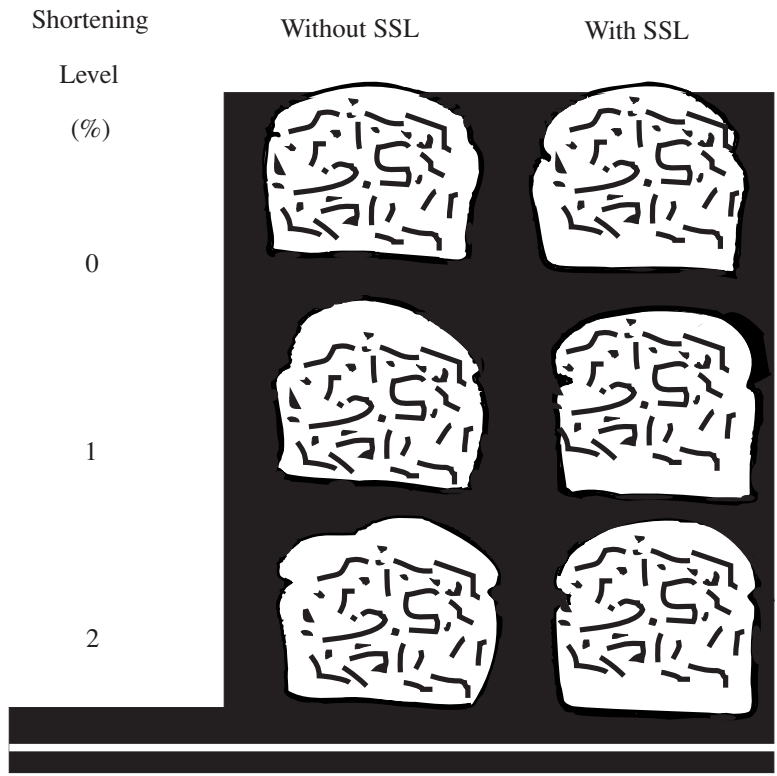

FIGURE 7.7 The effect of SSL on the volume of bread. (Y. Pomeranz; Private communication.)

citrate is used as an emulsion stabilizer in shortening.[126] Sodium stearoyl-2-lactylate, an anionic lipophilic food emulsifier, is very functional as a dough conditioner on a weight base since it has excellent dispersing characteristics in aqueous systems.[124] According to Bechtel and co-workers,[131] CSL changes the structure of wheat gluten, which was later explained by Thompson and Buddemeyer[132] as binding of the additive to the flour protein. Further studies indicated that CSL cannot be removed

TABLE 7.10
Shelf-Life of Standard Bread as Estimated from Compressor Readings (in grams load), in the Presence of Shortening (SH) and SSL at Various Levels (Weight Percentage on Flour Basis) After 1 to 5 Days of Storage at Room Temperature and in the Open

		Shelf Life from Compressometer Readings in Gram Load Storage Period (days)			
Additive	**wt%[a]**	**0**	**1**	**3**	**5**
—	0	40	61	118	125
SH[b]	1	35	74	109	122
SH	2	36	70	100	121
SH	3	32	64	106	115
SSL	0.25	33	62	91	111
SSL	0.5	38	62	81	105
SL	1.0	36	63	84	96

[a] On flour basis.

[b] SH stands for shortening from tallow.

by solvent extraction once the bread has been baked. When the emulsifiers complex with wheat gluten, as in yeast-raised doughs, a strong protein network is formed that allows the carbon dioxide produced during processing to be retained. The reaction results in better texture and increased volume of the baked products.

In addition to complexing with protein, CSL has also been shown to inhibit the transition temperature of dilute starch-water mixes in the amylograph and sharply increases the maximum paste viscosity.[131] These authors also suggested that the effect of starch gelatinization is associated with the observed reduction in the rate of firming in bread containing CSL. In general, stearoyl lactylates form complexes with both protein and starch. The latter property allows for effective antistaling agents in bread (Table 7.10), while their interaction with gluten results in a finer crumb structure, increased volume, and better crust in the finished loaf.[133] Furthermore, SSLs under certain conditions can be used in imitation sour cream to improve heat stability and resistance against melting down.[105] SSL has been found to be beneficial in many other applications.

Sorbitan Esters and Ethoxylated Sorbitan Esters

Sorbitol, a sugar alcohol, belongs to the group of hexahydric alcohols made by hydrogenation of glucose. When esterified, water is split off and the hydroxylated tetrahydrofuran, 1,4-anhydrosorbitan, is formed as the main product. The free hydroxyl groups can react with fatty acids to form sorbitan esters.[134] Another product formed is 1,5-anhydrohydroxypentahydropyran which can dehydrate further to isosorbitude. The synthesis of sorbitan esters was comprehensively reviewed over 30 years ago by Markley,[127] who described interesterification of triglycerides and sorbitol at several temperatures, with different catalysts, and under a variety of

conditions. He also reported on a series of fatty acid esters of sorbitan (mixed monoesters) that included lauric, palmitic, stearic, and oleic acids sold under the trade name SPAN.

By modifying sorbitan's pattern of esterification with ethers of ethylene oxide, products marketed under the trade name TWEENS are made. The industrial use of these compounds depends on the nature of the fatty acid esterified to the alcohols, the degree of esterification, and the formation of polyoxyalkylene derivatives.[127] When such products are ethoxylated (with 20 moles of ethylene oxide), a hydrophilic range of sorbitan esters called polysorbates is produced.[97] Consequently sorbitan esters can form a wide range of HLB values, from 1.8 to 16.7, which can be used as W/O or O/W emulsions for numerous applications.[134] Many polysorbates have been part of feeding studies in animals and appear no more toxic than many other foodstuffs.[125]

Sorbitan monostearate[136] is most often used in combination with polysorbates in cakes, cake mixes, whipped toppings, cake icings, fillings, confectionery coatings, and coffee whiteners.[123] Polyoxyethylene (20 EO) sorbitan monostearate 60 (Polysorbate 60) can be combined with lipophilic emulsifiers such as sorbitan monostearate and glycerol monostearate to provide cakes with greater volume and finer, more uniform grain, adding softness without acquiring fragility. In icings, these combined emulsifiers increase lightness by improved emulsification of the fat and hydrocolloidal phase to prevent oiling-off or sticking to the wrapper. Polysorbate 60 is also used in prepared dry mixes, whipped toppings, bread and yeast-raised products, and coffee whiteners.[124] Polysorbate (20) sorbitan monooleate (polysorbate 80) is used in ice cream and frozen desserts for resistance to heat shock and to improve texture and palatability. It is also used in non-standardized baked goods, prepared mixes, fillings, icings, toppings, and very often as a solubilizing agent for flavors. Polysorbate 65 (polyoxyethylene sorbitan tristearate) is also used in frozen desserts, whipped toppings, cakes, cake mixes, and coffee whiteners.[124]

Sorbitan esters of fatty acids are effective antiblooming agents (fat crystal modifiers) in confectionery products containing cocoa butter and cocoa butter substitutes. In chocolate, an undesirable property called bloom is inhibited by the use of sorbitan esters of fatty acids. Because of the complex composition of fat in cocoa, the product tends to crystallize in stages characterized by a Solid Fat Index (SFI) curve which quantifies the percentage of the solid phase content as a function of the temperature of the cocoa butter. At least six crystalline structures (six different polymorphs) have been isolated and characterized for cocoa butter. The different polymorphs differ in melting points, heat capacity, refractive index, and X-ray diffraction pattern. The polymorphs tend to transform from one to the other as the temperature is raised. The organoleptic properties of the chocolate depend on the nature of the polymorphs present in the product at any temperature and storage conditions. The polymorphic transformations lead to "instability" in the sugar-cocoa solid matrix resulting occasionally in the migration of one of the cocoa fat polymorphs from the interior, along the cocoa fiber to the chocolate's surface. This phenomona is known as bloom and is responsible for the dull-gray white appearance when chocolate has bloomed. In the absence of blooming, the chocolate is bright and shiny with a rich appearance.[135-137] Sorbitan esters modify fat crystals by retarding/inhibiting the reversion of crystalline fat to a more stable form.

Polyglycerol Esters

These are generally prepared by polymerization of glycerol under alkaline conditions at elevated temperatures (approximately 230°C)[140] by direct esterification of the polyol with selected free fatty acids. PGEs can also be made by interesterification of the polyol in the presence of an alkaline catalyst. In general, PGEs contain from two to ten glycerol moieties in length and are used in many food applications. To obtain the desired physical characteristics and functional properties, the polymerization and esterification reactions are carefully controlled and the final product can provide a broad range of HLB values.

PGEs are multifunctional, a property that allows them to be used as fat substitutes as well as emulsifiers. For example, they are used as surfactants in whipped toppings and frozen desserts, as anti-bloom agents in confectioneries, as anti-spattering agents in cooking oils, as flavor dispersants and stabilizers in beverages, as texture enhancers in prepared cake mixes, and as binders of ingredients and lubricants in extruded snacks. When used in liquid salad dressings, PGE can prevent oil and water separation, make products creamy, and aid color and flavor dispersion. They also aid in getting oil-based flavors into solution in oil-based mixes and often allow mixing of incompatible ingredients in hard to emulsify mixes.[141] PGEs (mainly di-, tri-, and tetraglycerols) are used in aerated foods containing fat, such as cakes and sponges. PGEs impart excellent properties to cake batter by increasing the cake volume and uniform structure when used in cake margarine in combinations with distilled MG. Triglycerol monostearate (4G1S), for example, can be used as a whipping agent and as an aerator in non-aqueous lipid systems. In this capacity it reduces costs by eliminating the need for egg whites and vegetable protein as foaming agents, eliminates an extra processing step, and enhances shelf stability since no water is required for functionality. By aerating readily in either batch or continuous automatic whipping equipment, 4G1S sets rapidly after whipping, which allows product extrusion through various shaped dies.

Octaglycerol monooleate (8G1O) and octaglycerol monostearate (8G1S) are also commercially available as emulsifiers. 8G1O is used as a viscosity reducer in high protein systems, as an emulsion stabilizer, and as a beverage clouding agent. It is also used in imitation cheese products to retard oiling-off or separation of fat and casein, and to improve the melt-down characteristics of the finished cheese with a dramatic reduction in the viscosity of the product. In ice cream toppings, 8G1O can be used to provide emulsion stability and improve gloss. 8G1O can also be used to reduce the cholesterol content of casein, which would make the product useful as an ingredient in imitation meats, cheeses, and other dairy products. In food analogs, such as imitation bacon, 8G1O can be used to prevent the vegetable oils from separating during storage and to improve the texture of the product. 8G1S is used as a whipping agent, and an emulsion and freeze-thaw stabilizer. In pet foods, 8G1S can be used to produce a glossy, moist appearance, which makes the product more appealing. Both products, 8G1O and 8G1S, can be used as polysorbate replacers. They are also used to disperse flavors and colors into aqueous systems as they are relatively free of any flavor character note.[142]

A new line of PGEs bland in taste, mild color, and with excellent melt color have become commercially available. These compounds range in appearance from

a soft white solid (triglyceride monopalmitate) to hard white beads (hexaglycerol dipalmitate and hexaglyceryl distearate) to a pale yellow liquid (decaglycerol dioleate). When these compounds were tested as emulsifiers in dessert toppings, results showed markedly superior organoleptic qualities, improved color characteristics, and enhanced emulsifying characteristics. Furthermore, the hexaglycerol distearate outperformed all other emulsifiers (including sorbitan monostearate and polysorbate 60) tested.[142-145]

Sucrose Esters (SE)

Sucrose esters, particularly mono- and diesters, are potentially very valuable emulsifiers and as such offer a number of unique advantages. They are non-toxic, odorless, tastless, non-irritating to the skin, and easily digested. They are biodegradable under both aerobic and anaerobic conditions, and unlike most other non-ionic surfactants they are normally solids, and may be used as powdered or spray-dried products. SEs have a chemical structure that is found in many of the typical emulsifiers, i.e., both polar and nonpolar groups in the same molecule. Since there are eight possible positions that can be esterified with fatty acids, the final product offers a wide range in HLB values, from one extreme (lipophilic HLB value of 1) to another (hydrophilic HLB values of 20).[145]

SEs compounds, formed from the reaction between sucrose and methyl esters of fatty acids, are used extensively in foods.[145,146] They are used in cakes, cookies, and breads; as emulsified oils and fats in coffee whiteners, whipped cream, recombined milk, shortening oil, ice cream, low calorie margarine; in instant foods such as curry, soybean curd, cocoa, and cake mixes; for confectionery use in biscuits, chocolate, chewing gum, rice cakes, and tablet candy; and for preventing starch retrogradation. One of the recent uses of SEs is as a low-calorie, cholesterol-free fat substitute. Sucrose polyesters have been reported to have a fat content of zero calories and an ability to lower cholesterol levels. If so, then these products could be used as a home-cooking oil as well as for making salted snacks, such as potato chips. They could also be used as cooking oils in restaurants and in the fast food industry as deep frying oils. Other food products that could benefit are ice cream, mayonnaise, salad dressings, desserts, and meats. One of the major advantages of these products is that they pass through the body without being absorbed. Furthermore, they are also claimed to eliminate some of the cholesterol already in the body. The manufacture of a sucrose polyester obtained FDA approval and is sold under the brand name Olestra. The metabolic role and possible future applications of sucrose polyesters have been reviewed by Toma and co-workers.[147]

The ultimate application of sucrose polyesters as food emulsifiers will depend on the HLB value, which is determined by the specific fatty acid used. For example, one such product made from stearic acid is used as an emulsifier in shortening or margarine and could have an HLB value of 2 to 3, whereas another product made from a different proportion of stearic acid and used as an emulsifier in ice cream or in other dairy products could have an HLB value of 11.

In bakery foods, SEs are used in many ways. In 1969, Pomeranz et al.[148,149] reported on the addition of wheat glycolipids and SE to wheat flour that allowed

fortification with up to 16% soy flour and other protein-rich additives without loss in the bread's physical properties. These would include an increase in water absorption and maximum hot-paste viscosity and volume, on crumb grain, and bread softness. In reference to loaf-volume in breads, Finney[150] described the ability of SE to (1) carry soy flour (protein), (2) spare shortening, and (3) strengthen dough, i.e., improve gas retention with faster proofing. Sucrose monopalmitate was also shown to be active in overcoming the volume-depressing effect of soy flour in bread.[151] These authors concluded that sucrose monoesters were very good dough-strengtheners. They showed that sucrose monolaurate and monopalmitate were more effective dough strengtheners than either monocaprylate and monoarachidate. The corresponding monomyristate, monostearate, and monooleate esters were almost as effective as the monolaurate ester, but the monocaprylate ester was somewhat less effective. These researchers also showed that the crumb-softening ability of sucrose monostearate was effective, but not as effective as sodium stearoyl-2-lactylates, one of the most widely used commercial bread softeners. The crumb-softening effect of sucrose monoesters is diminished as the fatty acid chain decreases from C-18 to C-8. Using surfactants, Tsen et al.[152] successfully made low-calorie cookies containing 25% shortening. In 1973–74, Tsen et al.[152,153] reported on the fortification of wheat flour with soy flour to which several fatty acid derivatives, including sucrose esters, fatty esters of polalkoxylated polyolglycosides, sodium or calcium stearoyl-2-lactylate, and ethoxylated monoglycerides and glycolipids were added. Generally, adding soy flour to wheat flour can produce adverse effects such as altered absorption, mixing, and machining properties, changed fermentation rates, poor crumb grain and color, reduced loaf volume, and a beany flavor. However, addition of the surfactants tested was found to eliminate many of these negative effects caused by fortification of soy flour. Chung and co-workers[153] reported that SE with an HLB value of 14 was very effective in replacing wheat-flour lipids and 3% shortening while those with an HLB value of 1 proved ineffective. Ebeler and Walker[155] reported that SE improved volume and softness of white layered cakes. SEs were subsequently incorporated as emulsifiers in sponge cakes.[156] By hydrating SE prior to addition, the resulting cake volume increased an average of 20 to 160 cc greater than when SEs were added in the powdered form. The SEs with HLB values of 11 and 15 (the more polar compounds) were the most effective.

NATURALLY OCCURRING EMULSIFIERS

PROTEINS

Proteins are natural polymeric surfactants. The importance of proteins to food colloids and emulsions is reflected by the thousands of studies reporting their action at various food interfaces.[157,158] This section will provide some general ideas and characteristics of proteins as emulsifiers. Halling[158] claimed that proteins form high viscoelastic films on surfaces which oppose the surface deformation (either in shear or dilution) needed for the later stages of drainage and for the rupture of lamellae. This theory faced considerable criticism as rheological interface properties depend on other factors such as bulk protein concentration, pH, film age, etc. so that this

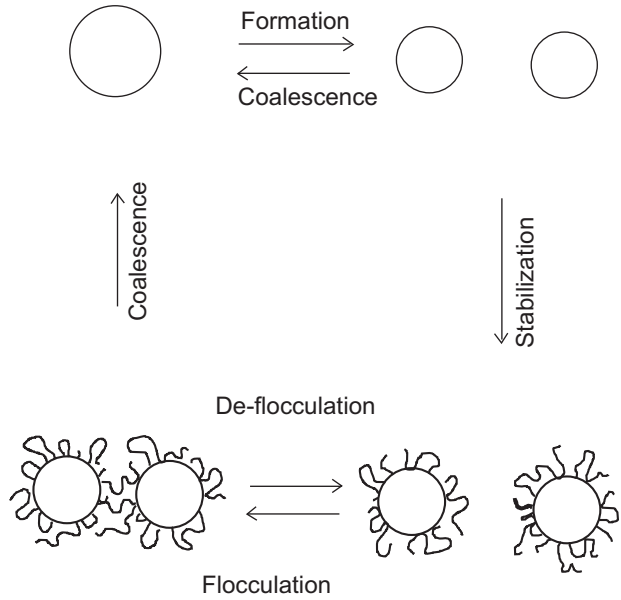

FIGURE 7.8 Kinetic description of emulsification in the presence of protein. The figure illustrates the steric stabiliation (deflocculation) and the interaction between droplets due to lack of steric stabilization. (From Darling, D. F. and Birkett, R. J., Food Colloids in Practice, in *Food Emulsions and Foams,* Dickenson, E., Ed., Royal Society of Chemistry, London, 1987, 1-29. With permission.)

criteria cannot solely explain the role of proteins at the interface. Proteins also have a significant effect on interdroplet forces, and while adsorbing at the oil-water interface (likely to be located mainly on the aqueous phase side), they will affect the Van der Waals forces via the strategic interactions between the adsorbed layers of proteins. Double layer repulsive forces can also be expected to exist, providing the radius of gyration of the adsorbed polymer is much less than the Debye length. As the molecular weight of the protein increases, the contribution of the steric stabilization effect will dominate the repulsive forces over the electrostatic forces.

Best steric stabilization (Figure 7.8) will occur provided the polymer fully covers the interface (sparse covering attracts forces) forming a thick non-raptured film. A good solvent will thoroughly dissolve the dangling chains of the polymer by ensuring the polymer chains stay apart. Other important aspects include liquid drainage between approaching emulsion droplets, droplets formation, transport of molecules in the bulk, rate of adsorption of the macromolecules, competition between surfactants, and possible chemical reactions.

In many food emulsions, the heterogenous milk protein "casein" is commonly the major macromolecular component of the adsorbed layer surrounding dispersed fat particles or oil droplets. The exceptional emulsifying properties of casein are attributed to a molecular structure highly disordered and substantially hydrophobic.[160] Milk casein exists as "casein micelles", polydispersed proteinaceous colloidal particles containing the four major monomeric caseins, α_{s1}, β, α_{s2}, κ (in approximate

proportions 4:4:1:1) linked by calcium ions and colloidal calcium phosphate. The caseins α_{s1}, β, κ have fairly similar molecular masses and isoionic points (pH = 5.2), but rather different molecular charges (–20e, –12e, and –4e) at neutral pH.[160] The commercial emulsifier sodium (or potassium) caseinate lacks calcium phosphate and is consequently less aggregated than the casein in milk, but has roughly the same protein composition. A comparison of the properties of emulsions stabilized by individual caseins, α_{s1}, β, and κ with those stabilized by sodium caseinate will provide insight into the competitive and/or cooperative aspects of protein adsorption and colloid stabilization in casein-containing systems.

Adsorption experiments at fluid interfaces by Michel et al.[160] and Dickinson et al.[161] showed that β-casein was more surface-active than α_{s1}-casein but direct evidence for preferential adsorption of β-casein in emulsions is rather limited.[163,164] Replacing a real complex emulsion system with a model system composed of monodispersed polystyrene latex particles enables adsorbed proteins to be studied in a systematic manner. Different monomeric caseins adsorbed separately on negatively charged latex particles.[164,165] Consistent with it having a higher net charge in solution than β- or κ-casein, α_{s1}-casein adsorbed on latex gave coated particles a higher electrophoretic mobility compared to the other caseins. However, in the presence of calcium ions, at concentrations greater than 1 mM, latex particles coated with κ-casein carry a higher effective negative charge than particles coated with either α_{s1}- or β-casein. This is interpreted as being due to the strong binding of calcium ions to α_{s1}- and β-caseins thereby reducing the net negative charge on the adsorbed protein layer. The adsorption characteristics of sodium caseinate on lattices are intermediate between those for α_{s1}- and β-caseins, suggesting that for solid surfaces, β-casein does not displace α_{s1}-casein over the short experimental time-scale.[164]

Many semi-empirical studies on the emulsifying and foaming behavior of other food grade proteins have been reported. Such semi-empirical information is essential to food technologists but gives little insight into the key physico-chemical factors involved. Many proteins have been reported to stabilize emulsions including lysozyme, bovine serum albumin, myosin, soy protein, β-lactoglobulin, gelatin, etc. Each of these proteins has some advantages in the key factors affecting stabilization such as adsorption (kinetics of diffusion, induction periods, ability to lower surface tension, number of adsorbing sites, thickness of the film, extent of coverage, etc.), desorption (reversibility of adsorption, competitive adsorption, conditions for interfacial replacement), and film properties (thickness of adsorbed protein film, denaturation on the surface, coagulation formation of mixed film with other proteins or with low molecular weight surfactants, surface rheology).

A basic knowledge of the physico-chemical properties of food proteins and an understanding of the many factors affecting the quality of foods is required to fabricate acceptable food products. While soy proteins have largely been considered as economical substitutes for more expensive protein ingredients, they should be viewed as vital functional components for use by food technologists to fabricate new foods.

The trend towards quick-service convenience foods requiring fabrication, dehydration, rehydration, etc., brought the versatility of textured proteins to the forefront in the mid and late 1960s. The economic performance of soy protein in chopped

emulsified meats, egg white replacement in cakes, and substitution of nonfat dry milk in baked goods, all pointed to a lower cost per unit of performance, while at the same time, maintaining the required level of consumer acceptance.

In the domestic market, soy protein products gained acceptance as useful and economical ingredients for the manufacture of conventional foods, and in the design of new foods. More food package labels show soy protein products in their ingredient listings. This is a trend that is expected to continue.

Chemically and Enzymatically Modified Proteins

Many physical and chemical properties of proteins can be modified to enhance their surface acivity.[165] Some of the most important characteristics, such as film strength, viscoelasticity, and colloid stability, can be changed by extrinsic factors (pH, ionic strength, temperature, etc.) while others such as hydrophobicity, flexibility of the polymer, net charge, etc. can be modified (chemically or enzymatically) by altering the intrinsic properties of the protein. Chemical modification of proteins affects both protein-protein interactions as well as surface activity.

It is beyond the scope of this chapter to elaborate on the different studies conducted on the various amphiphilic modified proteins. Some of the most common extrinsic modifications are[166-170] (1) change of pH which will affect the film-forming properties of BSA (rather than lowering the degree of electrostatic repulsions or steric stabilization) or will affect the hydrophobicity of BLG (which will result in improved emulsifying activity (Table 7.11); (2) the use of *cis*-parinaric acid[171] to

TABLE 7.11
Effect of pH on Some Film Properties (Surface Pressure, Surface Yield Stress, and Film Elasticity) and Foaming Properties (Drainage Half-Life) of Bovine Serum Albumin (BSA)

	Film			
pH	Surface Pressure[a] (mN m^{-1})	Surface Yield Stress[b] (mN m^{-1})	Film Elasticity (mN m^{-1})	Foam Drainage Half-Life (min)
4.0	2.8	3.0	2.2	5.0
5.0	15.0	3.8	5.0	8.0
5.5	19.0	4.0	5.2	9.6
6.0	14.0	4.3	5.4	8.5
7.0	10.0	3.0	2.3	6.3
8.0	2.0	2.2	1.8	6.0

[a] Surface pressure of protein solutions (5×10^{-3} wt% in 10 mM citrate) was measured after 5 min at a temperature of 23°C.

[b] Surface yield stress was estimated by measuring surface viscosity using a Brookfield viscometer: protein solutions of 0.1 wt% in 10 mM citrate (pH 3 to 5.5) and phosphate (pH 5.7 to 8) buffers at 3°C after 5 min.

From Kim, S. H. and Kinsella, J. E., *J. Food Sci.,* 50, 1526, 1985. With permission.

TABLE 7.12
Changes in Surface Hydrophobicity due to Thermal Denaturation[a]

Temperature (°C)	Hydrophobicity[b]				
	Ov[c]	7S[c]	κ-C[c]	BLG[c]	BSA[c]
20	10	260	430	2700	3200
50	10	260	480	2700	3100
60	10	270	600	2600	3000
70	15	400	750	2400	2500
80	1950	500	1050	1500	2000

[a] 0 to 2 wt% protein solutions in 0.1 M phosphate buffer (pH 7.4) heated at a rate of 1°C min^{-1} from 20 to 80°C; and then immediately cooled to 20°C after reaching the given temperature (data from Kato, A., Osako, Y., Matsudomi, N., and Kobayashi, K., *Agric. Biol. Chem.,* 47, 33, 1983).

[b] Determined by fluorescent probe method using *cis*-parinaric acid.

[c] Abbreviations: Ov = ovalbumin; 7S = 7S soy globulin; κ-C = κ-casein; BSA = bovine serum albumin; BLG = β-lactoglobulin.

TABLE 7.13
Consequences of Alterations of Net Charge in the Chemical Modification of Proteins, on the Surface Activity of the Protein

A. Isoelectric point changes.
B. Changes in protein associations, e.g., hydrophobic, electrostatic.
C. Randomness in protein structure due to electrostatic repulsion.
D. Stabilization against heat-induced denaturation (?).
E. Enhanced surface hydrophobicity or hydrophilicity.
F. Increased amphiphilic behavior of proteins, i.e., more surface-active (detergent-like).

affect the flexibility of the protein and as a result to change its hydrophobicity (BSA, lysozyme, ovalbumin, κ-casein, and BLG);[172] and (3) thermal denaturation, which affects the protein folding and protein association ability and alters the hydrophobicity of the protein (Table 7.12).

Among the intrinsic modification it is worth noting that some chemical derivatizations serve as a way of enhancing many of the functional surface characteristics of the protein (Table 7.13).

Some of the most common chemical modifications[7,173,174] are listed in Table 7.14 and a good example of the effect of the activity of BSA is shown in Table 7.15. Of all the chemical processes, acylation of lysine residues has been studied by many investigators. The effect of both of these procedures is to increase the solubility of proteins, thereby improving their functional properties in general, and their emulsifying and foaming properties in particular (Figure 7.9).

TABLE 7.14
Amino Acid Side-Groups in Proteins for Chemical Modifications

Group	Modification
Amino (lys)	Acylation; akylation
Carbonyl (asp, glu)	Esterification; amide formation
Disulfide (cystine)	Reduction
Sulfydryl (cys)	Alkylation; oxidation
Thioether (met)	
Imidazole (his)	
Indole (trp)	
Phenolic (phe)	Acylation
Guanidino (arg)	Condensation by dicarbonyls

TABLE 7.15
Parameters Relating to BLG Adsorption at the Air-Water Interface: Average Area Cleared Per Protein Molecule, dA, and Apparent Number of Amino Acid Residues Penetrating Interface (N_τ)[a]

pH	Time/min	dA/nm^2 [b]	N_τ
4.06	0.8–8	2.9	19
4.06	60–360	20.6	137
5.05	0.4–10	5.1	34
5.05	6–360	13.1	87
5.85	6–360	11.9	79
6.36	2–60	7.7	51

[a] Adapted from Waniska and Kinsella.[174]

[b] The quantity dA is calculated from the equation

$$\ln(\mathrm{d}\prod/\mathrm{d}t) = \ln(Kc) - \prod \mathrm{d}A/kT,$$

where K is the first-order rate constant of adsorption (or rearrangement), c is the effective concentration of active groups, k is Boltzmann's constant, and T is the temperature.

Arai and Watanabe[175] have written an excellent review on the properties of enzymatically modified proteins and compared chemical and enzymatic processes on various proteins. Enzymatic processes are generally carried out under much milder conditions providing safer experimental conditions. Proteolytic enzymes have been shown to improve the solubility of proteins such as soy, leaf protein concentrates, fish protein concentrates, meat proteins, egg proteins, milk proteins, and blood

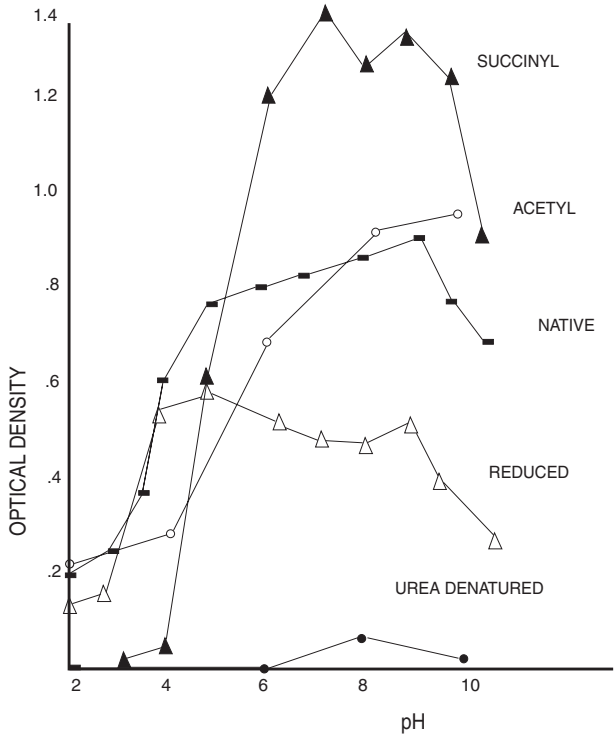

FIGURE 7.9 Effect of pH on the emulsifying activity of various types of modified BSA. Optical density at 550 nm is plotted against pH for diluted solution (pH 7.0, 0.1M NaCl) and an oil phase volume fraction of 0.6. The urea-denatured sample was obtained by treating BSA with urea for 6 h at 37°C. The reduced sample was obtained by treating BSA with 0.01 M dithiothreitol for 4 h at 40°C. Acylation was accomplished with 10:1 ratio by weight of anhydride to protein at pH 8 to 9 for 1 h at 25°C. (From Waniska, R. D., Shetty, J. K., and Kinsella, J. E., *J. Agric. Food Chem.,* 29, 826, 1981. With permission.)

proteins. Partial hydrolysis of these proteins under well-controlled conditions produce emulsifying and whipping agents for food processing, and many of these are produced on an industrial scale. Some of the industrial produced protein hydrolysates exhibit excellent emulsifying properties.[176] This is evaluated by gradually adding soybean oil to an aqueous solution of protein hydrolysate under controlled conditions until the resulting emulsion reverts from an oil-in-water to a water-in-oil system. Other protein hydrolysates have functional properties such as whipping expansion and foam stability.[177]

More sophisticated processes include attachment of amino acid esters to improve hydrophobicity.[178] An excellent example is the dramatic change in enzymatically modified gelatin (EMG). Such modified proteins (EMG-1 and EMG-6) were shown to significantly improve the quality of food products such as mayonnaise, baked bread, and ice cream by changing the emulsification capacity (efficiency) of the proteins.

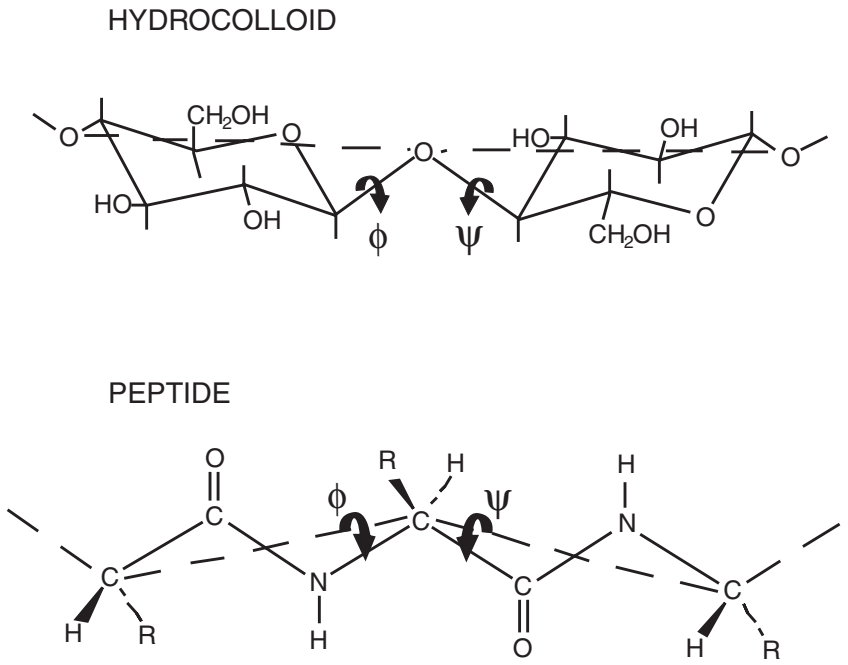

FIGURE 7.10 Structure of a typical hydrocolloid emphasizing its rigidity in comparison to the structure of the flexible proteins with the ability to rotate.

HYDROCOLLOIDS

Most hydrocolloids are polysaccharides with high water solubility and significant structural rigidity; therefore, most investigators consider hydrocolloids as gelling agents or viscosity builders (Figure 7.10). The ability of polysaccharide hydrocolloids to stabilize colloidal dispersions and emulsions is usually explained in terms of a modification of the structure and rheological properties of the aqueous continuous phase.[179] Due to their predominantly hydrophillic character, most polysaccharides have low surface activity at air-water or oil-water interfaces, and therefore are not expected to form adsorbed layers in food colloids, which also contain proteins and low-molecular weight surfactants. However, at least three groups of hydrocolloids are well known to stabilize oil-in-water emulsions:

1. Gum arabic
2. Extracellular microbial "emulsan"
3. Chemically modified (containing auxillary groups such as methyl, ethyl, acetyl, etc.) hydrocolloids such as pectin and cellulose

Recently,[180,181] work was done on other untreated hydrocolloids, such as galactomannans (guar, LBG, fenugreek), and noticeable surface-activity was recorded for some of these gums even after removal of most of the contaminating proteinaceous material. Surface activity at the air-water interface was reported with solutions of

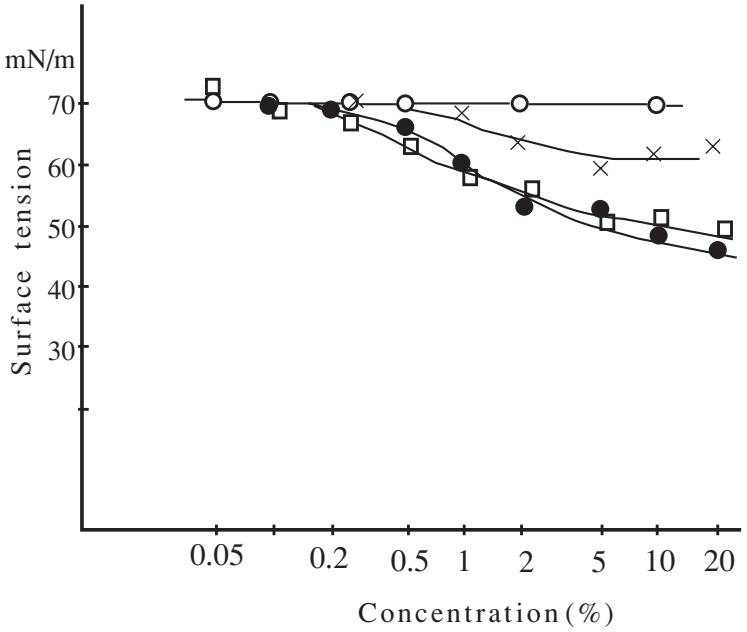

FIGURE 7.11 Plot of the surface tension vs. the concentration of the hydrocolloid. (○) Dextrin; (X) κ-carrageenan; (□) gum arabic; (●) guar gum (diluted 1:20). (From Reichman, D., Ph.D. thesis, Hebrew University of Jerusalem, Israel, 1990. With permission.)

other gums as well (Figure 7.11). For example, a 1 wt% solution of xanthan gum[182] and a 0.1 wt% solutions of gum tragacanth or sugar beet acetylated pectin.[183]

Gum Arabic

In terms of the properties of its adsorbed films, the most widely studied hydrocolloid (after gelatin) is gum arabic. It is a highly complex branched polysaccharide carboxylic acid (ca. 2.5×10^5 Da) occurring naturally in the form of its potassium, magnesium, and calcium salts. The surface activity of arabic acid and its salts (also called gum acacia), and their ability to form thick viscoelastic films at oil-water interfaces, was demonstrated almost 30 years ago.[184] Subsequently, Nakamura et al.[185,186] studied the dependence on molecular weight of the surface rheology of gum arabic films at coconut oil-water interface and observed a correlation between surface viscosity and emulsion stability. The values of the surface modulus and surface viscosity for 24-h-old films adsorbed on the oil-water interface (pH 7, 30°C) from solutions at various samples of commercial gum arabic.[186] The data clearly showed that the rheological parameters are increasing functions of the weight-average molecular weight, whereas interfacial tension under the same experimental conditions was essentially independent of molecular weight.

The pioneering work by Anderson[187] over the past 20 years showed that gum arabic, in common with other gums (sugar, tragacanth, xanthan, etc.), contained a low level of nitrogen which was attributed to bound protein (or polypeptide). If a

TABLE 7.16
Surface Pressure II and Surface Viscosity η of Adsorbed Films of Gum Arabic After 24 h at the *n*-Hexadecane-Water Interface[a]

	Π/mN m^{-1}				
	10^{-3} wt%			10^{-3} wt%	
	pH 7.0	pH 2.3	pH 7.0	pH 7.0	pH 2.3
(I) *A. eriopoda* (5.27% N)	19	16	—	220	200
(II) Commercial sample (0.36% N)	4	4	11	10	1
(III) *A. ampliceps* (0.10% N)	<1	<1	9	<1	<1

[a] 0.005 M, 25°C.

From Anderson, D. M. W., in *Gums and Stabilizers for the Food Industry,* Phillips, G. O., Wedlock, D. J., and Williams, P. A., Eds., Elsevier Applied Science, London, 3, 79, 1986. With permission.

good proportion of this protein is bound to the periphery of the gum molecules, then it may make the major contribution to surface activity. It might be speculated that the variability in surface activity of commercial gum arabic could be due to the differing amounts of accessible protein in the various samples.

A strong correlation between surface properties of adsorbed films and the nitrogen content of gum arabic samples was observed based on analytical measurements.[187] These results, shown in Table 7.16, indicate that gum arabic (sample I) with a high nitrogen content produced a film with surface pressure and surface viscosity levels higher than gelatin under the same conditions.[188] It was also shown that as the temperature of the gum solutions was raised, the proteins present denatured resulting in a significant reduction of emulsification. It was concluded that the nitrogen content of gum arabic samples was likely an important indicator of the strength and thickness of gum arabic films adsorbed at the oil-water interface. These results were consistent with recent studies on the adsorption of gum arabic on polystyrene latex particles,[189] which showed that only the high molecular weight proteinaceous component of the gum was capable of stabilizing the particles against flocculation. Mechanistically, it is easy to visualize that the viscoelasticity of a film of proteoglycan molecules (e.g., gum arabic) is determined primarily by the polysaccharide component, with the polypeptide moiety providing an anchor to the interface (Figure 7.12). Further characterization of the internal product distribution of gum arabic[190] showed the gum was composed of at least three different glycoprotein fractions each differing in molecular weight and surface activity. Only one fraction was amphiphilic and mostly responsible for the adsorption power of the gum.

"Emulsan" — A Microbial Hydrocolloid

Emulsan is a naturally occurring surface active hydrocolloid produced by the oil-degrading bacterium *Acinetobacter calciaceticus*.[191] It is a microbial bipolymer with good emulsifying properties and a strong affinity for the oil-water interface.[192,193] In part, its emulsifying properties arise from the presence of fatty acids (C_{12} to C_{18})

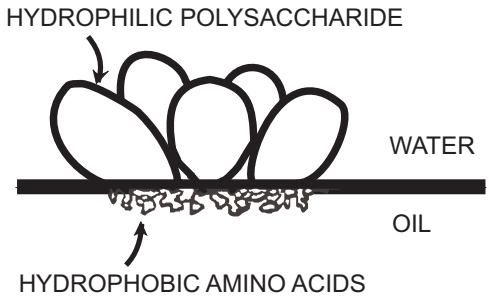

FIGURE 7.12 Schematic illustration of the structure of the arabinogalacto-protein complex at the oil-water interface. (From Zosim, Z., Gutnick, D. L., and Rosenberg, E., *Colloid Polymer Sci.,* 265, 442, 1987. With permission.)

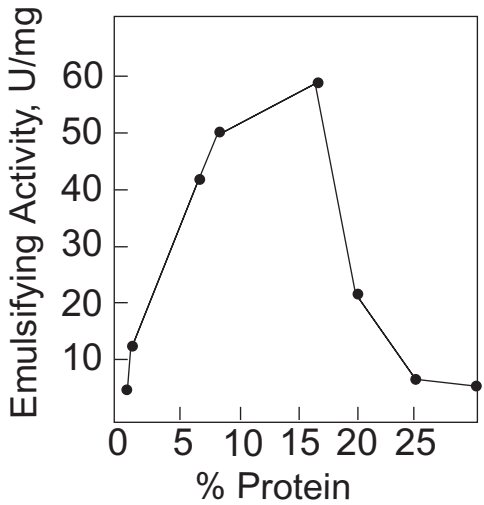

FIGURE 7.13 Emulsifying activity (U/mg) as a function of percentage of protein in gum arabic. (From Zosim, Z., Gutnick, D. L., and Rosenberg, E., *Colloid Polymer Sci.,* 265, 442, 1987. With permission.)

linked to the amino-sugar backbone of the anionic polysaccharide. Its surface activity is no more than moderate,[194] but it exhibits good stabilizing ability for emulsion droplets due to the formation of films that are thick (≥2 nm) and (presumably) viscoelastic. More importantly, however, emulsan is known to exist as a complex of lipohetero-polysaccharide (apoemulsan) and protein. Apoemulsan, or emulsan treated with proteolytic enzymes, has little surface activity and emulsifying capacity. The latter is optimized in emulsan samples containing 5 to 15% protein, which can be produced by mixing protein-rich and deproteinized preparations together.[193] The synergistic effect can only be explained in terms of the protein moiety lowering interfacial tension and the contribution of polysaccharide component to the stability of film formation (=10^6 Da) (Figure 7.13).

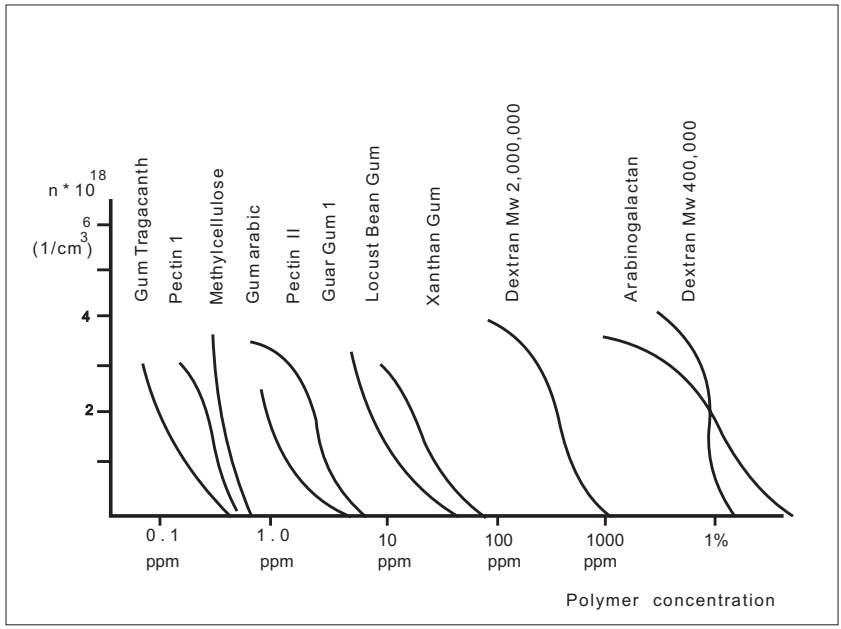

FIGURE 7.14 The flocculation rates as a function of amount of added gum. The theoretical rate, if rapid conditions are assumed, in a 0.05 M $MgCl_2$ solution at 25°C is about 12×10^{-18} ($1/cm^3$).

Non-Proteineous Gums

Many attempts have been made to evaluate the basic mechanisms behind the dispersion stabilizing properties of so-called "non-proteineous gums". The classical steric stabilization (steric hindrance created by a layer of adsorbed polymer at the interface) did not appear to exist for some gums (rigidity, water solubility, weak adsorption).

Burgenstahl et al.[195] examined a number of technical gums and showed that the gums that affect flocculation rates of monodispersed polystyrene latex are absorbed on the latex and infect steric stabilization in the mechanism that enhances the stability of dispersed particles. Their conclusions are supported by surface tension measurements (Figure 7.14). Using surface tension measurements, the gums were absorbed on the latex modifying the steric stabilization in the mechanism that enhances stability of the dispersed particles. According to Bergenstahl[196] gums adsorb very weakly on solid particles like polystyrene but will not adsorb directly on oil/water interfaces. If monomeric emulsifiers or proteins are used in combination with gums, the gum adsorbs onto the first layer of the emulsifier behaving like a protective colloid. The traditional term "protective colloid" is only used for surface active gums like gum arabic.

Studies by Garti et al.[197-200] demonstrated that all three galactomannans (guar, LBG, and fenugreek) have surface activity and reduce both surface and interfacial tensions even after almost complete removal of proteinaceous matter. Guar and

fenugreek protein content was reduced to levels below 0.5 wt% but surface activity was retained. Furthermore, they demonstrated that unlike gum arabic the protein-rich fractions had very limited surface activity.

Oils emulsified with the pure galactomannans (at concentrations below 0.7 wt%) produced stable, unflocculated emulsions. The oil droplets exhibited strong birefringence (under polarized light) indicating formation of a "liquid crystalline" type of organization around the droplets, possibly with "limellar structure". The gums were found to bind "weakly" to the oil droplets but with good coverage and significant thickness. This was the first study to show that water-soluble, rigid hydrophilic polysaccharides have significant emulsification capacity. The authors noted that while gum arabic efficiency is very low (20 wt% is required for 5 wt% oil-in-water emulsions), the optimum levels for guar and fenugreek did not exceed 0.5 to 0.7 wt% under similar oil concentrations.

Xanthan gum is also widely used in formulations such as salad cream, low calorie dressings, mayonnaise sandwich spreads, etc. Gordon[201] reported that xanthan gum is used in some salad dressings not as a stabilizer but as an improver of the yield value. The yield value is referred to as the shear stress applied to the solution in order for flow to occur. It is this property that allows the dressing to cling to the salad and to appear to have body and not a thin appearance. The greater the yield value, the greater the suspending powers. These unique properties have been attributed to some interfacial properties that the gum exhibits in the presence of oils. These properties can be related to the ability of a gum to adsorb onto oil droplets. Additional work is required to further clarify the emulsification power of xanthan gum.

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8 Effects of Sulfur Dioxide on Food Quality

B.L. Wedzicha

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INTRODUCTION

Sulfur dioxide and sulfites have been used as direct additives in food preservation since ancient times. They are still regarded as indispensable in many antimicrobial applications and unique in their ability to control most types of chemical (enzymic and nonenzymic) food spoilage. This success depends on the relatively low oral toxicity of sulfite; use of this preservative is approved in most countries of the world. Sulfites are unusually reactive among food additives; the concentration in a given food at the time of sale is often half, or less, of the amount added at the time of production. Such reactivity is both specific to the intended action of the additive (e.g., inhibition of browning) and non-specific as a result of its broad spectrum of reactivity. This raises questions about the toxicology of sulfited foods. However, the specific reactions of sulfites with key intermediates in chemical spoilage are now being used to provide a fundamental understanding of those spoilage mechanisms. Moreover, the chemical reactivity of sulfite can be used to probe for the nature of the aqueous phase in food as a medium for chemical reactions, particularly under non-ideal conditions, e.g., high ionic strength, low water activity. This chapter will

explain the success and versatility of sulfites as food additives, and discuss the use of these species as probes in a new approach to the study of Maillard browning.

A general review, to 1980, of the chemistry of sulfite relevant to its enzymology, microbiology, and food applications has been published by Wedzicha.[1] More recently, Taylor et al.,[2] Rose and Pilkington,[3] Wedzicha, Bellion, and Goddard,[4] and Wedzicha[5,6] have discussed the subject with various emphasis. The review by Taylor et al. is of particular interest as this is one of the most detailed accounts of safety aspects of the use of sulfites in foods. On the other hand, Rose and Pilkington mainly consider the mechanism of the antimicrobial action of SO_2 and is a key contribution to this field of study.

CHEMICAL NATURE OF THE SPECIES

The terms *sulfur dioxide* or *sulfite(s)* refer to oxospecies of sulfur in oxidation state (IV); they are all derived by the dissociation of the so-called sulfurous acid H_2SO_3. Despite the wide use of the term *sulfurous acid* in chemical literature, it is acknowledged[7,8] that this species does not exist as such, or is present at very low concentrations in equilibrium with aqueous (dissolved) SO_2. Spectroscopic data show the interaction between SO_2 and H_2O to be immeasurably weak. Thus, it has become conventional to represent the dibasic acid as $SO_2.H_2O$, while recognizing that it has the properties of H_2SO_3. The following equilibria need to be taken into account in the discussion of the reactivity of sulfites in food:

$$SO_2(g) + H_2O(1) \rightleftharpoons SO_2{\cdot}H_2O(1) \qquad K_H = 1.2 \times 10^{-3} \text{ mol kg}^{-1} \text{ bar}^{-1} \qquad (8.1)$$

$$SO_2{\cdot}H_2O \rightleftharpoons HSO_3^- + H^+ \qquad pK_1 = 1.89 \qquad (8.2)$$

$$SO_2{\cdot}H_2O + X^- \rightleftharpoons SO_2X^-(H_2O) \qquad (8.3)$$

$$HSO_3^- \rightleftharpoons SO_3^{2-} + H^+ \qquad pK_2 = 7.18 \qquad (8.4)$$

$$HSO_3^- + M^+ \rightleftharpoons MHSO_3 \qquad (8.5)$$

$$SO_3^{2-} + M^+ \rightleftharpoons HSO_3^- \qquad (8.6)$$

$$2HSO_3^- \rightleftharpoons S_2O_5^{2-} + H_2O \qquad K = 0.05 \text{ mol}^{-1}\,1 \qquad (8.7)$$

$$S_2O_5^{2-} + M^{n+} \rightleftharpoons MS_2O_5^{(2-n)-} \qquad (8.8)$$

where the values of all equilibrium constants given for the specific reactions are at 25°C and infinite dilution, K_H is Henry's constant, and M^{n+} represents a metal ion.

The dissolution of SO_2 in water obeys Henry's law, but the apparent value, $(K_H)_{app}$, of Henry's constant (i.e., the total concentration of S(IV) species in solution divided by the pressure of SO_2 in equilibrium with this solution) depends on temperature,[9] and it is easy to show that the value depends on pH as follows:

$$(K_H)_{app} = K_H\left\{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}\right\} \quad (8.9)$$

where K_1 and K_2 are the first and second dissociation constants of sulfurous acid. The value of Henry's constant is also sensitive to the presence of solutes which can form complexes with SO_2. The SO_2 molecule is able to accept electrons from nucleophiles; complexes, such as SO_2I^-, SO_2Br^-,[10] and SO_2Cl^-,[11] have been identified and their stabilities determined. In principle, amines (e.g., amino acids, lysine residues on proteins) and other nitrogenous bases are expected to form such complexes. However, at pH values sufficiently low for significant concentrations of SO_2 to be present, these bases are protonated and do not behave as nucleophiles; hence, the significance of such interactions in the food matrix is likely to be small. Nevertheless, the stability of SO_2Cl^- (dissociation constant = 7.1 mol l^{-1}) is sufficient for there to be a reduction in the vapor pressure of SO_2 above solutions of the gas,[11] upon the addition of NaCl, as illustrated in Figure 8.1.

In the normal pH range of food, pH 3 to 6, the principal species is HSO_3^-, in equilibrium with small but pH-sensitive amounts of $SO_2{\cdot}H_2O$ and SO_3^{2-}. These minor species are responsible for the preservative action and chemical reactivity of the additive. However, it is important to appreciate that, in some instances, the pK values of $SO_2{\cdot}H_2O$ are sensitive to the composition of the medium, other than its pH. The addition of salts tends to reduce both pK_1 and pK_2 as illustrated in Figures 8.2 and 8.3, in accordance with theoretical predictions based on the variation of solute

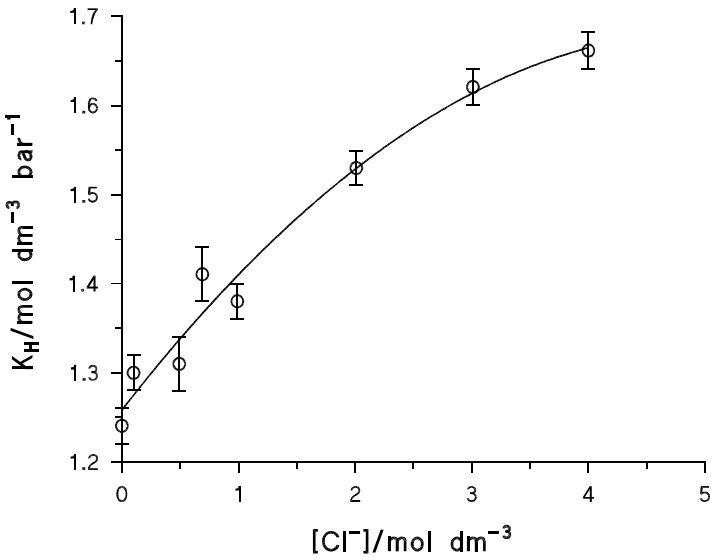

FIGURE 8.1 The effect of [Cl^-] on the value of Henry's constant K_H for SO_2 in the headspace above a solution of the gas in water at 25°C. The error bars represent standard deviations obtained from 15 replicate experiments. Reproduced from Wedzicha, B. L. and Webb, P. P., *Food Chem.*, 55, 338, 1996. © 1996 Elsevier Science Ltd. With permission.

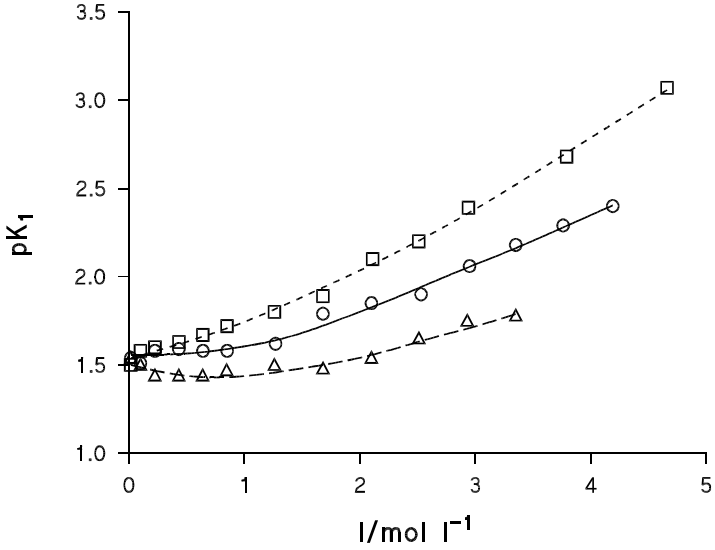

FIGURE 8.2 The effect of ionic strength, I, on pK_1 of $SO_2 \cdot H_2O$ at 30°C for the addition of sodium halides at [S(IV)] = 50 mmol l^{-1}. Δ, NaCl; ○, NaBr; □, NaI. Reproduced from Goddard S. J. and Wedzicha, B. L., *Food Chem*., 52, 218, 1995. © 1995 Elsevier Science Ltd. With permission.

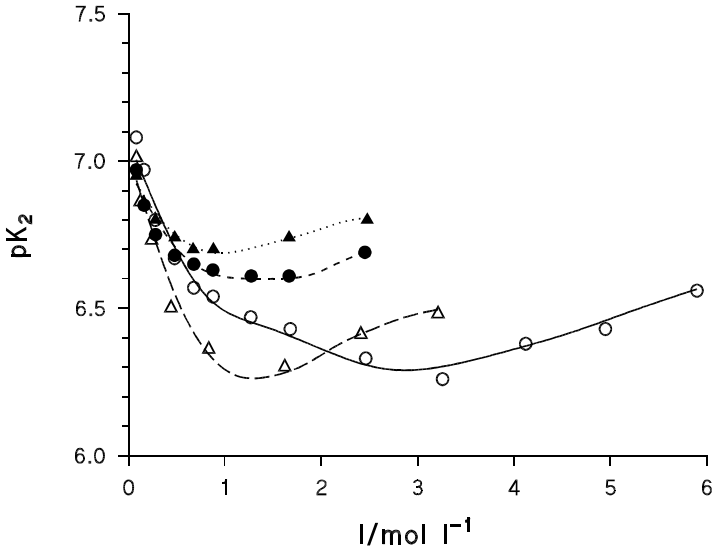

FIGURE 8.3 The effect of ionic strength, I, on pK_2 of $SO_2 \cdot H_2O$ at 30°C for the addition of the group 1 halides at [S(IV)] = 50 mmol l^{-1}. ○, LiCl; Δ, NaCl; ●, KCl; ▲, CsCl. Reproduced from Goddard S. J. and Wedzicha, B. L., *Food Chem*., 52, 219, 1995. © 1995 Elsevier Science Ltd. With permission.

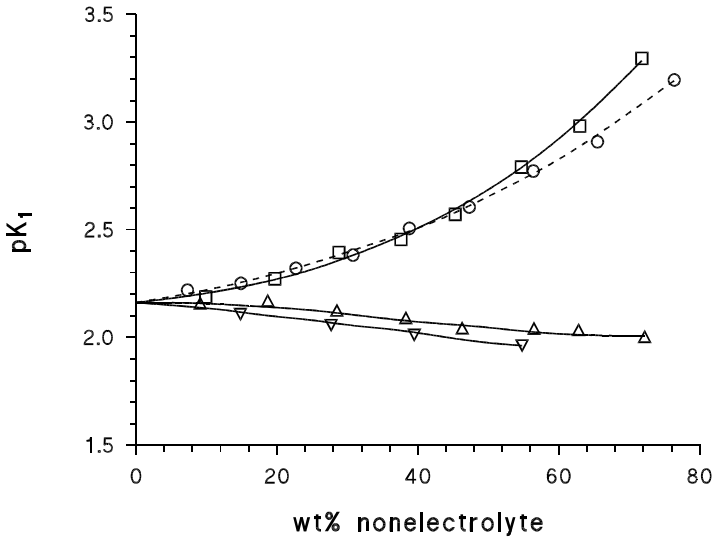

FIGURE 8.4 The effect of concentration of non-electrolyte on pK_1 of $SO_2{\cdot}H_2O$ at 30°C and [S(IV)] = 45 mmol l^{-1}. ○, Ethanol; Δ, Glycerol; □, PEG400; ∇, Sucrose. Reproduced from Wedzicha, B. L. and Goddard, S. J., *Food Chem.*, 40, 131, 1991. © 1991 Elsevier Science Ltd. With permssion.

activity coefficients with ionic strength, according to the extended Debye-Huckel theory.[12,13] However, a critical comparison of the behavior of a range of metal halides on pK_1 also confirms that they have specific effects on the dissociation constant and the observed pK value of $SO_2{\cdot}H_2O$ is affected by SO_2X^--type complexes with the halide ions.

The addition of certain non-electrolytes, e.g., ethanol, has a very marked effect by increasing both pK values by up to 2 units, illustrated in Figures 8.4 and 8.5. On the other hand, high concentrations of sucrose have very little effect.[13] In general, the presence of humectants and non-electrolytes is expected to displace equilibria in the direction of the non-ionic or the least charged species. Conversely, low to intermediate concentrations of electrolytes favor the ionic or most highly charged forms. Thus, the correct pK values of $SO_2{\cdot}H_2O$ in a given food situation are subject to some debate. There is no simple rule to estimate these and they are likely to be dependent on the food matrix itself.

As the concentration of a solution of HSO_3^- increases, the tendency to form disulfite (metabisulfite) ion increases,[15,16] e.g., the extent of conversion of HSO_3^- to $S_2O_5^{2-}$ increases from 1 to 14 mol% as the concentration of S(IV) is increased from 0.1 to 1.0 mol l^{-1} as illustrated in Table 8.1. This is predictable from the law of mass action expression for the equilibrium:

$$K = \frac{\left[S_2O_5^{2-}\right]}{\left[HSO_3^-\right]^2} \tag{8.10}$$

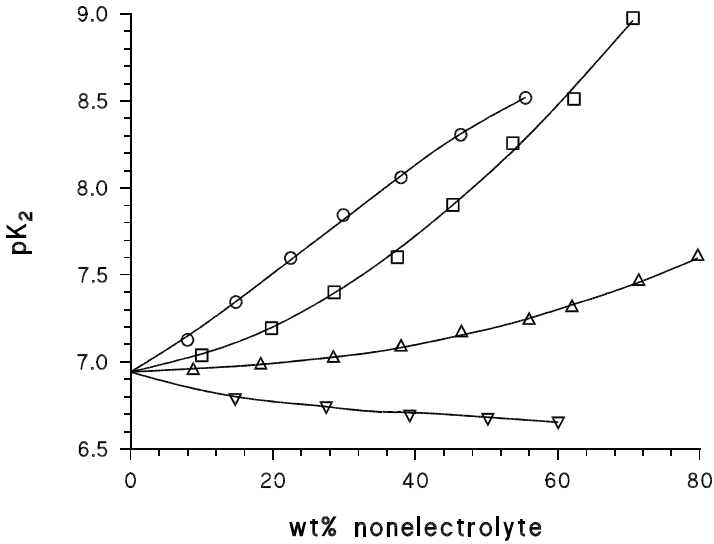

FIGURE 8.5 The effect of concentration of non-electrolyte on pK_2 of $SO_2{\cdot}H_2O$ at 30°C and [S(IV)] = 50 mmol l^{-1}. ○, Ethanol; Δ, Glycerol; □, PEG400; ∇, Sucrose. Reproduced from Wedzicha, B. L. and Goddard, S. J., *Food Chem.*, 40, 129, 1991. © 1991 Elsevier Science Ltd. With permission.

TABLE 8.1
Calculation of the Percentage (mol/mol) of HSO_3^- Remaining in Equilibrium with $S_2O_5^{2-}$, in a Solution Initially Consisting of HSO_3^- at a Concentration *s*

s/mol l^{-1}	0.5	1.0	1.5	2.0	2.5	3.0	3.3
I/mol l^{-1}	0.5	1.0	1.6	2.2	2.9	3.6	4.0
	2	7	6	8	3	0	1
mol% HSO_3^-	93.7	86.3	78.9	72.0	65.7	60.1	57.0

I is the ionic strength at equilibrium.

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and the expected variation in solute activity coefficients with ionic strength.[16] However, increased concentration also leads to anion-cation (ion-pair) interactions becoming significant. Thus, even the singly charged alkali-metal ions interact significantly with doubly charged anions such as SO_3^{2-} and $S_2O_5^{2-}$, as illustrated in Table 8.2, and the stability of these ion-pairs is increased markedly when non-aqueous solvents and humectants are added to aqueous solutions of S(IV). For example, the formation constant of the ion-pair $NaS_2O_3^-$ is 4.8, 71.4, and 143 mol^{-1} l in water,[17,18] 44 and 50 wt% ethanol,[19] respectively; these results are expected to be applicable to SO_3^{2-} because the thiosulfate ion shows solute-water interactions

TABLE 8.2
Calculation of the Percentage (mol/mol) Conversion of SO_3^{2-} to the Ion-Pair $NaSO_3^-$ in a Solution of Na_2SO_3 Whose Total S(IV) Concentration is in the Range 0.01 to 1.00 mol l^{-1}

[S(IV)]/mol l^{-1}	0.0 1	0.0 2	0.0 3	0.0 4	0.0 5	0.1 0	0.2 5	0.5 0	1.0 0
mol% $NaSO_3^-$	8	13	17	20	22	32	46	56	66

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similar to sulfite ion.[1] Multiple charged cations form much more stable ion pairs, which cannot be disregarded, even in dilute systems. All these interactions tend to reduce the activity of S(IV) species, they perturb the acid-dissociation equilibria, and the equilibrium for the formation of $S_2O_5^{2-}$.[20] Surprisingly, little attention has been paid to these interactions in the past.

It can be seen that the state of sulfur dioxide or sulfite in food is complex, but all the forms that have been identified thus far are readily and rapidly interconvertible. Regardless of the chemical form in which S(IV) is added to food (e.g., gaseous SO_2, sodium, or potassium metabisulfites), the actual composition of this preservative depends on the pH of the food, the concentration of S(IV), the ionic strength and the presence of non-electrolytes.[21] During analysis, all these forms of S(IV) are converted either to SO_2 (as in the Monier-Williams distillation technique, or its adaptations), or to some other well-defined species (e.g., SO_3^{2-} for ion chromatography).[22]

In view of the complex specification of the additive in any given situation, the convention adopted in this chapter refers to the mixture of sulfur(IV) oxospecies, in all forms which are readily converted to SO_2 on acidifying, as S(IV). Only where it is necessary to refer to a given species will the actual name of the species be used.

CHEMICAL REACTIVITY OF S(IV)

Sulfur(IV) oxospecies show two distinct types of reactivity; sulfite ion is an excellent nucleophile, whereas all the S(IV) species behave as reducing agents.

Nucleophilic Reactions

The main reason for reactions between S(IV) and food components is the nucleophilic reactivity of SO_3^{2-}, leading to the formation of C–S and S–S covalent bonds. Sulfite ion is one of the best nucleophiles available, with reactivity similar to that of the thiolate ion, acting both as a carbon- and a sulfur-nucleophile.[23] Hydrogen sulfite ion is less nucleophilic.[13] The structure of $S_2O_5^{2-}$ involves an S–S bond, which can be imagined to result from the nucleophilic attack by SO_3^{2-} on a SO_2 molecule; this species shows no significant nucleophilic reactivity. Thus, the tendency for the conversion of HSO_3^- to $S_2O_5^{2-}$ at high concentration and low water activity, the formation of ion pairs involving metal ions and SO_3^{2-} or $S_2O_5^{2-}$,[24] and the preferred

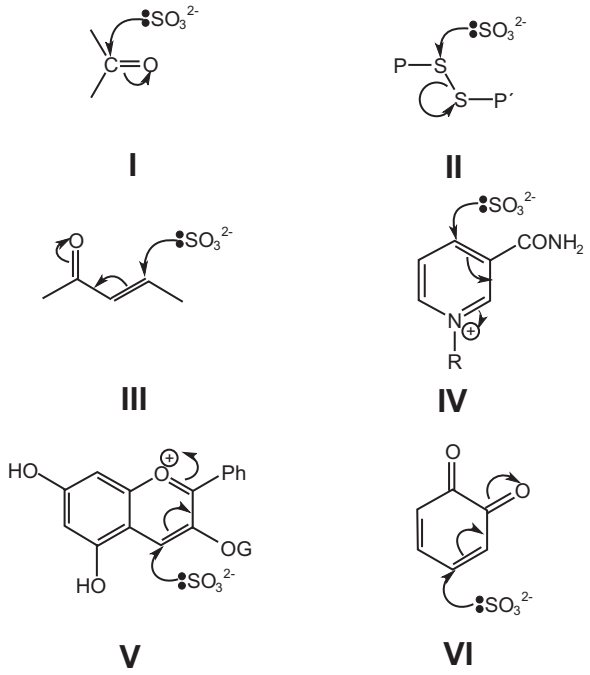


FIGURE 8.6 Examples of nucleophilic reactions of sulfite ion with food components. (I) Attack at a carbonyl group as the first step in the formation of hydroxysulfonates. (II) Cleavage of a disulfide bond. (III) Addition to α,β-unsaturated carbonyl compounds. (IV) Addition of sulfite ion to the nicotinamide moiety of NAD^+. (V) Addition to the benzopyrrylium structure of anthocyanins and responsible for the bleaching of the red colors of many fruits. (VI) Addition to *o*-benzoquinone as an example of the first step in the inhibition of enzymic browning by converting *o*-quinones to the corresponding sulfodiphenols.

conversion of SO_3^{2-} to HSO_3^- in the presence of certain non-electrolytes, all lead to a reduction in the nucleophilic reactivity of S(IV).

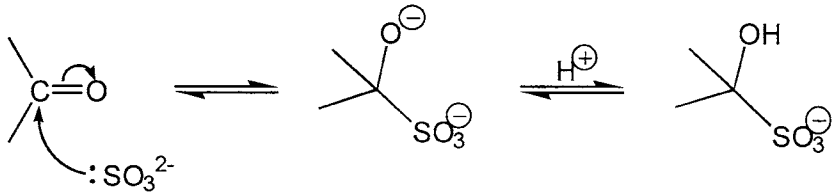
A selection of nucleophilic reactions of SO_3^{2-}, of direct relevance to food quality, is given in Figure 8.6,[25] and these will now be considered in relation to the two most important functions of S(IV) in food, that of an antimicrobial agent and as an inhibitor of nonenzymic browning.

Chemistry of the Antimicrobial Behavior of S(IV)

It has long been recognized that undissociated $SO_2 \cdot H_2O$ is the effective antimicrobial species in S(IV) mixtures, and the general use of the term "molecular SO_2" in this context is remarkably well informed, at a time when few are still aware of the unlikely existence of sulfurous acid as H_2SO_3. Definitive experiments carried out by Rose and his group demonstrated that uptake of SO_2 by yeast cells involves passive transport (diffusion) of SO_2 across cell membranes.[26-28] The initial rate of accumulation of S(IV) in cells is related logarithmically to the pH of the suspension medium (range pH 3 to 5) confirming SO_2 as the important species. Woolf-Hofstee plots are

nearly vertical suggesting the absence of a reversible interaction between SO_2 and other species involved in the transport process.[26] Further evidence for the non-involvement of a conventional protein-assisted membrane transport process comes from the fact that none of the reagents, carbonyl cyanide *m*-chlorophenylhydrazone, DNP, iodoacetamide, or *p*-chloromercuribenzoate inhibit the transport of SO_2, despite being able to inhibit lysine transport under the same conditions. Cell death occurs after a loss of ATP[29] either as a result of the activation by S(IV) of an ATP-hydrolyzing enzyme, the inactivation of glyceraldehyde-3-phosphate dehydrogenase, or by using up ATP to pump H^+ out of the cell in order to restore the pH within the cell. The last of these possibilities also applies to the majority of the carboxylic acid food preservatives. However, S(IV) inhibit a wide range of metabolic enzymes by reaction with disulfide bonds (Figure 8.6, reaction III), coenzymes (as illustrated in Figure 8.6 reaction IV for NAD^+) and cofactors, substrates and intermediates in enzyme reactions.[3] Similarly, the cleavage of disulfide bonds may result in the denaturation of structural proteins.

Perhaps the best known interaction between components of living systems and S(IV), and of considerable significance in foods, is the addition of HSO_3^- to carbonyl groups (Figure 8.6, reaction I). Its mechanism[30,31] involves two steps; first there is nucleophilic attack at the carbonyl group by SO_3^{2-} after which the reaction is completed by the addition of H^+, as follows:



The rate of the forward reaction depends on pH in so far as this determines the concentration of the nucleophile, which is approximately proportional to $[H^+]$ when the pH is well below the pK_a value of HSO_3^-, i.e., within the pH range of most foods. On the other hand, the first step in the decomposition of hydroxysulfonates is the ionization of the OH group (pK_a = 10.7); the rate of the reverse reaction is inversely proportional to $[H^+]$ over this pH range. The equilibrium constant for the dissociation of hydroxysulfonate (HS) is given by

$$K = \frac{[\mathrm{S(IV)}][\mathrm{C=0}]}{[\mathrm{HS}]} \tag{8.11}$$

and corresponds to the ratio of rate constants for the reverse and forward reactions, k_r and k_f, respectively, i.e.,

$$K = \frac{k_r}{k_f} \tag{8.12}$$

The effects of pH on k_f and k_r are almost in balance over the pH range 3 to 6 such that the value of K remains almost constant. However, the adducts become progressively labile (formed and decomposed more rapidly) as pH is increased. Outside this range of pH, hydroxysulfonates are less stable; as pH is reduced to below 3, the increasing conversion of S(IV) to SO_2 results in the apparent value of K passing through a minimum at around pH 2, although the rates of formation and decomposition of the adducts are very slow. On the other hand, they decompose rapidly at pH > 7.

The classification of S(IV) in foods into *free* and *bound* S(IV) is well known[25] and referred to often in the food industry, but there are many instances where its significance is poorly understood. *Bound* S(IV) is sometimes referred to as *reversibly bound*. Free S(IV) is the term used to describe the additive present in the form of SO_2, or any S(IV) species (e.g., SO_3^{2-}, $S_2O_5^{2-}$) which are converted rapidly to SO_2 upon acidifying. This term is, therefore, synonymous with the more accurate (in the chemical sense) use of the term S(IV) in this chapter. Bound S(IV), which is now regarded as mostly in the form of hydroxysulfonates, was defined originally in terms of the different stability and the rates of formation and decomposition of these products. Thus, bound S(IV) is the amount of the additive that is converted to the free form by raising the pH of a sample to at least pH 10, whereas free S(IV) is usually analyzed at pH ≈ 2, under which conditions hydroxysulfonates are most stable. The standard method of analysis of S(IV) in food, based on that devised by Monier-Williams,[32] involves prolonged boiling of the sulfited sample in a strongly acidic solution. Under these conditions the very low pH and the high temperature assist in the decomposition of any hydroxysulfonates present in the sample and allow all the S(IV) to be desorbed from solution as gaseous SO_2.

Hydroxysulfonates are usually decomposed in the human gastrointestinal tract and their toxicity is equivalent to that of free S(IV);[33] thus legislation stipulates the total (i.e., free + bound) S(IV) present in food at the time of sale. The reason for taking an interest in the relative amounts of free and bound S(IV) in food is that bound S(IV) does not exhibit the antimicrobial properties of the free additive. The author has often observed that foods (particularly fruit juices) containing acceptable amounts of total S(IV) spoil because the level of free S(IV) is too low; often the quality assurance protocol adopted during food manufacture does not include the monitoring of free S(IV) levels. It is important, therefore, to understand the factors that determine the S(IV)-binding capacity of different types of foods.

Hydroxysulfonate dissociation constants for a wide range of carbonyl compounds, representing the extremes of stability normally encountered for food components, are given in Table 8.3.[34-36] The value for a simple aldehyde, e.g., acetaldehyde, is of the order 10^{-5} to 10^{-6} mol l^{-1} representing a very stable adduct. On the other hand, the carbonyl group of reducing sugars exists in equilibrium with cyclic structures, and the values of their hydroxysulfonate dissociation constants depend on the proportion of acyclic form present. Thus, the values 0.9 and 15 mol l^{-1} for glucose and fructose hydroxysulfonates, respectively, indicate unstable adducts; fructose is regarded as not forming such an adduct to a significant extent, in practice.

Problems stemming from S(IV) binding are seen most commonly in fruit products, particularly beverages, and most of our understanding of the underlying reasons has come from investigations on fermented beverages.[36-38] Acetaldehyde, 2-ketoglutaric

TABLE 8.3
Dissociation Constants *K* of Carbonyl-S(IV) Adducts (Hydroxysulfonates)

Carbonyl Compound	*K*/mmol l^{-1}
Glucose	900
Fructose	15000
Arabinose	40
2-Ketoglutaric acid	0.5 (pH 3)–0.7 (pH 4)
Pyruvic acid	0.14 (pH 3)–0.22 (pH 4)
L-xylosone	1.4
D-Threo-2,5-hexodiulose	3.4
2,5-Diketogluconic acid	0.4
Galacturonic acid	17
Acetaldehyde	0.002

The value of *K* was calculated from the total carbonyl and S(IV) species concentrations in solution. Unless otherwise indicated, values are nominally for the pH range 3 to 4.

Sources of the data are Burroughs, L. F. and Sparks, A. H., *J. Sci. Food Agric.*, 24, 187, 1973; Würdig, F. W. and Schlotter, H.-A., *Wein Wiss.*, 24, 67, 1969; Beech, F. W., Burroughs, L. F., Timberlake, C. F., and Whiting, G. C., *Bull. O.I.V.*, 586, 1001, 1979.

and pyruvic acids are formed in normal yeast fermentation, the yields being dependent on fermentation time, the extent of aeration, the type of yeast, and the presence of thiamin. The mechanisms for the production of these compounds are well understood. Pyruvic acid is the final product of glycolysis. 2-Ketoglutaric acid is an intermediate in the tricarboxylic acid cycle while acetaldehyde is formed as a result of the decarboxylation of pyruvate. Increased acetaldehyde production is associated with a reduction in pyruvate formation. 2-Ketoglutaric acid may also be formed from 2,5-diketogluconic acid by *Acetobacter melanogenum*. Diacetyl and acetoin are metabolic products of lactic acid bacteria. The ketogluconic acids (2-ketogluconic, 5-ketogluconic, and 2,5-diketogluconic) are the result of the action of gluconobacter and pseudomonas on glucose and gluconic acid. Chromobacteria also metabolize gluconic acid to ketogluconic acids. Fructose may be converted by gluconobacter to D-*threo*-2,5-hexodiulose. Ascorbic acid is converted to L-xylosone. In general, the presence of S(IV)-binding compounds cannot be avoided in fermented beverages, but significantly higher concentrations occur when fruit has undergone microbial spoilage through the action of molds or bacteria.

For mixtures of carbonyl compounds, the distribution of S(IV) between the various hydroxysulfonates can be calculated, for the general case, by solving the series of simultaneous equilibria:

$$S(IV) + (C{=}O)_2 \rightleftharpoons (HS)_1 \quad K_1 \tag{8.13}$$

$$S(IV) + (C{=}O)_2 \rightleftharpoons (HS)_2 \quad K_2 \tag{8.14}$$

$$\vdots \qquad \vdots \qquad \vdots \qquad \vdots$$

$$S(IV) + (C{=}O)_n \rightleftharpoons (HS)_n \quad K_n \tag{8.15}$$

made possible by computer successive approximation methods. The most satisfactory calculations of the S(IV)-binding capacity of whole beverages are still those published for wine samples by Burroughs and Sparks,[34,39,40] who summed the contributions from S(IV) bound to D-*threo*-2,5-hexodiulose, L-xylosone, acetaldehyde, galacturonic acid, pyruvic acid, 2,5-diketogluconic acid, and 2-ketoglutaric acid. Würdig and Schlotter identified 5-ketogluconic and 2-ketogalacturonic acids as additional components but, even after including these, the authors[35] were unable to account quantitatively for the S(IV)-binding power of the wines they had available. These investigations all reveal that even at the lowest concentrations of free S(IV) used in the calculations (6.4 ppm free SO_2, 150 ppm total SO_2), essentially all the acetaldehyde is present in the form of hydroxysulfonate, and is by far the most important contributor to S(IV) binding in beverages. Both the aroma and taste of white wine is degraded by the presence of acetaldehyde and the "anti-acetaldehyde" property of S(IV) is considered to be one important reason why this additive is indispensable. Any attempt to reduce the levels at which S(IV) is added in wine-making has to be focused on a reduction in the concentrations of S(IV)-binding compounds. However, the accumulation of some acetaldehyde is unavoidable even when the best quality fruit is used, and fermentation in the presence of S(IV) causes the yeasts to produce increased levels of the aldehyde.

The relative resistance of specific strains of yeast to S(IV) is important for the selection of "desirable" strains for fermentation. Strains of *Saccharomyces cerevisiae* have long been regarded as particularly resistant organisms, but *Saccharomycodes ludwigii* have frequently been isolated from alcoholic beverages that had been treated with S(IV) at levels that prevent the growth of *S. cerevisiae*. One reason[27] for the higher resistance of *S. ludwigii* is that this organism produces acetaldehyde at a higher rate (mirrored by a reduction in free S(IV)) and the amount of S(IV) accumulated in the cells is one-third that of *S. cerevisiae*.

In contrast to the detrimental quality characteristics of acetaldehyde in wine, it is often used as a flavor additive in soft drink formulations to provide "impact". Despite the known S(IV)-binding characteristics, many beverage manufacturers are still using acetaldehyde in S(IV)-preserved products. Figure 8.7 shows the calculated concentration of free acetaldehyde at different total acetaldehyde and S(IV) concentrations, to demonstrate the very low equilibrium concentrations of the aldehyde which might result in such a situation. Thus, the addition of acetaldehyde to flavor a sulfited beverage is inadvisable for two reasons. First, it does not represent good manufacturing practice to use increased levels of food additives to compensate for losses as a result of chemical reactions between those additives. Second, the long-term

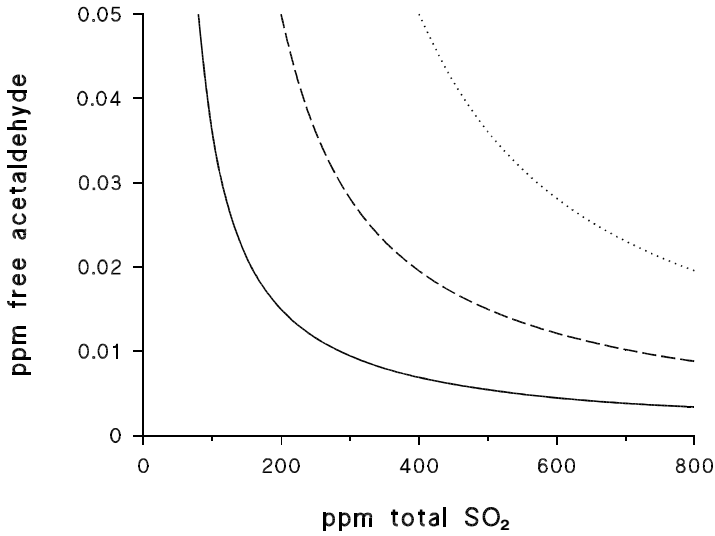


FIGURE 8.7 Calculated concentrations of free acetaldehyde as a function of total S(IV) concentration in mixtures containing various total concentrations of acetaldehyde. Data were obtained using a dissociation constant of acetaldehyde hydroxysulfonate of 2×10^{-6} mol l^{-1}. Total acetaldehyde concentrations were 20 ppm (——), 50 ppm (------), 100 ppm (·····).

flavor stability of such a food may be degraded. It is well known that the S(IV)-content of most sulfited foods decreases with time, particularly when beverages are exposed to air repeatedly. Since hydroxysulfonate formation is reversible, free acetaldehyde is released as the concentration of free S(IV) falls.

In the U.K., S(IV) may be added as an antimicrobial agent to certain meat products, e.g., to preserve and impart a unique characteristic to pork sausage. The additive is effective[41] against Salmonellae even at the relatively high pH of this meat product, and any spoilage is restricted initially to Gram-positive microflora consisting of Lactobacilli and *Microbacterium thermosphactum*. Thus, the spoilage of sulfited meat tends to be associated with a sour odor, unlike the spoilage of untreated meat which leads to a "putrid" smell. The important microbial contaminants identified in sausages which are sensitive to S(IV) include *Brochothrix thermosphacta*, *Enterococcus* spp., *Lactobacillus* spp., *Pseudomonas* spp., Enterobacteriacea, and *Salmonella* spp.; the concentrations of S(IV) (pH 7 in batch culture medium) to inhibit growth decrease in the above order of microbial contaminant. On the other hand, yeasts are not inhibited significantly at concentrations of S(IV) found in sausages. The total S(IV)-content of sulfited pork sausage falls with time during storage, e.g., a sample to which 450 ppm SO_2 had been added could lose 70 to 80 ppm SO_2 after 8 d at 4°C, but the level of free S(IV) falls to 100 ppm SO_2 or less under the same conditions. The principal S(IV)-binding compound is, again, acetaldehyde of microbial origin, and is an important reason for the reduced storage life of this product.

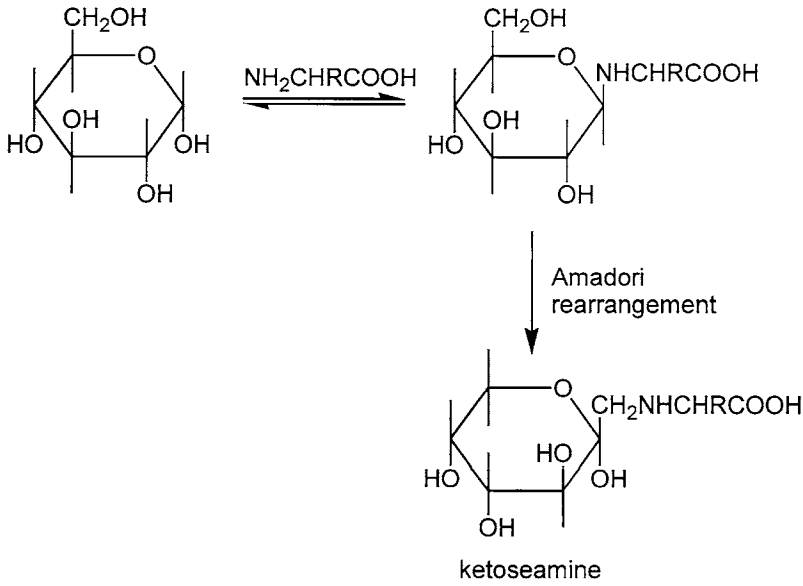
While there is much interest in acetaldehyde, important food flavors arise from a wide range of volatile aldehydes, e.g., through enzymic oxidation of unsaturated fatty acids in plant products such as tomato, cucumber, and peas. The known antioxidant properties of this additive are often confused between its ability to react with oxygen or by inhibiting enzymes in one of many ways, with the reaction of S(IV) with the final carbonyl products of oxidation. In many situations it is difficult to distinguish between these possibilities. A particular example where current thinking suggests that S(IV) inhibit the sensory impact of an oxidation product is through their reaction with the carbonyl moiety of *trans*-2-nonenal to form involatile hydroxysulfonate salts. The aldehyde is alleged to be the cause of a "cardboard"-like taint (staleness) when beer undergoes oxidative spoilage, e.g., after beer is allowed to stand in air or when beer containers are defective in so far as they allow ingress of oxygen.[42] Evidence for the important role played by nonenal in eliciting the sensation of staleness is demonstrated by spiking beer with the aldehyde to obtain a stale product. When acetaldehyde is added to sulfited stale beer, which has no taint, the taint promoting substance is released.[43] It is found that nonenal is released in preference to other aldehydes as a result of competition between it and acetaldehyde for the S(IV) in solution.[44]

The defect may be avoided or removed by the addition of S(IV); levels as low as 2 ppm SO_2 are found to be sufficient with concentrations of nonenal at the ppb level. While low levels of acetaldehyde appear to reverse the anti-staling effect of added S(IV), there are no reliable values of nonenal-S(IV) hydroxysulfonate dissociation constants. One reason is that the very low solubility of this aldehyde in water means that equilibrium measurements require correspondingly low concentrations of S(IV) which are difficult to measure accurately. However, since in this situation, hydroxysulfonate formation is the conversion of a relatively non-polar species into a species with significant polarity, i.e., because of the sulfonate group, it is likely that the hydroxysulfonate of nonenal is at least as stable as that of acetaldehyde. The addition of an excess of acetaldehyde to a solution of nonenal hydroxysulfonate should compete successfully for the S(IV). The same considerations could apply also to hexanal and other hydrophobic aldehydes derived from the oxidation of unsaturated triglycerides. Sulfite ion can also react with nonenal by nucleophilic attack on the α,β-unsaturated carbonyl moiety (as in Figure 8.6, reaction II). There is no evidence of this reaction in beer; the reason it apparently does not take place is important because such a reaction would obviate the need for residual S(IV) in beer. While any explanation can only be speculative, it is reasonable to suggest that the highly efficient formation of nonenal hydroxysulfonate (which is not attacked by SO_3^{2-} at the C=C bond) reduces the effective concentration of the α,β-unsaturated carbonyl moiety to such an extent that the overall rate of its biomolecular reaction with S(IV) is too slow to be significant. The synthesis of this hydroxysulfonate is carried out usually at high concentration in water–non-aqueous solvent mixtures.

Inhibition of Nonenzymic Browning

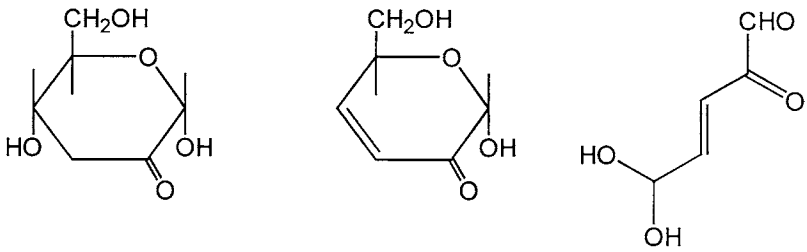
To most individuals, the term nonenzymic browning is synonymous with the Maillard reaction, i.e., the reaction between reducing sugars and amino acids, peptides, and proteins. Current understanding of its mechanism stems from that proposed by

Hodge in 1953.[45] The reaction is initiated by the formation of a glycosylamine which undergoes an irreversible rearrangement to a ketoseamine shown below for the reaction of glucose with an amino acid:



ketoseamine

At the pH of most foods, the 1,2-enolamine precursor of the ketoseamine decomposes to give a key α-dicarbonyl nitrogen-free intermediate, 3-deoxyhexosulose (DH),[46,47] which is present in a variety of cyclic and acyclic isomeric forms; essentially, this molecule consists of glucose with one molecule of water removed and for this reason the term "amine-assisted dehydration" has been coined for the overall process of the conversion of glucose to DH.[48] This, in turn, dehydrates to *cis*- and *trans*-3,4-dideoxyhexosulos-3-ene (DDH) which are by far the most reactive known intermediates in browning.[49,50] The structures of these important intermediates in browning are as follows:



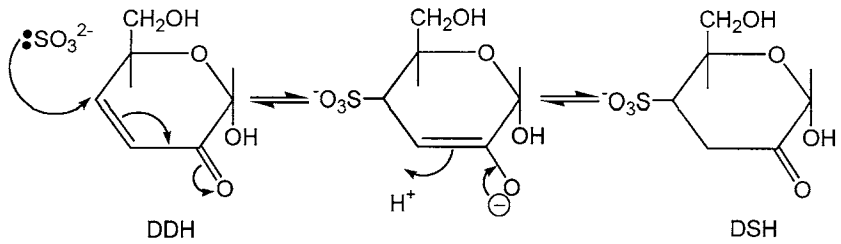
DH cis-DDH trans-DDH

DDH is converted to other intermediates in the route to color. It behaves as an α-dicarbonyl compound in the Strecker degradation of amino acids while a proportion

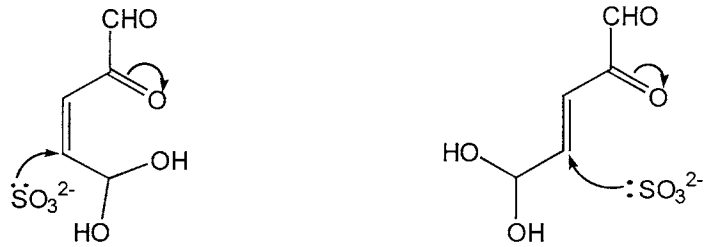
dehydrates further to hydroxymethylfurfural HMF, which has a low potential for browning.

In general, the major products of the irreversible combination of S(IV) with food components are organic sulfonates[1] most often formed as a result of the inhibition of nonenzymic browning reactions. It is the only permitted additive for this purpose and, consequently, this property is of considerable technological importance. In food dehydration, the additive is used to prevent spoilage in the intermediate moisture phase of the process, at which stage the rate of browning is at a maximum, and to protect the dehydrated food from browning while in storage. The effect of S(IV) is to delay the onset of browning[51] but, once it commences, browning continues at the same rate in sulfited and unsulfited systems. In most dehydrated foods, the additive continues to react after dehydration and the shelf-life of the products is determined by the time required for the level of the additive to fall to such an extent that it no longer inhibits browning. This is a complex function of temperature and moisture content for a given food product.

The principal mechanism of inhibition of browning involves the nucleophilic addition of SO_3^{2-} to the α,β-unsaturated carbonyl moiety of DDH (Figure 8.6, reaction II), leading to 3,4-dideoxy-4-sulfohexosulose (DSH) as follows:[51]



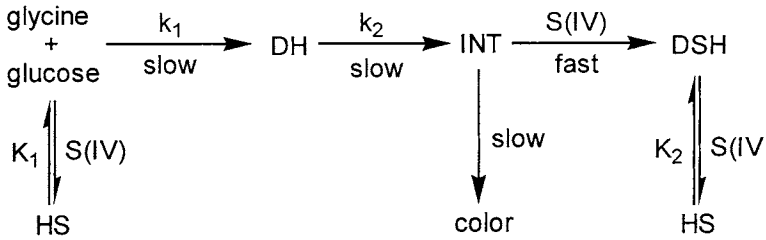
where the reaction could equally involve the acyclic *cis*- or *trans*-isomers of DDH, i.e.,



The formation of DSH results in the binding of S(IV) in such a way that it cannot be recovered by conventional analytical procedures, and is usually referred to as *irreversibly* bound S(IV). However, a proportion of S(IV) present in reducing sugar–amino acid–S(IV) mixtures is also bound reversibly as hydroxysulfonates. Initially this binding is to glucose, and in a typical model reaction mixture where [glucose}>>[S(IV)], pH 5.5, the concentration of hydroxysulfonate is given by

$$[\mathrm{HS}] = \frac{[\text{glucose}][\mathrm{S(IV)}]}{K + [\text{glucose}]}, \tag{8.16}$$

i.e., for a glucose concentration of 1 mol l^{-1} approximately half of the S(IV) is reversibly bound.[52,53] As the reaction proceeds, the fraction of the total S(IV) which is bound increases with time, indicating that carbonyl compounds that bind S(IV) more strongly than glucose are being formed. It has been shown that, in the glucose–glycine–S(IV) reaction, the principal S(IV)-binding component is DSH (hydroxysulfonate dissociation constant = 4 mmol l^{-1}).[53-55] The kinetics of the irreversible binding of S(IV), the formation of hydroxysulfonates, and the relationship of this mechanism to the route taken by the Maillard browning reaction are described by the following model:



where INT is an unspecified intermediate common both to browning and the reaction with S(IV), k_1 and k_2 are rate constants, and K_1 and K_2 are equilibrium constants. This kinetic model has been tested rigorously and found to apply to a wide range of sugars and amino acids, and under a variety of reaction conditions.[56-62] The unspecified intermediate could well be DDH and the success of S(IV) as an inhibitor of browning rests with its ability to compete effectively for this intermediate. In model systems, e.g., containing aqueous solutions of glucose (1 mol l^{-1}), amino acid (0.5 mol l^{-1}), and S(IV) (0.05 mol l^{-1}), the concentration of S(IV) becomes limiting in the conversion of the intermediate to DSH, only after some 75% of the additive is irreversibly lost. In this situation a very high proportion of what remains is reversibly bound and the concentration of free S(IV) to react with the intermediate is very small. This explains why the reaction becomes dependent on S(IV) concentration. On the other hand, most evidence concerning the loss of S(IV) from foods suggests that the kinetics are of first order with respect to this additive.

The high molecular weight colored products in Maillard browning (melanoidins) are partially bleached by the addition of S(IV). Those (with $M_r > 12{,}000$) formed in the glucose-glycine reaction combine irreversibly with S(IV) such that one C=C bond for every two glucose-derived residues is lost; it is envisaged that here SO_3^{2-} could be adding to an α,β-unsaturated carbonyl compound or a Schiff base derived from it.[63] As the melanoidin reacts with S(IV), the sulfur to carbon ratio of the product increases, but this is related linearly to its absorbance. Melanoidins are polymers and this result suggests that the chromophores in these products behave independently[64] and they are not the extensively conjugated structures suggested by some authors.

Even in a simple Maillard model system consisting of a single reducing sugar and amino acid, there are thousands of reaction products which may contribute to color and flavor. Control and optimization of the Maillard reaction are major research themes within the food manufacturing and food ingredients industries but the complexity of the reaction and the many ill-defined products, places considerable limitations on the ability to study the kinetics of the rate-determining steps. For this reason little real progress has been made to identify the factors that determine browning in specific foods or to devise food flavor formulations from first principles. An important way forward is illustrated by the painstaking analytical work of van Boekel's group,[65] who have measured the concentrations of known intermediates in the early stages of the browning of sugars with casein and have attempted to relate these to an overall kinetic model for the reaction.

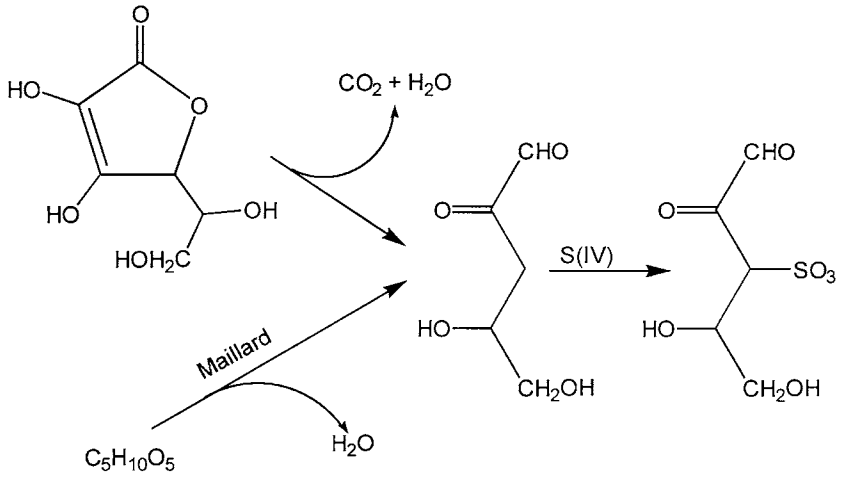
Understanding of the kinetics of the reactions of S(IV) during its inhibition of Maillard browning now offers a new and focused approach to understand better the mechanism of the Maillard reaction.[61,62,66,67] The approach is based on the premise that since S(IV) reacts quantitatively and irreversibly with a precursor of color and flavor, the rate at which S(IV) reacts in this way is a measure of the rate at which reducing sugars are converted to Maillard intermediates up to the point where S(IV) exerts its inhibitory effect. Thus, measurement of the rate of loss of S(IV) allows the rate constants k_1 and k_2 to be obtained in relation to variables of interest in Maillard browning, e.g., concentration, pH, water activity, and the presence of different amino acids.[57,59,61,67,68] It has been shown that k_1 can have an overall controlling effect on the rate of browning,[60] and a combination of k_1 and k_2, with a third rate constant for color formation, can be used to model accurately the rate of browning of glucose-glycine mixtures.[61,62]

The inhibition of the browning of fructose by S(IV) also results in the formation of DSH. The kinetics of the fructose-amino acid-S(IV) reaction are simpler than those of the Maillard reaction, and may be described by

$$\text{fructose} \xrightarrow[\text{slow}]{k} \text{INT} \xrightarrow[\text{fast}]{\text{S(IV)}} \text{DS}$$

the major difference being the absence of an early amino acid-dependent step.[69] This point is adequately demonstrated by the fact that the rate of reaction of S(IV) in the absence of amino acid ([fructose] = 1.0 mol l^{-1}; [S(IV)] = 0.05 mol l^{-1}; pH 5.5, 55°C) is 53 μmol l^{-1} h^{-1} and 53, 55, and 50 μmol l^{-1} h^{-1} in the presence of 0.5 mol l^{-1} glycine, glutamic acid, and arginine, respectively. The reaction is, however, catalyzed by the components of buffer systems, e.g., acetate ion,[70] and these results all provide insight into the reported discrepancies between the rates of browning of fructose and glucose in buffered and unbuffered media.

The browning of ascorbic acid is a form of nonenzymic browning reaction, which is related to the Maillard reaction. In the absence of oxygen, the reaction is inhibited by S(IV) and so leads to the formation of 3,4-dideoxy-4-sulfopentosulose (DSP), the 5-carbon analog of DSH.[71,72] In this respect, the reaction shares a common step in the S(IV)-inhibited Maillard reaction of pentoses, i.e.,

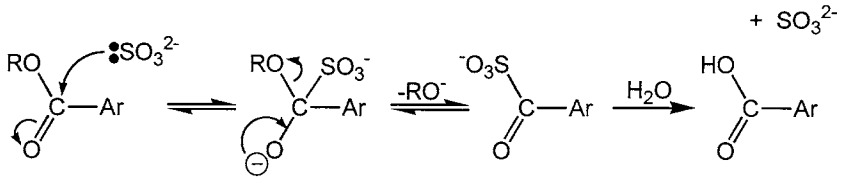


but the mechanism of the formation of melanoidins appears very different, with those derived from ascorbic acid and glycine having a much larger number of 3-deoxypentosulose-derived subunits per glycine residue, than those from the pentoses.[73]

In the presence of air, ascorbic acid is converted to dehydroascorbic acid; this reaction takes place by way of an unusually stable free radical intermediate, monodehydroascorbic acid, which is very difficult to trap or react with conventional antioxidants. In food and model systems, it is usually impossible to avoid the oxidation of ascorbic acid unless oxygen is strictly excluded from the system. While dehydroascorbic acid exhibits vitamin C activity, it is much more susceptible to browning and its presence is regarded as undesirable. The use of ascorbic acid as an inhibitor of enzymic browning results in the coupled oxidation of the vitamin by the quinone intermediates; the widely seen practice of adding ascorbic acid to apple and pear juice for fermentation is probably of no consequence with respect to the browning of ascorbic acid in long-term storage because these beverages are also treated with S(IV) which protects also against the browning of dehydroascorbic acid. The mechanism of the inhibition of dehydroascorbic acid browning is still not well understood, but dehydroascorbic acid forms a stable monohydroxysulfonate (K = 5.7×10^{-4} mol l^{-1}), which should be much less reactive.[74] On the other hand, dehydroascorbic acid decarboxylates and dehydrates to L-xylosone which also forms a stable carbonyl-S(IV) adduct ($K = 1.4 \times 10^{-3}$ mol l^{-1} from Table 8.3).

The kinetics of the irreversible binding of S(IV) in the ascorbic acid-S(IV) reaction, under anaerobic conditions, suggest that S(IV) catalyze the conversion of ascorbic acid to 3-deoxypentosulose (DP) or some other intermediate which reacts with S(IV) to give DSP.[75] Indeed, at millimolar concentrations of S(IV), and at pH 3 to 5, the rate of the catalyzed reaction far exceeds the rate of spontaneous decomposition of the vitamin. The possibility that S(IV) is able to increase the rate of hydrolysis of the lactone moiety of ascorbic acid, which is regarded as the first step in the mechanism of its decomposition to DP, cannot be tested directly on ascorbic acid, but it is found that the rate of hydrolysis of δ-gluconolactone (which can be measured using optical rotation) is, indeed, increased by S(IV).[75]

The pK_a of HSO_3^- is similar to that of $H_2PO_4^-$, and it is reasonable to expect that the S(IV) species could act as general acid–base catalysts in chemical reactions, as do phosphate species. Usually, this occurs when proton transfer takes place in the rate-determining step of the reaction and the catalyst is either the proton donor or acceptor. It is significant that S(IV) catalyze the mutarotation of glucose,[76] presumably by speeding up the rate of ring opening, although there is no evidence that it increases *per se* the concentration of the acyclic structure. Insight into the mechanism of the catalysis of the hydrolysis of gluconolactone by S(IV) comes from an investigation of the hydrolysis of *p*-nitrophenyl acetate.[77] In this case, SO_3^{2-} is 16,000 times more effective a catalyst than HPO_4^{2-}; this is attributed to the ability of SO_3^{2-} to initiate the hydrolysis by nucleophilic attack at the carboxyl-carbon atom, as follows:



The implications of this reactivity have not yet been explored fully, but it is possible that esters which are components of natural or additive food flavors could be "destabilized" by S(IV).

This discussion of the effects of S(IV) on the stability of ascorbic acid appears to contradict the normally accepted role of S(IV) as a protector of the vitamins in food. However, the chief cause of its degradation is oxidation and S(IV) is capable of preventing this from taking place by scavenging oxygen, reducing the monodehydroascorbic acid intermediate, or stabilizing dehydroascorbic acid. It is suggested that the rate of the S(IV)-mediated degradation of ascorbic acid is slow in comparison with the other reactions that lead to its loss from food, and the overall effect is, therefore, that S(IV) stabilizes the vitamin.

Homolytic Reactions

It is well known that sulfite ion is a good reducing agent which reacts with oxygen and numerous inorganic and organic oxidizing agents. The oxidation of S(IV) takes place by a one- or two-electron transfer mechanism. Here we will focus on the one-electron route because this leads to free radical intermediates and has the greater potential impact on food quality.

The primary step in the one-electron oxidation of S(IV) is the formation of the sulfite radical $^{\bullet}SO_3^-$ as a result of the transfer of one electron from the sulfite ion to a suitable electron acceptor, such as a transition metal ion in one of its higher oxidation states.[1] Whereas the oxidation of S(IV) is inhibited by low concentrations of organic compounds such as alcohols, it is striking that high concentrations of ethanol appear to increase the rate of autoxidation. There is evidence that this increase is due to the participation of transition metal complexes, including those

with ligands such as EDTA which normally inhibit oxidation.[78] Sulfite radicals can also be formed by the reaction of sulfite ion with radicals derived from other reactions.

Much of the interest in radicals derived from S(IV) stems from the fact that they can react with oxygen ($k = 1.5 \times 10^9$ mol^{-1} l s^{-1}) to form a peroxyl radical ($^{\bullet}SO_5^-$) which is a more powerful oxidizing agent[79] than $^{\bullet}SO_3^-$. Species such as $^{\bullet}OH$, $^{\bullet}O_2^-$, and $^{\bullet}SO_4^-$ are formed by the reduction of $^{\bullet}SO_5^-$ by metal ions, e.g., Fe^{2+}, by S(IV), or as a result of the decomposition of $^{\bullet}SO_5^-$. It is observed that S(IV) in food is converted partly to sulfate and oxidation of S(IV) is, therefore, taking place. Hence, it is clear that the oxidation of S(IV) in food can lead potentially to oxidative spoilage.

An impressive demonstration of this fact is an experiment whereby a solution (in ethanol-water-chloroform) of β-carotene is decolorized in a few seconds upon the addition of a solution of S(IV). This reaction results in as many as 17 atoms of oxygen becoming incorporated for every molecule of β-carotene oxidized.[80] Other demonstrations (carried out by the author but unpublished) include the accelerated development of rancidity in emulsified unsaturated triglyceride mixtures in the presence of S(IV). Such observations have led to the term "sulfite-mediated" oxidation being used to describe the process. A most interesting example of such an oxidation is the "oxidative depolymerization" of starch first identified by Hill and co-workers[81] and illustrated in Figure 8.8. This shows that low concentrations of S(IV) dramatically reduce the swelling volume and viscosity of starch, and there is

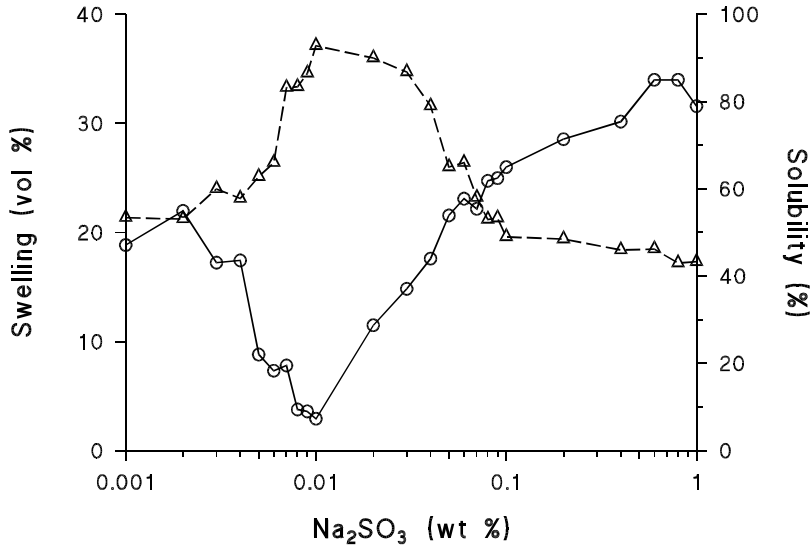


FIGURE 8.8 Effect of sodium sulfite concentration on the swelling volume (——) and solubility (------) of cassava starch (1% w/v) and held at 95°C for 1 h. The solubility is the total weight of carbohydrate found in the supernatant expressed as a percentage of the dry weight of starch in the whole system. These graphs have been plotted using the data shown in Mat Hashim, D. B., Moorthy, S. N., Mitchell, J. R., Hill, S.E., Linfoot, K. J., and Blanshard, J. M. V., *Stärke,* 471, 1992, and kindly provided by Dr. S.E. Hill.

a reduction of molecular weight. The effect is reversed at high concentration. Evidence for a pathway that involves oxidation comes from the fact that this effect is inhibited by conventional antioxidants. The absence of oxidative depolymerization at high S(IV) concentration has not yet been explained satisfactorily.

These observations lead to a most intriguing question regarding the generally accepted role of S(IV) in food as an antioxidant, whereas its behavior in model systems can often be seen to be that of a pro-oxidant. Currently we have no evidence that S(IV) acts as a pro-oxidant in any real food system despite the fact that S(IV) is oxidized in food and should, therefore, provide oxidizing free radicals. There is no scientific evidence to reconcile the anti- and pro-oxidant behavior, but such knowledge would be fundamental to our understanding of food as a medium for chemical reactions. The reason for the apparent contradiction is likely to lie in the multiphase structure of food and the availability of different antioxidants in the different phases.

FOOD APPLICATIONS

The subject of this chapter has been slanted towards the principles underlying the interactions of sulfur dioxide with food components to provide generic understanding of the chemistry. The reader is made aware of specific food examples to illustrate the relevance of the chemistry throughout this chapter, but is directed to the significant reviews[1-6] for more detailed information.

It should be recognized that many "traditional foods" cannot be prepared without this additive. They include wine, cider, perry and other fermented beverages, burger meat with a minimum vegetable and/or cereal content of 4%, breakfast sausages, longanzia fresca and butifarra fresca, jams, jellies, and marmalades made with sulfited fruit, sultanas and other dehydrated fruits such as apricots, dehydrated vegetables, barley water, and capilé groselha.[82] The production of beer with a second fermentation in the cask (cask-conditioned or real ale) requires a relatively high level (50 ppm) of sulfur dioxide, and is a process unique to Europe.

ALTERNATIVES TO SULFUR DIOXIDE

The Need for Alternatives

This chapter describes a large number of chemical reactions involving S(IV) in foods and it is understandable that they have caused concern about the safety of the use of sulfur dioxide as a food additive. The toxicity of ingested S(IV) is regarded as low on account of the very efficient sulfite oxidase detoxifying system present in the liver of all animals.[83,84] The major reaction product from the inhibition of Maillard browning, DSH, is metabolically inert.[85,86] The destruction of thiamin[87] is the "classical" example of an adverse effect of a food additive on the nutritional quality of a food, but this loss of vitamin is not regarded as being of any significance in practice. It has, however, led to the view that sulfur dioxide should not be used in foods that are an important source of thiamin. On the other hand, the products formed when disulfide bonds in proteins react with S(IV) (Figure 8.6, reaction II) either in food

or *in vivo* are metabolized quickly and harmlessly.[84] Thus, from the available evidence, we see that despite the somewhat indiscriminate reactivity of S(IV) in foods, this is not associated with any known toxicological hazard to humans.

A critical appraisal of the evidence presented by Til and Feron,[83] on whose research the acceptable daily intake (ADI) of S(IV) has been set by the European Community, indicates that there are a number of minor toxicological effects that have not yet been explained. Of particular interest is the observation that toxic effects are sometimes associated with the oxidized lipid fraction of sulfited diets, and it is speculated here that sulfite-specific oxidation products may be involved. However, the compelling reasons why much effort has turned to seeking alternatives to sulfur dioxide is the fact that some individuals are abnormally sensitive to low concentrations of SO_2[88] in the head space above sulfited foods, or as a result of eructation after a sulfited food has been eaten and come into contact with the acid environment of the stomach.

Estimates[89] of the per capita consumption of sulfur dioxide (taking account of all the possible forms in which it may be added to food) in the U.S. by reference to 782 food products reveal a daily intake that is 44% of the ADI (210 mg per person per day based on an individual weighing 60 kg). On the other hand, a total diet survey suggests that the typical intake is probably somewhat lower at 18 mg per person per day, with extreme users reaching some 64 mg per person per day. Alcoholic beverages, soft drinks, sausages, and hamburgers are the most significant contributors to the dietary intake of sulfur dioxide. Dehydrated fruits can also be a significant source of dietary sulfur dioxide for those who eat them. These levels of consumption of sulfur dioxide are among the highest intakes of food additives in relation to their ADI values. This evidence, taken together with the abnormal sensitivity of certain individuals to gaseous SO_2, has been the principal reason why there has been particular interest in reducing the levels of the additive and finding suitable replacements.

The Alternatives

There have been a number of comprehensive reviews outlining the main issues that need to be considered for replacing sulfur dioxide in foods.[90,91] The role of S(IV) in controlling the diverse spoilage reactions in food as an antimicrobial agent, an inhibitor of browning, and an antioxidant are recognized and our ability to offer suggestions for replacements is based on a good understanding of the mechanisms of its preservative action. It is often said that the complete role of S(IV) in food extends beyond the obvious chemical reactions associated with its preservative action, and includes as yet unknown contributions to subtle changes in quality. For this reason there is a need to understand the full range of contributions of S(IV) to the quality of preserved foods.

Whereas S(IV) is unique in its ability to control *simultaneously* several forms of food spoilage, there are possible replacement food additives to control *individual* spoilage processes. Thus, alternative antimicrobial agents could include benzoates and sorbates, and antioxidants include citrate, tocopherol, and BHT, among the wide range of food additives currently available for these purposes.

It is often said that S(IV) is unique in its ability to control browning in food. The mechanism of inhibition of enzymic browning is the reaction of sulfite ion with the *o*-quinones which are formed by the enzymatic oxidation of *o*-diphenols. Essentially the quinones are reduced to the sulfonated phenols (Figure 8.6, reaction VI). In the case of catechol oxidation, it has been shown that the 4-sulfocatechol which is formed upon reduction of the quinone is unreactive towards polyphenol oxidase and so represents a relatively stable product.[92] In general, inhibitors are expected to work in two ways. Either they inhibit the enzyme or, in the same way as S(IV), they react with the quinone intermediates. Ascorbic acid is probably the best known reagent that acts by the latter of these mechanisms. However, there has been a long-standing interest in specific inhibitors of the enzyme which work at much lower concentrations than typical concentrations of S(IV) added to food. Hydroxycinnamic and benzoic acids,[93] kojic acid (5-hydroxy-2-(hydroxymethyl)-γ-pyrone),[94] 4-hexylresorcinol,[95] ficin,[96] C_3-C_5 aliphatic primary alcohols,[97] and even honey[98] are examples of potentially useful inhibitors of varying degrees of effectiveness. Of course, where applicable, a very simple solution to prevent enzymic browning when foods are stored is the action of heat (e.g., by blanching), since polyphenol oxidase is relatively heat labile. When foods are treated in this way, only nonenzymic browning is said to occur.

The search for alternative antibrowning agents against nonenzymic browning has proven to be particularly difficult. It has long been known[99] that thiol compounds inhibit the Maillard reaction in a way similar to S(IV), but the practical use of such additives was not advocated seriously until Friedman conducted a series of studies involving N-acetyl cysteine and glutathione.[100-102] It is believed that thiols react with intermediates such as DH in much the same way as sulfite ion[103,104] because the thiol group is similarly nucleophilic. Studies in the author's group suggest that N-acetyl cysteine is not sufficiently stable in acid solution and is converted slowly to cysteine which reacts with Maillard intermediates to form characteristic "meaty" odors. On the other hand, dipeptides of cysteine (N-substituted cysteine) with another amino acid are an excellent choice.[105] Figure 8.9 shows a comparison of the rates of browning of a glucose-glycine mixture in the presence of S(IV), mercaptoethanol, the dipeptides, and glutathione. These results indicate that the dipeptides are generally as effective as S(IV) except that S(IV) is better at controlling the formation of low levels of color in the early stages of the reaction. Thiols are seen to be highly reactive towards cabbage on blanching and during dehydration,[106] but unpublished evidence suggests that the mixture of products is much more complicated than that obtained when S(IV) is used as the antibrowning agent. There is as yet no evidence regarding the nature of the products formed when thiols inhibit such browning reactions.

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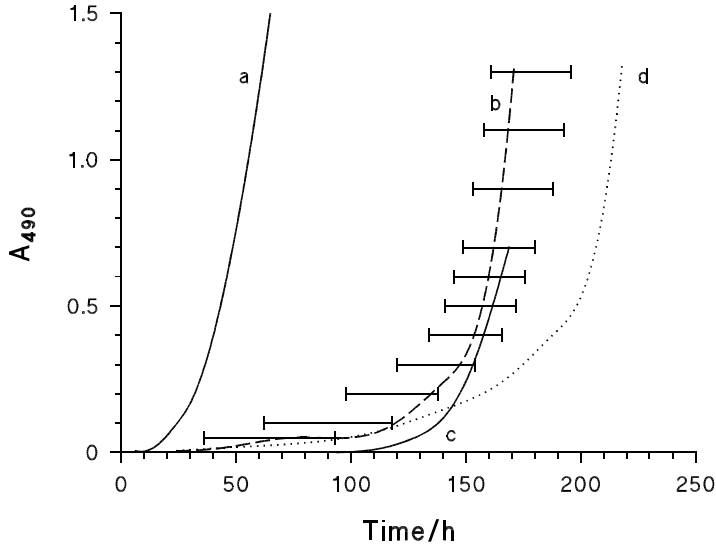

FIGURE 8.9 Absorbance-time curves for the browning of glucose+glycine in buffered solutions (a) in the absence of inhibitor and in the presence of (b) mercaptoethanol, (c) S(IV), and (d) glutathione and the dipeptides. The curves for reactions in the presence of gly-cys, leu-cys, val-cys, aba-cys, and glu-cys are within the limits of the horizontal bars. Reaction conditions: 0.5 mol l^{-1} acetate buffer, pH 5.5, 55°C, [thiol] or [S(IV)] = 20 mmol l^{-1}, [glucose] = 1 mol l^{-1}, [glycine] = 0.5 mol l^{-1}. Reproduced from Edwards, A. S., Wedzicha, B. L., and Wedzicha, B. L., *Food Chem.*, 51, 389, 1994. © 1994 Elsevier Science Ltd. With permission.

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Section III

Biochemical Factors

9 The Effect of Oxidative Enzymes in Foods

David S. Robinson

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INTRODUCTION

Occurrence in Foods

Oxidative enzymes through the action of oxygen are of increasing interest to the food scientist due to their effect on both the color and flavor of plant foods. Polyphenol oxidases are responsible for enzymic browning often coupled to the loss of vitamin C in a wide range of vegetables, fruits, and juices. Ascorbic acid oxidase also catalyzes the oxidation of ascorbic acid to dehydroascorbic acid which can then degrade further to non-enzymic browning products. The effects of peroxidases, although visually less obvious, may be equally damaging to food quality as this group

of enzymes in many instances is thermostable and generates free radicals. Nevertheless, both polyphenol oxidases and peroxidases are extensively and beneficially involved during the manufacture of tea, coffee, and cocoa. Another group of enzymes, the lipoxygenases, catalyze the oxidation of polyunsaturated fatty acids to produce hydroperoxides which can be broken down by other enzymes to form desirable and characteristic aroma compounds. Alternatively, the latter group of enzymes may also be responsible for off-flavors, especially in seed products. A large number of compounds contribute to the aroma of fruits and vegetables and are mainly produced when the cellular structure of the foodstuff is damaged during preparation in the kitchen or during processing. Hexanal is believed to be one of the major compounds responsible for off-flavor in soybeans. It is also possible that lipoxygenases might have a role in the formation of oxidative rancidity of animal food products. Likewise, lipoxygenase may also catalyze the cooxidation of carotenoids including β-carotene resulting both in the loss of essential nutrients and the development of off-flavors.

Oxygen Reactivity

Unlike the numerous enzymes involved in metabolism, the oxidative enzymes that are of prime importance to the food scientist are those able to incorporate either both or one atom of molecular oxygen into susceptible substrates. Molecular or triplet O_2 has two unpaired electrons, each located in a different outer orbital. These two electrons have the parallel spins, so if O_2 attempts to oxidize another atom or molecule by accepting a pair of electrons from it, both of the new electrons must be of anti-parallel spin so as to fit the vacant spaces in the orbitals. Thus, a spin restriction is an impedance on oxidation, which tends to make O_2 accept electrons one at a time in order to allow time for spin inversion. Thus, O_2 is poorly reactive towards non-radical species, and thus permits the existence of many organic substances in aerobic environments. Transition metals are found at the active sites of many oxidases and oxygenases, including polyphenol oxidases, peroxidases, lipoxygenases, and ascorbic acid oxidase; their ability to accept and donate single electrons overcomes the spin restriction. In common with other enzymes, oxygenases also exist as families of isoenzymes with small structural variations and slightly different catalytic properties. Enzyme systems may initiate free radical mediated oxidation in foods, resulting in changes in the sensory quality of the food; for example, peroxidases, lipoxygenases, and microsomal enzymes may be involved not only in lipid peroxidation but more so in the generation of free radicals capable of reacting with a wide range of other substances. However, within fresh foods, indigenous antioxidants may inactivate free radicals, although our current knowledge of free radical mediated biochemical changes within foods and their inhibition is incomplete.[1] The potential to preserve quality by limiting the manifestations of free radical activity during storage and processing has not been fully exploited by the food industry.

Effects on Shelf Life

Unprocessed foods are biological systems often composed of aerobically respiring cells. The dissociation of organelles and the breakdown of cellular structure during

harvesting and storage, or during homogenization, emulsification, or dispersion of food ingredients must initiate many biochemical reactions, whereby especially those reactions involving transfer of single electrons may be uncoupled resulting in the release of free radicals. In aerobically respiring living cells, most of the oxygen absorbed is catalytically reduced to water by cytochrome c oxidases or blue copper oxidases in the respiration cycle, but up to 5% of the total O_2 may be only univalently reduced to $O_2^{\cdot-}$.[2] In fresh postharvest foods and certainly in those stored and processed, the tight compartmentalized control of active oxygen species, including superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) is gradually lost. Thus, under these conditions free radical intermediates including active oxygen species may accumulate. In complex food systems it has not yet proved possible to always define in precise chemical terms how such released free radicals react with the very large number of compounds present in food materials, although it is now becoming increasingly accepted that free radicals damage the main quality attributes — texture, flavor, and color. Some enzymes like peroxidases and lipoxygenases are often able to initiate oxidation directly through the involvement of free radicals. Some of these enzymes can also withstand thermal processing and therefore in processed foods, where other protective enzymes have been denatured, they may still be sufficiently active to initiate changes in quality during long-term storage. Plant cultivars have been identified that lack a particular isoenzyme and, for example, plants grown from seeds lacking two lipoxygenase isoenzymes showed no obvious deleterious effects.[3] The literature abounds with studies on oxygenases in a wide range of fruits and vegetables including potatoes, avocados, olives, bananas, mangoes, tea, coffee, cocoa, and some exotic species. However, there is a need for detailed studies at the molecular level to determine both the precise reasons for the occurrence of such a wide range of isooxygenases within a given plant species and the relative mode of action of the isoenzymes on various natural substrates. During fruit development and ripening, the activity of many enzymes including peroxidases, polyphenol oxidases, and lipoxygenases, as well as the concentration of their substrates, changes dramatically. Peroxidases may play important roles in the color changes associated with the ripening of fruits and the senescence of fruits and vegetables. For instance, it has been claimed[4,5] that peroxidase activity and concentrations of hydrogen peroxide increase during the senescence and ripening of fruits. Abeles et al.[6] reported studies that implicate the biosynthesis of a 33-kDa peroxidase during ethene-induced senescence in cucumber cotyledons. As fruits age enzymes also become more soluble and activation can occur. During senescence colored compounds are formed, which are not the direct result of the primary oxidation reaction. The formation of such colored compounds, often brown or black, is most obvious in products and spoiled foodstuffs and is initiated by cellular damage and release of previously bound enzymes.

LIPOXYGENASES

Occurrence

Lipoxygenase (EC 1.13.11.12, linoleate: oxygen oxidoreductase) is an iron-containing dioxygenase which catalyzes the oxidation of polyunsaturated fatty acids containing

cis,cis-1,4-pentadiene units to first produce free radicals and subsequently after oxygenation conjugated unsaturated hydroperoxy acids. The occurrence and the mode of action of lipoxygenases have been reviewed recently.[1,7-9]

Lipoxygenases have been found in plants, animal tissues including marine products, and more recently in mushrooms and other fungi. In plants the enzyme has been found in various organs and a comprehensive list of plant sources where lipoxygenase has been identified has been compiled.[8] Lipoxygenase activity has been reported to be higher in leaves used for making high quality black tea than in those used for lower quality products. It is not known whether lipoxygenase is essential for the survival of plants, although in animals lipoxygenases and similar oxidizing enzymes such as cyclooxygenases give rise to important physiologically active compounds. It is not known whether a lipoxygenase-free plant or seed will be viable, although the technology to develop such products is available.

Oxidation of Polyunsaturated Fatty Acids

A very large number of different types of compounds, volatile and non-volatile, contribute to the aroma of fruit and vegetables. Some aroma compounds are present in the intact product, being produced during the normal metabolism of the plant. However, many of the volatile aroma compounds are produced only when the raw fruit or vegetable is subjected to chewing, cutting, or processing which causes mixing of enzymes and substrates that are normally compartmentalized. Lipoxygenases are of interest to the food scientist both due to their role in the genesis of flavor and aroma compounds in plant products and their ability to form free radicals that can attack other constituents such as vitamins, colors, phenolics, and proteins. Lipoxygenase-produced aroma compounds are desirable in many foods but may also give rise to off-flavors, particularly in soybean products. Fujimaki et al.[10] showed that hexanal is primarily responsible for the green-bean-like flavor of defatted soy-flour owing to its extremely low flavor threshold. Lipoxygenase-2 (LOX-2) is believed to be the isoenzyme mainly responsible for the generation of n-hexanal in soybeans.[11] Enzyme systems degrading linoleic acid and linolenic acid to hexanal and cis-3-hexenal, respectively, plus isomerization of the latter to trans-2-hexenal, have been reported in tea leaf chloroplasts.[12,13] Off-flavors in sweet corn have been attributed to lipoxygenase activity and it has been claimed that the enzyme is responsible for off-flavor development in cottonseed oil.[14] Furthermore, addition of purified lipoxygenase to blanched peas produced similar off-flavors.[15] Although heat treatment can be used to inactivate lipoxygenase activity, the functional properties of other protein constituents may be affected. In unblanched stored vegetables, lipoxygenases might also be responsible for the bleaching of chlorophyll. However, during pasta manufacture, the bleaching action on carotenoids can be beneficial.

Different lipoxygenases from various plant and animal species oxidize polyunsaturated fatty acids stereospecifically. The insertion of molecular oxygen is chiral and positionally specific.[9] Following oxidation of linoleic acid by many plant lipoxygenases, the hydroperoxide group may be located at carbon-9 or carbon-13, depending on the type of lipoxygenase. The hydroperoxides are then degraded further by

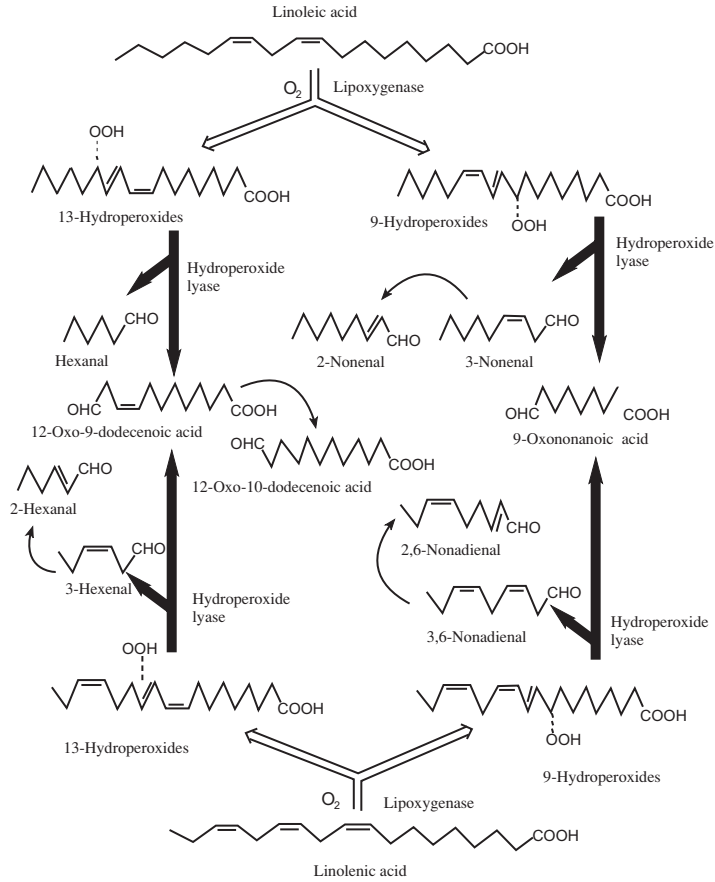

FIGURE 9.1 Possible products of the oxidation of linoleic acid catalyzed by lipoxygenase.

the action of lyases to produce the C-9 and C-6 characteristic aroma compounds, including several aldehydes, ketones, and alcohols, many of which have low flavor thresholds (Figure 9.1).[16]

Also it is now being increasingly realized that there are other mechanisms by which hydroperoxides can be converted into more stable products. Cleavage of the hydroperoxide O–O bond has been observed with metalloporphyrins[17] and heterolytic cleavage takes place during the reaction of cytochrome P-450 enzymes.[18] Wilcox and Marnett[19] have shown in the presence of porphyrins that cleavage of the O–O bond can give rise to both heterolytic and homolytic products which include the corresponding alcohols, aldehydes, and ketones. More recently, another type of enzyme, a peroxygenase, has been described which uses unsaturated acyl hydroperoxides as an oxidant that catalyzes the reduction of hydroperoxides by a heterolytic mechanism leading to a ferryl-oxo complex analogous to peroxidase compound I. Soybean peroxygenase is a ferrihemoprotein and has been shown to catalyze both

the reduction of 13(S)-hydroperoxyoctadeca-9(Z),11(E)-dienoic acid, (13-HPOT) to 13(S)-hydroxyoctadeca-9(Z),11(E)-dienoic acid and the formation of 9,10-epoxy-13(S)-hydroxyoctadec-11(E)-enoic acid.[20] A cytochrome P-450 enzyme[21] has also been described which dehydrates 13-HPOT to form an allene-oxide as a precursor of ketones. These enzymes, like the hydroperoxide lyases, illustrate a co-operative action with lipoxygenases to form further products from polyunsaturated fatty acids.

For foods like bananas the spectrum of volatile compounds depends on the stage of ripeness of the fruit. Green bananas contained *trans*-2-nonenal, *trans*-2,*cis*-6-nonadienal and 9-oxonanoic acid. Hultin and Proctor[22] identified 2-hexenal as the main carbonyl compound in banana volatiles. *Trans*-2-hexenal is also present in banana volatiles.[23,24] Stored bananas treated with ethylene have been shown to contain hexanal, *trans*-2-hexenal, and 12-oxo-*trans*-dodecenoic acid.[25] In cucumber both C-9 and C-6 volatiles are present and are believed to be formed from linoleic and linolenic acids.[26] The most important volatile compound in cucumber is believed to be *trans*-2, *cis*-nonadienal.[27] The volatiles are formed during peeling, cutting, and chewing, which cause disruption of the tissues. Cucumber peel has been claimed to contain twice as much lipoxygenase activity as the fleshy tissue[28] and a cucumber hydroperoxide lyase has been isolated.[29] Nona-*cis,cis*-3,6-dienal and nona-*cis*-6-enal have been claimed to be important aroma compounds in melons.[30,31] Three types of enzymes (lipoxygenases, lyases, and isomerases) are thought to be involved in their formation.[32–34]

Mode of Action

Soybean seed lipoxygenase "type I" (LOX-1)[8,35] is the best characterized enzyme. "Type I" enzymes have an optimum activity at approximately pH 9, whereas "type II"(LOX-2 and LOX-3) enzymes, which include soybean seed LOX-2 and LOX-3, are most active between pH 6.5 and 7. It has been reported that LOX-3 is the most abundant isoenzyme in mature soybeans on a protein basis; LOX-1 is almost as abundant, with LOX-2 the least abundant. However, LOX-2 has the highest specific activity, so that, on the basis of enzymic activity, similar amounts may possibly be present in soybeans.[36] All of the soybean lipoxygenase isoenzymes are monomeric, have a molecular weight of the order of 100,000 and contain one atom of iron per mole of protein. The amino acid sequences of lipoxygenases, predicted from DNA sequences, from different plants have been compared and assessed in relation to enzyme specificity and the three-dimensional structure of soybean LOX-1.[9] The substrate should contain a cis-cis-pentadiene moiety with an activated methylene group at w-8 located between the double bonds. It is suggested that the native enzyme exists as E-Fe^{II} and that activation is by either a small amount of hydroperoxide naturally occurring in lipid substrates or by nanamole quantities of hydrogen peroxide to generate the E-Fe^{III} form. The iron in oxidation state III[37] is believed to initiate the reaction[8,38,39] and abstract stereospecifically an electron from the activated methylene group of the substrate to form an enzyme-linked pentadienyl resonance stabilized radical with the Fe atom then reduced to oxidation state II. Addition of oxygen to pentadienyl radicals results in the formation of chiral and regio-specific

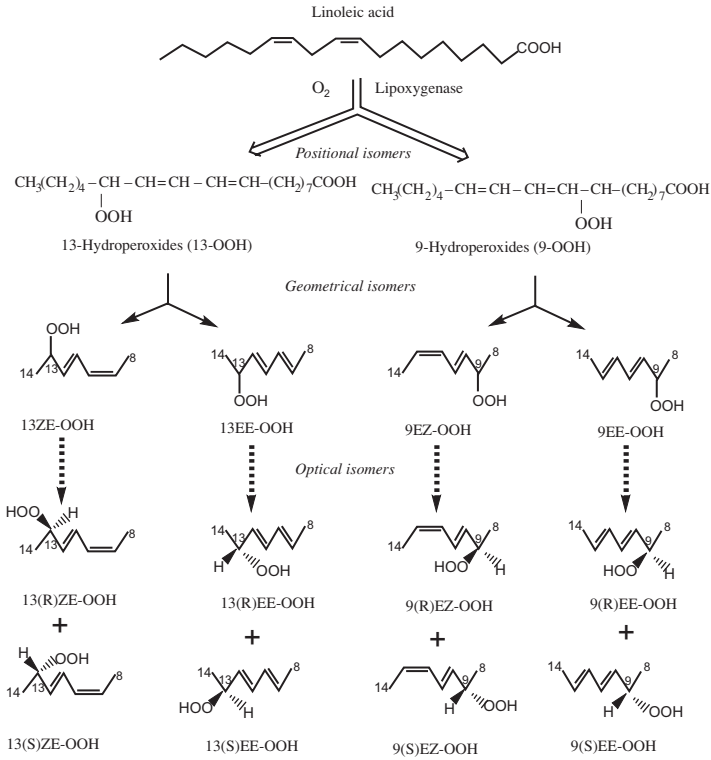

FIGURE 9.2 Positional, geometrical, and optical isomers of peroxy linoleic acid.

hydroperoxyl isomers. Theoretically such isomers are possible from each cis-1,4-pentadiene configuration (Figure 9.2).

In an "anaerobic" environment due to shortage of oxygen, the E-Fe^{II}-pentadienyl radical complex is believed to dissociate to a free pentadienyl radical which may then polymerize or propagate the formation of free radicals from other substrates. However, in the anaerobic pathway the products might be oxygenated by interaction of the pentadienyl radical with small amounts of residual hydroperoxyl or lipoxyl radicals.

Soybean LOX-I forms the 13(S)-hydroperoxy 9(Z), 11(E)-octadecadienoic acid from linoleic acid and the 13(S)-hydroperoxy-9(Z),11(E),15(Z)-octadecatrienoic acid from linolenic acid. The C-20 tetraenoic acid (arachidonic acid) found in animal tissues is surprisingly oxidized to the respective 15-hydroperoxy-5-cis, 8-cis, 11-cis, 13-trans-eicosatetraenoic acid.[40] Furthermore, a range of secondary products including dihydroperoxides arising from dioxygenation at a second site and other compounds normally considered to be of mammalian origin (leucotrienes and lipoxins) also arise from the oxidation of arachidonic acid catalyzed by soybean and potato lipoxygenase.[41] For the type II enzymes it has been claimed for soybean LOX-II that the 9R-hydroperoxide is formed, while for maize germ LOX has been claimed

to remove a hydrogen radical from the pro-R position of the w-8 carbon coupled to insertion of oxygen from the opposite side of the fatty acid chain to form the 9S-hydroperoxide.[41,42] Ketodienoic acids absorbing at 270 to 280 nm have been detected and identified as another group of major reaction products from the incubation of linoleic acid or arachidonic acid with pure pea LOX-1[43] and of linoleic acid with pea LOX-2 and LOX-3.[44] It has been claimed that the three lipoxygenase isozymes in soybeans have slightly different substrate/product specificities. LOX-I has been claimed to be more active on linoleic acid, while LOX-2 was more active on arachidonic acid than on linoleic acid and LOX-2 and -3 were somewhat more active on methyl linoleate than on linoleic acid.[45,46] Furthermore, soybean LOX-I has been said to react with the water-soluble linoleyl sulfate, while types-2 and 3 show only limited activity on this substrate.[5]

Co-Oxidation

Lipoxygenases can be used to bleach carotenoids through a co-oxidation reaction in wheat flour during bread making. The co-oxidative activity of lipoxygenases may be source-dependent; lipoxygenases in peas and beans have been claimed to have a high co-oxidation activity.[47] For soybean and peas the "type 2" isoenzymes which have optimum activity at pH 6 to 7, as opposed to the "type I" isoenzymes with an optimum at pH 9, have been claimed to be more effective at co-oxidation.[48] Recently a high co-oxidation activity was reported for one of two chickpea "type 2" isoenzymes[49] and oxidation by LOX in nonconventional media has been reported.[50] Purified tomato lipoxygenase has been reported to oxidize β-carotene faster than α-carotene and lutein, while lycopene the main tomato pigment remained unaffected.[51] Given the choice of natural substrates available and the occurrence of different isoenzymes types in any given source, it is not surprising that there have been a number of reports in the literature relating loss of carotenoids to lipoxygenase activity.

Lipoxygenases are known to catalyze the oxidation of carotenoids and chlorophyll by a free radical mechanism but still require the presence of a polyunsaturated fatty acid. It is possible that the enzyme is an integral part of the system for co-oxidation of carotenoids through the involvement of an enzyme pentadienyl radical-complex.[52–54] The co-oxidation reaction may arise from abstraction of a hydrogen atom from a carotenoid resulting in the formation of a resonance stabilized radical able to combine with oxygen to produce carbonyl compounds.[52] Further products may arise either by decomposition of the radicals or condensation from dimers or higher polymers. However, little is known of the chemistry of the degradation products.[51] One potential mechanism involves the leakage of a peroxyl radical from the enzyme which can then attack the carotenoid, presumably at positions adjacent to double bonds. A second mechanism is that an enzyme-bound hydroperoxide is the oxidizing species. A third mechanism which may operate could be the generation of free radicals in reactions catalyzed by anaerobic cycling of lipoxygenase. Whatever the primary mechanism, the net effect generates carotenoid moieties containing a free radical center which can then react with oxygen to cleave an adjacent double

bond to give two carbonyl fragments. Such processes, if continued, may lead to the formation of odorous molecules causing off-flavor and loss of color. Some work with tomato LOX has indicated that cleavage can occur at a number of the double bonds in lycopene and that 6-methyl 2-heptanone may be one of the products. For the apparent associated co-oxidation of other substances, including thiol groups and inhibitors, it is possible that free radicals are first dissociated from the enzyme. Concentrations of β-carotene higher than 14 μM inhibited oxidation by chickpea lipoxygenases,[49] which may be due to the formation of an irreversible enzyme — β-carotene complex.[55] Endogenous inhibitors of lipoxygenase in plant sources include chlorophyll, α-tocopherol, and phenolic compounds. These substances could act as scavengers for released, or possibly enzyme-bound radicals, and it has been suggested that the mechanism for bleaching of chlorophyll differs from that for carotenoids.[54]

Manipulation of Factors to Enhance Shelf Life

The first and principal approach to the reduction of suspected enzymic off-flavor development in foods is always inactivation or inhibition of the suspected offending enzymes. The alternative approaches that are becoming increasingly feasible are the development of cultivars deficient in the undesirable isoenzymes. As many fruits and vegetables contain different types of lipoxygenases, and a range of lyases and isomerases capable of further degrading hydroperoxides, the manipulation of flavor volatiles in foods should be possible. Aromas derived from polyunsaturated fatty acids arise from the concerted action of this group of enzymes. For lipoxygenases there are a number of examples to be found in the literature where lipoxygenases have been found to be absent. In some cases, as for soy flour and soy milk, a lack of LOX-2 has resulted in less rancid products,[56] whereas, for soybean varieties lacking LOX-1, no significant changes in the flavor or stability of the soybean oil have been found.[57]

Homogenates prepared from LOX-1-deficient, LOX-3-deficient, and LOX-1 plus -3-deficient soybeans generated significant amounts of n-hexanal, whereas only the LOX-2-deficient line had reduced amounts of hexanal and removal of LOX-3 increased n-hexanal amounts.[58,59] Lines lacking all three isoforms, although not in isogenic backgrounds, still had 70% of the normal amounts of n-hexanal,[59,60] indicating that there are other pathways to hexanal. However, for soybean lipoxygenase LOX-2 Hildebrand et al.[58] have claimed this isoenzyme to be more effective for hexanal formation, although these authors also claimed that LOX-1 was involved. Soybean varieties lacking some or all of the lipoxygenase isoenzymes should theoretically be less susceptible to lipoxygenase-mediated oxidation and the production of undesirable off-flavors. Soybean LOX-1 and -3 are inherited independently of each other[61] and soybean LOX-2 is closely linked to the LOX-1 gene.[62] Naturally occurring soybean varieties lacking more than one isoenzyme have not been identified.[63] However, using appropriate crosses, near-isogenic soybean seeds have been developed that lack either isoenzymes-1 and -3 or isoenzymes-2 and -3. Plants grown from seeds lacking two lipoxygenase isoenzymes have shown no obvious deleterious

effects when grown under glasshouse conditions,[3] and the agronomic performance of mutant plants in the field is unaffected by the absence of specific seed lipoxygenase isoforms.[64] Although lipoxygenases may be and seem to be dispensable in seeds, a potato variety lacking a specific lipoxygenase isoform has been described,[65] lipoxygenases are likely to be important in other (non-storage) plant organs. Indeed it is believed that lipoxygenases are part of the biosynthetic pathways for the plant growth regulators abscisic acid[66,67] and methyl jasmonate.[68] Both of these substances play significant roles in responses to environmental stress, including drought, wounding, and pest/pathogen attack, and in animals lipoxygenases and other similar oxidizing enzymes such as cyclooxygenases also give rise to physiologically active compounds.

PEROXIDASES

Free Radical Generators

Peroxidases are enzymes whose primary function is the oxidation of phenolic moieties at the expense of hydrogen peroxide. All the various isoperoxidases present in plant foods and also animal products catalyze the overall reaction:

$$2AH + H_2O_2 \text{ ---------- } 2A^{\cdot} + 2H_2O$$

A very significant feature is the formation of $A^{\cdot}$ as free radicals, as these are able to react with a large number of susceptible compounds including phenolics, vitamins, and many other substances from which an electron or hydrogen radical can easily be abstracted to form other radicals. Phenolic compounds represent one of the most prominent classes of natural products in plants. They are very reactive and easily subject to oxidation, substitution, and coupling reactions. Hydrogen abstraction readily occurs from phenolic substrates because of the resonance stablization of the resultant phenoxyl radicals. These separate cationic radicals are then able to react non-enzymically with a wider range of other susceptible substrates to generate other radicals and promote a large number of separate reactions. In this way peroxidases have a propensity for initiating the oxidation of a wide range of compounds generally not exhibited by other enzymes. It is this propensity to stimulate the oxidation of a large number of sometimes ill-defined substrates which makes it difficult to ascribe precisely the action of peroxidases, not only in foods but also in living plants. Because of the ease of hydrogen abstraction, many of the natural susceptible substances must include phenolics, like coumaric and caffeic acids and anthocyanins. In the same way flavins, pyridinium compounds, and other aromatic compounds are likely to be oxidized by the peroxidase-generated free radicals. Peroxidases also have an important role during the formation of lignin. Asparagus undergoes lignification and hence increases in toughness during storage. Smith and Stanley[69] have suggested that a free radical mechanism may be involved that may be promoted by peroxidases. Tracer experiments have shown that lignin is synthesized from phenylalanine or tyrosine via cinnamic acids and the corresponding hydroxycinnamyl alcohols. These compounds are directly dehydrogenated by peroxidase with hydrogen

peroxide to produce phenoxy radicals. Vitamin C is oxidized by peroxidases generally and sometimes by a specific ascorbic acid peroxidase to produce resonance stabilized ascorbate radicals that can disproportionate to dehydroascorbate which then can degrade chemically to form non-enzymic browning products. Nevertheless in foods the amount of hydrogen peroxide present is generally small and therefore the rates of oxidation are likely to be low, but yet become apparent during long-term storage at ambient temperatures. When test substrates, such as guaicol and o-dianisidine, are used the generated radicals are present at high concentrations and are more likely to combine to form dimers or higher polymers.

Peroxidase Activity

Fundamental investigations began in 1920 with the work of Onslow[70] who observed that the activity of oxidizing enzymes varied with the ripeness of fruit. In 1926 Willimott and Wokes[71] found that peroxidase was present in both the flavedo and albedo of citrus fruits. During the 1930s, Keilin and co-workers[72] observed the importance of the hem component. With advances in spectral techniques and protein purification methods in 1951, Chance[73] discovered the oxidized peroxidase derivatives, compound I and compound II. These are important intermediates formed between peroxidase and the oxidant H_2O_2 in peroxidase-catalyzed reactions.[74] Peroxidase receives two oxidation equivalents from a hydroperoxide and shows very broad specificity for aromatic hydrogen donors which are subsequently released as free radicals. Hydrogen peroxide is generally, but not necessarily, the oxidant that causes the formation of the activated compound I from the native peroxidase enzyme, which in turn oxidizes the electron donor substrate, AH, by abstraction of a single electron to form a free radical and compound II. Both compounds I and II where the hem Fe exists in the higher oxidation states, 5 and 4, respectively, are able to accept electrons from a wide range of substrates and especially phenolics.

$$\underset{\text{compound I}}{\text{Per-Fe}^{V} \text{=== O}} + e^- + H^+ \rightleftharpoons \underset{\text{compound II}}{\text{Per-Fe}^{IV} \text{--- OH}}$$

$$\text{Per-Fe}^{IV} \text{ - - - OH} + e^- + H^+ \rightleftharpoons \text{Per-Fe}^{III} \text{ -- } H_2O$$

Therefore, peroxidases have a potential for a wide range of biological functions such as initiating polymerization, depolymerization, and the removal of hydroperoxides. Furthermore, the presence of several isoenzymes, some of which are thermostable, also makes it difficult to define the function of peroxidases in foods and indeed also in living cells.

Effects on Shelf Life

The relationship of peroxidase activity to off-flavors and off-colors in raw and unblanched vegetables has not and furthermore cannot easily be attributed to any particular constituent. Nevertheless, for the reasons given above, peroxidases are

believed to be partly responsible for a more general deterioration in flavor, color, texture, and the loss of some nutritional qualities in raw and processed foods[75,76] through free radical initiated reactions. Indeed a direct relationship between peroxidase activity and off-flavor development in peas was claimed in 1958 by Wagenknecht and Lee[77] and later by Pinsent.[78] Active peroxidase is believed to spoil fruits and vegetables at temperatures as low as –18°C and at low moisture levels the development of off-flavors is often associated with the oxidation of indigenous lipids and the phenolic constituents of foods.[79] It has been suggested that a significant negative correlation between peroxidase activity and the flavor scores of high- and low-yield orange juices indicates that it might be possible to use peroxidase activity as an index of potential adverse flavor.[80] For string beans, the qualitative peroxidase test was found to be the best index of adequate blanching.[81] Wagenknecht and Lee[77] found that off-flavors developed when horseradish peroxidase was added to pea slurries and Zoueil and Esselen[82] also showed that the addition of horseradish peroxidase to sterilized green bean puree caused an increase in the amount of acetaldehyde in stored samples. Similarly, earlier work in 1936 by Arighi et al.[83] and later in1949 by Joslyn[84] established the accumulation of volatile aldehydes in the raw and unblanched peas. In 1952 Joslyn and David[85] claimed that peas containing more than 10 ppm of volatile aldehydes were objectionable.

Thermostability

High correlations between off-flavor development in frozen vegetables during storage and the residual peroxidase activity[86–88] have shown peroxidase activity to be useful as an index for the degree of blanching. Effective blanching of some vegetables is particularly difficult, not only because of the presence of thermostable enzymes, but also because of either their large size, shape, or density. For instance, for Brussels sprouts, due to their compact structure, inactivation of enzymes throughout the sprout may be particularly difficult to achieve.[89] Similarly, whole corn-on-the-cob is one of the most difficult vegetables to blanch effectively because of its large size and the structural characteristics of its different parts.[90] Lee and Hammes[91] determined significant correlations between residual peroxidase activity in the outer cob and kernels blanched at 100°C and the off-flavor development detected by a panel after 9 months storage at –18°C. The thermostability of peroxidase activity has been investigated in other vegetables, e.g., turnips[92] and cabbages[93–95] and in fruits, e.g., apples,[96,97] pears,[98] grapes,[99] and citrus products[100] and mango.[101] The flavor of mango is very delicate and is easily affected by thermal processing and mango is marketed in the form of juice, pulp, or slices canned in syrup. The thermal processes followed at present for these canned products are mainly empirical.[102] Studies on the thermal inactivation of peroxidases have generally shown that plots of residual enzyme activity against heating time are non-linear indicating deviation from first order kinetics. The most obvious explanation for non-linearity is the presence in the whole foods and crude extracts of a mixture of thermostable and heat-labile isoperoxidases.[103-109] However, while it seems very likely that some of the deviation is probably accounted for by the presence of

isoperoxidases with different susceptibilities to heat, this has not yet been clearly established as the main cause of the deviation from linearity.

Regeneration of enzymic activity after heat denaturation is unusual for enzymes generally, although it is a well-recognized property of peroxidases. The ability of peroxidases to regenerate after heat denaturation varies not only between different plant species but also between the isoenzymes that occur within a single variety. Restoration of peroxidase activity is generally observed over a period of a few hours after heat treatment of either test solutions of enzyme or whole vegetables. For a purified apple isoperoxidase, A2, up to 80% of the original activity was restored when the heat-treated isoenzyme was held at 30°C.[100] Cationic isoperoxidases in crude extracts from *Brassica* spp. and apples[89,96,100] have been shown to be incapable of regeneration, which implies that the molecular structure of the protein may primarily determine this property. Naveh et al.[109] elucidated an interesting linear relationship between residual enzymic activity immediately after heat treatment and subsequent regenerated peroxidase activity. A large slope of approximately 3.0 for the graph implied that more drastic heat processing would not only increase inactivation but also markedly decrease the extent of regeneration. Also, the proportionality between increasing thermostability and the enzyme's ability to regenerate supports the general observation that the more thermostable enzymes tend to exhibit a greater ability to regenerate. At the molecular level, the mechanism of regeneration of peroxidases is not fully understood. However, in general terms it is considered that regeneration must require a reversal of denaturation with concomitant changes in the conformational structure of the protein moiety. As it has been shown that added hematin increases the amount of restored enzymic activity,[107] it has been generally accepted that the hem moiety can recombine with the apoprotein to form active enzyme. Tamura and Morita[110] observed that, after heat treatment, cooling for 20 h at room temperature resulted in a recovery in the absorbance at 404 nm which indicates that the native molecule with bound hem can be reassembled under such conditions.

Commercial methods, other than direct conductive heating, such as microwave and gamma-irradiation, can justifiably be expected to affect enzymic activity and has been reviewed by Thomas.[111] Microwave heating causes rapid changes in temperature, not dependent on conduction, and may therefore be particularly useful for heterogeneous foods. Nevertheless, the potential for rapid heating and control that microwave processing offers may not yet have been sufficiently exploited for the blanching of plant foods. Irradiation of pure solutions of horseradish peroxidase using ^{60}Co gamma rays caused a loss of enzymic activity which was highest in the presence of oxygen-saturated water where it is suggested that hydroxyl and peroxy radicals were responsible for the inactivation.[112] It is reasonable to suppose that such inactivation was the result of a secondary attack on peroxidase by free radicals produced from water during the irradiation process. However, the species involved are likely to include free radicals which will have a similar effect to those radicals generated by the oxidative enzymes for which the process is designed to destroy.

The use of peroxidase activity as an indicator of blanching treatment is continuously being appraised in view of the higher temperatures required to inactivate

peroxidases, although it is frequently suggested that such temperatures are not needed for the blanching of vegetables. Nevertheless, Halpin et al.[113] have recently restated that other less stable enzymes are not more appropriate as blanching indicators. Therefore, it still seems likely that peroxidase activity will continue to serve this requirement. In this context it is important to realize that peroxidase catalyzes oxidative reactions through the involvement of free radicals, which by their very nature can initiate and propagate other chain reactions and thus allow a wide range of substrates to be oxidized. For this reason peroxidases should be inactivated in processed foods, even though some of their degradation products containing hem may still initiate chemical reactions, but obviously at much slower rates.

Oxidatic Activity

Peroxidases can also catalyze an oxidatic reaction without the need of hydrogen peroxide. This type of reaction, accompanied by the uptake of oxygen, is most frequently demonstrated by the oxidation of the synthetic substrate dihydroxyfumaric acid (DHF) by compounds I and II,[114] although other naturally occurring oxidatic reactions may involve ascorbic acid, thiols, and hydroquinones. All of these are characterized by the lack of need for added hydrogen peroxide as the oxidizing substrate. Based on the powerful inhibition of the reaction by superoxide dismutase, as opposed to hydroxyl radical scavengers,[114] an oxidative scheme involving the superoxide radical and the continual oxidation of DHF has been widely accepted:

$$\underset{\text{native peroxidase}}{\text{Per-Fe}^{III}} + H_2O_2 \rightleftharpoons \underset{\text{compound I}}{\text{Per-Fe}^{V}}$$

An initial amount of H_2O_2, if not naturally present, which is required for the formation of compound I, is believed to be generated by the non-catalytic autoxidation of DHF

$$2DHF + O_2 \rightleftharpoons 2DHF^{\cdot} + O_2^{2-}$$

Peroxidase then as compounds I or II catalyzes more rapidly the production of greater amounts of $DHF^{\cdot}$

$$\text{Per-Fe}^{V} + DHF \rightleftharpoons DHF^{\cdot} + \underset{\text{compound II}}{\text{PerFe}^{IV}}$$

$$\text{PerFe}^{IV} + DHF \rightleftharpoons DHF^{\cdot} + \underset{\text{native peroxidase}}{\text{PerFe}^{III}}$$

Further reactions result in the formation of diketosuccinate (DKS) by dismutation of $DHF^{\cdot}$

$$2DHF^{\cdot} \rightleftharpoons DHF + DKS$$

As shown in the above, other reactive oxygen species including H_2O_2 and superoxide, $O_2^{\cdot}$ and hence also compound III ($\text{Per-Fe}^{II}O_2^{\cdot}$) can be formed by autoxidation of DHF.

Furthermore, horseradish peroxidase in the presence of reducing equivalents of dihydroxyfumarate and oxygen may catalyze the hydroxylation of a wide range of aromatic compounds including tyrosine, phenylalanine, and other phenolic compounds. For the hydroxylation reaction compound III is said to be formed from $O_2^{\cdot}$, which itself is generated from the reduction of molecular oxygen by dihydroxyfumarate. The hydroxyl radical ($^{\cdot}OH$) is believed to be the hydroxylating species.

In plants and probably animal tissues a similar scheme is believed to operate for the oxidation by compounds I and II for appropriate substrates like NADH, pyridoxal compounds, indole-3-acetic acid,[115] and possibly ascorbic acid. In NADH oxidation, as for oxidation of DHF, peroxidases act according to a peroxidatic cycle to produce NADH radicals:

$$\text{compound I} + \text{NADH} \rightleftharpoons \text{NADH}^{\cdot} + \text{compound II}$$

$$\text{compound II} + \text{NADH} \rightleftharpoons \text{NADH}^{\cdot} + \text{native peroxidase}$$

The propensity of peroxidases to react with a wide range of compounds is also illustrated by the peroxidase-catalyzed oxidation of malonaldehyde (MA) a well-known product derived from autoxidation of fats. It is suggested that malonaldehyde radicals are generated by reaction of malonaldehyde with compounds I and II.

$$\text{Per-Fe}^{V} = O + MA \rightleftharpoons \text{Per-Fe}^{IV} - OH + MA^{\cdot}$$

$$\text{Per-Fe}^{IV} - OH + MA \rightleftharpoons \text{Per-Fe}^{III} + MA^{\cdot}$$

It is proposed that the malonaldehyde radicals react directly with oxygen to form peroxy radicals and then through the involvement of Mn^{II} form malonaldehyde hydroperoxide.

This mechanism proposed by MacDonald and Dunford[116] is similar to that described above for the oxidation of DHF by compounds I and II with overall consumption of oxygen. The above reactions indicate how further oxidation of malonaldehyde might be mediated by peroxidase. Likewise, other carbonyl compounds, such as those present as flavor substances, e.g., hexanal, might also be susceptible to oxidation catalyzed by peroxidase.

POLYPHENOL OXIDASES

Enzymic Browning

The browning reaction is a most obvious detrimental change occurring in many fruits and plant tissues damaged by improper handling resulting in bruising, compression, or indentations. The formation of such colored compounds, often brown or black, is one of the reasons for the great interest in polyphenol oxidases in food products. However, whole tissues and extracts may darken due to a variety of reactions, not all of which are enzymic. For instance, browning during the drying of fruits such as apricots or in heat processed products may be due to Maillard

chemistry. Nevertheless, it is generally accepted that much of the browning in fresh fruits is caused by the enzymic action of *o*-diphenol oxidoreductases now classified as O_2: *o*-diphenol oxidoreductase (EC.1.10.3.1). Apples are one of the most common fruits worldwide and hence there is considerable interest in apple polyphenol oxidases as a cause of excessive enzymic browning in juices, purees, and dried powder. However, for the manufacture of cocoa, coffee, and tea, enzymic browning is not only beneficial but is essential. In green coffee beans the main precursors of browning identified by HPLC and UV-absorbtion spectra are the chlorogenic acids, 5-*O*-caffeoylquinic acid (5-CQA) and 3-CQA and 4-CQA.[117] In tea catechin, epicatechin and their gallate esters are oxidized to quinones that then undergo condensation and polymerization to form a range of colored compounds including theaflavins and thearubigens. Undesirable enzymic browning caused by PPO on the surface of foods is of great concern, as the products are less acceptable to consumers. It is also thought that enzymic browning contributes significantly to undesirable color formation in sugar cane juice and that PPO is the main enzyme involved.[118] The enzymes are often referred to as PPO, catechol oxidases, phenolase, tyrosinase, and cresolase. A survey of recent reports on the enzyme in fruits and vegetables and their products adds several new species to an already long list of those previously reported.[119,120] The recent literature abounds with more studies on catechol oxidases in deciduous fruits, grapes and wine, potatoes, avocados, olives, bananas, mangoes, tea, coffee, cocoa, and mushrooms. The enzymes are abundant in tubers, storage roots, and fruits. High levels of enzyme are usually found in tissues that are also rich in phenolic compounds. The levels of PPO and its substrates often change markedly during the life cycle of the plant particularly in fruits and vegetables. Multiple forms of polyphenol oxidase are invariably found in extracts of fresh fruits and vegetables, juices, and concentrates. Although post-translational modifications might be responsible for the formation of multiple forms, the observations described suggest that at least part of the multiplicity encountered may be artifactual. Kiwi fruit[121] and DeChaunac hybrid grapes[122] have been reported to possess from 3 to 14 isoenzymes of various sizes. The multiple forms are easily observed on PAGE and IEF electrophoretograms. Although multiplicity in a hybrid could result from genetic differences between the parent species, it is probably not the only reason for the multiple forms observed. The isolation of PPOs is fraught with problems especially as the quinones react with the enzyme and thus cause inactivation to varying degrees. The membranous location of the enzyme in many cases necessitates the use of harsh extraction procedures. Tanning often seems to affect the multiple forms of the enzyme as well as the apparent molecular weight, specificity for substrates (especially the hydroxylation reaction), enzyme kinetics, and sensitivity to inhibitors. PPOs are often "tanned" proteins to which phenols and their oxidation products are attached. Antibodies prepared against such preparations are likely to give erroneous results if related to the phenolic epitope. The removal of phenolics and prevention of browning has been shown to markedly reduce the number of "multiple forms" of PPO.[123]

Although catechol oxidase is apparently not ubiquitous in plants,[119] there are few fruits and vegetables in which it has not been found. Histochemical methods using 3,4-dihydroxyphenylalanine (DOPA) may be used to locate the cellular distribution of PPO and distinguish them from laccases detected by their oxidation of

syringaldiazine or phenylenediamine. Care must be taken to test for peroxidase activity as this enzyme, arising from its ability to generate free radicals with aromatic compounds, may also oxidize these reagents. PPOs are frequently found in thylakoids in higher plants, whereas in fungi the enzyme seems to be present in the cytosol and not membrane-bound organelles. In higher plants, e.g., potato tubers, sugar beet roots, and bananas,[119] the enzyme is mainly believed to be membrane-bound in non-senescing tissues. However, lack of enzyme activity should not be taken as proof of the absence of catechol oxidase which may be due to the presence of endogenous inhibitors.[119,120,124] Zawistowski et al.[125] consider that cytochemical investigations show that PPO is solely a plastid enzyme localized in a diverse series of organs and tissues. It is generally accepted that the enzyme is not active until released from the organelle when it comes in contact with the polyphenol substrates located in the vacuole. Clearly, although it is universally accepted that the enzyme has a deleterious effect on the quality of fresh fruits and vegetables, it is nevertheless regarded by botanists as part of a natural plant defense system against insects and pathogens. Defense mechanisms in higher plants, attack mechanisms in fungi, pigment formation, or free radical scavenging are all possible candidates for functions of PPOs. Activation of PPO occurs during cellular disruption and therefore is caused by infection and mechanical injury. The enzymic products are primarily quinones which then react with other constituents including amino acids and possibly membrane bound peptides to form higher polymers to provide a barrier against further infection as well as with the infective agents themselves.

Monophenolase and Diphenolase Activity

Enzymic browning is caused by the oxidation of *o*-diphenols by the O_2: *o*-diphenoloxidoreductase activity resulting in the formation of *o*-quinones which may polymerize and also react further with amino acids and peptides to form melanins. Spread through the literature are claims for very high levels of enzyme, but these must be treated with considerable caution as this may be due more to greater amounts of polyphenols or in other cases lower activity might be due to the presence of inhibitors, such as ascorbic acid and other reducing agents. A wide range of phenolic compounds is found in many fruits, all of which are potential substrates for polyphenol oxidases. Recently, Murata et al.[126] have reported that the Japanese apple cultivar "Tsugaru" contained smaller amounts of polyphenols than the browning variety "Matsu" and was due to the oxidation of catechins rather than chlorogenic acids. However, the most common natural substrates are chlorogenic acid, catechin, epicatechin, and 3,4-dihydroxyphenylalanine (DOPA) and in bananas 3,4-dihydroxyphenylethylamine (dopamine). *p*-Coumaroyl and caffeoyl derivatives of tartaric acids have been stated to be oxidized by grape diphenolases, while dates contain a range of caffeoyl-shikimic acids.[127] The flavonoid pigments have been claimed to be poor substrates, but can possibly undergo co-oxidation in the presence of chlorogenic acid,[127] although a mechanism for this reaction, which may involve the release of free radicals as with lipoxygenases catalyzed oxidation of carotenoids, has not been elucidated. All *o*-diphenol oxidases require the *o*-diphenol group. Suitable test substrates are catechol, 4-methyl catechol, and 1,4-dihydroxytoluene. As referred to

above, the *o*-diphenolases are unusual because many also possess a monoxygenase property which results in *ortho* hydroxylation of monophenols such as *p*-cresol, *p*-coumaric acid, and tyrosine. Thus, it seems likely that many of the naturally occurring diphenols, for example, chlorogenic acid and catechin, are formed from the monophenol precursors. Sometimes polyphenol oxidase activity is determined by reference to specific test substrates, e.g., chlorogenic acid oxidase, catechin oxidase, or DOPA oxidase, but this does not necessarily imply the presence of specific isoenzymes for each of the test substrates. Polyphenol oxidases are enzymes now widely accepted as catalyzing two distinct, but related reactions, where the products of the first reaction undergo dehydrogenation by the second enzyme catalyzed reaction to produce quinones that then yield brown and black melanin pigments. Therefore, the polyphenol oxidases are also classified as monophenol monoxygenase, EC 1.14.18.1. However, there is no set ratio of monoxygenase activity to diphenolase activity and the relative activities vary with the plant source.[125]

The typical oxidation of polyphenols catalyzed by catechol oxidase is considered as either a two-step reaction, starting with a monophenol, or a one-step reaction, in which just a dihydroxyphenol is oxidized. The monophenol oxygenase activity may be called cresolase activity and the enzyme for the dehydrogenation reaction has been referred to as catecholase. Polyphenol oxidases are copper enzymes and the oxidation reaction involves changes in valency of the transition metal which acts as a single electron carrier:

$$\text{PPO-2Cu}^{+} + \text{O}_2 + 2\text{H}^{+} + 2\text{e}^{-} = = = = = = = = = \text{PPO-2Cu}^{2+} + \text{H}_2\text{O} \quad (9.1)$$

$$\text{PPO-2Cu}^{2+} + 1/2\ \text{O}_2 = = = = = = = = = \text{PPO-2Cu}^{+} + \text{H}_2\text{O} \quad (9.2)$$

The provision of two electrons to form the Cu(I) state of the enzyme from reaction (9.2) is believed to be coupled to the reduction of oxygen to bring about hydroxylation in reaction (9.1) with the reduction of one atom of oxygen to water. Thus, the reactions are coupled with the recycling of electrons through oxidation states I and II of the transition metal. Reaction (9.2) can be reversed by reduction of the quinones with ascorbic acid or sulfite. The coupled reduction by ascorbic acid will continue in fresh fruits and juices until oxidation to dehydroascorbic is complete, at which stage browning will be observed. In fresh fruits and vegetables, ascorbic acid delays the onset of enzymic browning to varying degrees depending on the amount of ascorbic acid present. Consequently, the natural content of vitamin C, or its deliberate addition, offers a useful commercial means for the control of enzymic browning. Where this is desirable, as in the manufacture of buff-colored apple puree, the oxidation may be allowed to commence before blanching. Quinones are very reactive compounds and may form additional products with peptides and amino acids resulting in a range of colored compounds commonly described as melanins. All polyphenol oxidases are now thought to contain two atoms of Cu per mole which have been suggested to exist in close proximity to each other at approximately 3.5 Å. The oxy form of the enzyme,[125] where two electrons have been passed from the 2Cu(I) to O_2 to form 2Cu(II) peroxide, has been proposed as the active oxidizing species (Figure 9.3).

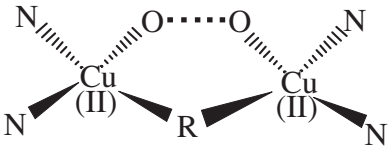

FIGURE 9.3 Polyphenol oxidase peroxy complex. (From Zawistowski, J., Biladeris, C. G., and Eskin, N. A. M., Polyphenol oxidase, in *Oxidative Enzymes in Foods,* Robinson, D.S. and Eskin, N. A. M., Eds., Elsevier Applied Science, London, 1991, Chap. 6. With permission.)

Through such a copper peroxide structure, Zawistowski et al.[125] have been able to account for both the hydroxylation of monophenols and the dehydrogenation of o-diphenols. It has been suggested that the hydroxylation or the dehydrogenation reactions are determined by slightly different modes of binding of the monophenol and the diphenol substrates to the peroxy form of the enzyme. Whether such geometrical structures are universal for all polyphenol oxidases is unknown.

Assays and Inhibitors

For the hydroxylation reaction, tyrosine and *p*-coumaric acid are probably the natural substrates, where tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) and *p*-coumaric acid is oxidized to caffeic acid. Chlorogenic acid (caffeoylquinic acid) is formed by the hydroxylation of *p*-coumaroylquinic acid. A suitable test substrate for the hydroxylation reaction is *p*-cresol. Oxygen uptake measured with an oxygen electrode will measure both the hydroxylation and subsequently dehydrogenation of the diphenol to form the quinone. Diphenolase activity can be assayed using catechol or 4-methyl catechol as substrates. Initial rate measurements are best made with the oxygen electrode, although other enzymes such as lipoxygenases, ascorbic acid oxidase, and possibly peroxidases present in crude extracts may interfere. For comparative measurements for different cultivars, the end product of the enzymic reaction, the *o*-quinone, can be detected by its condensation with amino acids like proline to form readily observed colored products. However, this end reaction is not strictly quantitative, as the enzyme is irreversibly inhibited by its own generated product, the *o*-quinone, naturally occurring reducing agents such as ascorbic acid and thiols. Competitive inhibition by the substrate analogs cinnamic, *p*-coumaric, and ferulic acids is possible.[127] There is a general trend towards avoiding the use of chemical inhibitors, such as sulfite, and therefore an increasing effort is being made to find further natural inhibitors[128] even from fungi and *Lactobacillus* species, some of which have been reported to be low molecular weight peptides.[127] Effective chemical inhibitors, especially for use in the laboratory for purification of PPOs are cyanide, carbon monoxide, polyvinyl pyrrolidone (PVP), 4-hexyl-resorcinol, and salicylhydroxamic acid (SHAM).

The closely related enzyme laccase[129] also contains Cu but may contain two or four atoms per mole. Laccases are distinguished from polyphenol oxidases by their ability to also catalyze the oxidation of *p*-diphenols as well as *o*-diphenols. The enzyme is found in Basidiomycetes and fungi and was first discovered in the sap of

the Japanese lac tree.[127] Although generally not found in higher plants, the presence of laccases has been claimed in peaches and apricots.[127,130,131]

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10 Biotechnology to Improve Shelf Life and Quality Traits of Foods

Angel Gabriel Alpuche-Solís and Octavio Paredes-López

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INTRODUCTION

Consumers require the highest quality for available food products at the fairest price, but their definition of food quality has changed dramatically. First, "freshness" has replaced "price" as their primary food product concern. Second, consumers have identified the absence of certain single food additives/ingredients as determinants of product quality; sodium, cholesterol and saturated fats, artificial food colors and flavorings, preservatives, and caffeine are some of the ingredients that consumers feel should be avoided. Another way consumers evaluate quality in food products is by their shelf life. In general, consumers appear to be satisfied with packaging practices.[1]

Quality and convenience are two main characteristics that consumers today need from their food products and apparently will continue to be the keys to food-product success for many years to come. Consumers will accept any new technology that extends shelf life, if they perceive that the process does not diminish product quality and if a long shelf life does not deteriorate both the quality and convenience of the product.[1] The average consumer today has a higher level of education, more knowledge about nutrition, and more money to spend on food. He defines "high-quality" food as a fresh, safe, and natural product with the capability to prevent health and body weight problems.[1]

Changes in the traditional family structure have affected family eating patterns, food choices, and methods for food preparation. Today's family lifestyle is related to lack of time, often with each family member on a different schedule. That is why snacking and the microwave oven have been so successful; the main reason for snacking and popularity of the microwave oven is the convenience. A family may need several different types of food products: single-serving, convenience products for the two or three weekdays when members eat at different times; family-sized products for those evenings when the entire family comes together; and gourmet foods for special days. All these products would be more convenient and useful to consumers

TABLE 10.1
Optimum Range of Storage Temperature for Fresh Fruits and Vegetables

Optimum Storage Temperature (°C)	Fruits/Vegetables
0–5 (Cold storage)	Apples, apricots, artichokes, asparagus, beets, broccoli, Brussels sprouts, cabbage, cantaloupes, carrots, cauliflower, celery, cherries, collards, corn, dates, figs, grapes, green onions, kiwifruit, lettuce, lima beans, mushrooms, nectarines, oranges, peaches (ripe), pears[a] (ripe), peas, plums, radishes, rhubarb, spinach, strawberries, turnips
5–10 (Cool storage)	Avocados (ripe), blueberries, cranberries, cucumbers, eggplant, melons (ripe), okra, peppers, pineapple (ripe), snap beans, summer squash, tangerines
10–18 (Slightly cool storage)	Bananas, coconuts, grapefruit, limes, lemons, mangoes, melons (unripe), nuts, papayas, pears[a] (unripe), pumpkins, sweet potatoes, tomatoes, winter squash
18–25 (Room temperature storage)	Avocados (unripe), dry onions, nectarines (unripe), peaches (unripe), potatoes, watermelons

[a] The optimum storage temperature for pears is 3–7°C (ripe) and 16–20°C (unripe).

Adapted from Floros.[2]

if they had a longer shelf life. One reason that extended shelf life will become more important in the future is that it will provide consumers with essential convenience.[1]

Temperature is the most important environmental factor in the postharvest life of fruits and vegetables; its effect is dramatic not only on respiration and transpiration rates, but on other biological and biochemical reactions as well.[2] Therefore, it is important to know the best condition of storage temperature for fruits and vegetables in order to extend their shelf life (Table 10.1).[2] Several processing methodologies have been used for the preservation of plant foods. Figure 10.1[2] shows the variation of shelf life of fruits and vegetables after being processed.

Novel technologies are being developed to extend shelf life and to improve the quality of plant foods. Table 10.2[3-19] shows several procedures which have employed plant genetic engineering to provide benefits such as better color, flavor, yield, shelf life, fewer unwanted traits, a special component production, and in general a more attractive plant food to the consumer. This chapter focuses on the traditional and novel technologies used to extend shelf life of foods while paying attention to their quality traits.

FRUITS AND VEGETABLES

General Aspects

Fruits and vegetables, the soft, fleshy, or leafy edible parts of plants, have for centuries improved the human diet by providing nutrients, pleasant flavors, and aromas. Nutritionally, fruits and vegetables contribute considerable amounts of vitamins A, C, B_6, thiamin, niacin, and minerals (i.e., magnesium, iron). In addition,

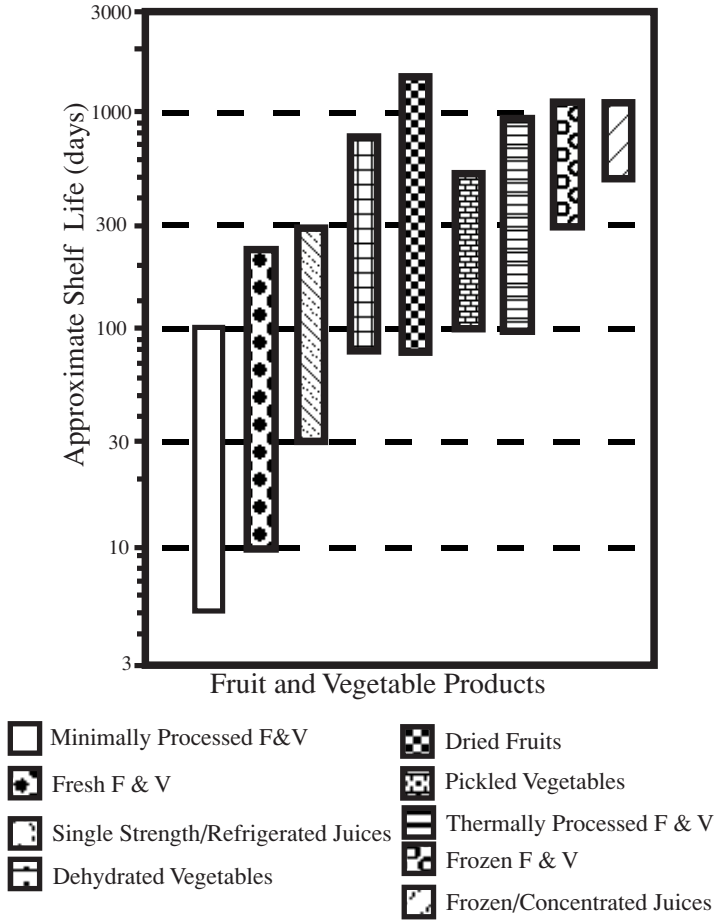


FIGURE 10.1 Approximate shelf life of various fruit and vegetable products. (Adapted from Floros.[2])

they supply proteins, starch, and sugars, and are important sources of dietary fiber. From a sensory viewpoint, fruits and vegetables are valued for their supreme flavor and aroma, crisp texture, attractive colors, and their overall appeal to the human senses of smell, taste, touch, and sight.[2]

Fresh fruits and vegetables are living dynamic systems even after detachment from the parent plant. As living biological entities, they respire and transpire. Respiration is the process of O_2 uptake and oxidation of energy-rich cellular organic substances such as starch, sugars, and organic acids to produce CO_2, H_2O, and energy, whereas transpiration is the process of water loss. Before harvest, when fruits and vegetables are attached to the parent plant, losses due to respiration and transpiration are replaced by water, photosynthates, and minerals. After harvest, when they are removed from the plant, losses of respirable substrates and moisture are not replaced

TABLE 10.2
Biotechnological Procedures Commercially Available to Improve Shelf Life and Quality Traits of Foods

Product	Technology Applied	Benefit	Ref.
Broccoli	Insertion of a non-plant gene to control ethylene production	Slow ripening so the crop stays green longer	3, 4
Canola oil (rapeseed)	A combination of classical breeding and RFLP screening	Composition control of erucic acid	4, 5
	Genetically engineered with 12:0-acyl-carrier protein (ACP) thioesterase from undomesticated California bay	High laurate and stearate composition	6, 7
	Genetically engineered	High content of hydrogenated oil for margarine	6
	Classical breeding, microspore (anther) culture, and RFLP screening	High temperature frying oil, low in saturated fat (high oleic, low palmitic)	6
Canola and other oils	Insertion of gene for stearoyl ACP desaturate	Shift in fat composition toward more unsaturation	8, 9
Chicory	Insertion of antisense gene	Increased availability of fructans which are normally converted to fructose immediately after harvest	10
Coffee	Gene insertion into *Coffea arabica* (proprietary)	Better flavor, better yields, better pest resistance, lower caffeine	8, 11
"Euromelon"	Insertion of antisense gene (pMEL1) which encodes the final enzyme for ethylene synthesis	Extended shelf life; high quality fruit, ripened on demand	8, 11
Oilseed rape	Insertion of leuenkephalin sequence into the 2S albumin protein gene of *Arabidopsis thaliana*	Pharmaceutical applications	12
Potato	Introduction of a starch production gene from *E. coli*	The genetically altered potatoes will have 30–60% higher starch and lower moisture; they will absorb less fat on frying; chips with less fat	10
	Inserted antisense gene which blocks ADP-glucose pyrophosphorylase	Sucrose is not converted to starch; sweetness enhancement	10
	Genetically engineered	Synthesis of human serum albumin	12
	Inserted antisense RNA of starch synthase	Potato starches devoid of amylose and containing only amylopectin	12
Raspberries	Recombinant ethylene control	Increase the shipping radius	4
Soybean	Two soybean lines with distinctive genes were crossed	Low palmitic acid content (3.5% vs. normal soy oil at 10%)	13
	Brazil nut gene insertion to enrich methionine	Higher methionine so protein is complete for chicken feed or for Third World countries	6, 12, 14, 15

TABLE 10.2 (continued)
Biotechnological Procedures Commercially Available to Improve Shelf Life and Quality Traits of Foods

Product	Technology Applied	Benefit	Ref.
Sunflower, canola	Application of recently acquired Allelix technology	Altered fatty acid composition and more nutritious oils with lower saturated fat content	4, 6
Tobacco	Genetically engineered	Production of immunoglobulins, not contaminated by human pathogens	12
Tomatoes	Expression of antisense RNA for 1-aminocyclopropane-1-carboxylic acid (ACC) synthase which blocks ethylene production	Slow ripening of tomatoes which stay green, firm, and unflavored until exposed to exogenous ethylene	16
	Expression of new genes, traditional bleeding, somoclonal variation, and RFLP analysis	High solids and high viscosity (pectin); less cooking for canned products: paste, catsup, etc.	4, 16, 17
	Expression of antisense RNA for polygalacturonase which would otherwise degrade pectin	Slow softening of tomatoes which can vine ripen and stay more firm in shipment or in home storage	4, 6, 12, 17
	Somoclonal variation	Ripening of "breaker" tomatoes continues after harvesting without ethylene	4
	Rearrange or duplicate existing genes	Control rate of softening, color, and flavor balance	18
	Classical breeding with wild tomato *Lycopersicon cheesmanii*	Increase vitamin A 30 times	17
Tomato, lettuce	Transference of a gene for monellin	Sweetness enhancement	9, 19

and deterioration occurs. These physiological changes of respiration, transpiration, and biosynthesis are affected by intrinsic (i.e., climacteric vs. nonclimacteric commodities) and extrinsic (i.e., temperature, ethylene, O_2, and CO_2 concentration) factors, but in general they cause quality decline and limit the shelf life of fruits and vegetables.[2,20] Other types of deterioration, besides physiological, may also occur in fruits and vegetables. Chemical and enzymatic changes may cause tissue softening, off-flavors, pigment loss and off-colors, and an overall decline in nutritional value and taste. Similar effects are produced by physical damage, which is the result of improper harvesting, handling, processing, or packaging. Microbial deterioration also contributes significantly to quality decline and may have important safety implications for some products. Fruits and vegetables are prone to attack by macroorganisms (i.e., insects, rodents), which further promote deteriorative changes and quality decline.

The shelf life and availability of fruits and vegetables are limited by the factors cited previously. To extend the shelf life of fresh fruit and vegetables, transpiration and respiration rates must be reduced, which can be accomplished by minimizing

mechanical damage and optimizing storage temperatures, humidity, and gaseous atmosphere (O_2, CO_2, ethylene). Packaging techniques, food additives, or special treatments, such as irradiation, may also be utilized. The optimum treatment and storage conditions vary with products. Furthermore, extended shelf life products can be generated by traditional transformation technologies such as microbial fermentations and lately by molecular biology techniques.[2,20]

Quality of raw and processed fruit and vegetable products are sometimes elusive factors and may differ from person to person based on individual tastes. However, there is no doubt that consumers desire high-quality foods in a fast-moving society. Consumers demand convenient, fresh, light, and nutritious products for their diets. Fruit and vegetables fit in a healthy diet. The food chain related to fresh and processed fruits and vegetables must economically deliver high-quality products to consumers.[20]

Extending Shelf Life

According to estimations by the U.S. Department of Agriculture, nearly half of the fresh fruits and vegetables harvested annually is lost due to spoilage; such losses may be much higher in developing countries. This spoilage is mainly due to the formation of ethylene which triggers fruit ripening.[12] In order to prevent or delay fruit ripening, sequestrants of ethylene are used, or fruits are harvested well before they ripen on the plant. Both ways have their disadvantages; early harvest, as a rule, results in an unpleasant taste and sequestering ethylene may involve the use of chemicals and increase the price of the fruit.[12] When a tomato is bruised or wounded, certain genes responsible for ethylene biosynthesis such as LE-ACS2 and LEACS4 (from ACC synthase family) and pTOM13 (from ACC oxidase family), are turned on while others are turned off. As much as 30% of the tomato crop is lost annually from bruising during harvesting, shipping, and storage.[17] Ethylene, a plant hormone that causes aging, is produced when a tomato is bruised and preliminary research has indicated that it may influence gene expression.[17]

Thus, possibilities have been explored aiming at modifying the ethylene formation or content in plants and fruits.[12] Table 10.3 shows several strategies used in tomato for the inhibition of ethylene formation.[12,21] Ethylene is formed from S-adenosylmethionine (SAM) via the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC). The formation of ACC is catalyzed by the enzyme ACC synthase. The second step leading to ethylene formation is catalyzed by the ethylene forming enzyme (EFE) or ACC oxidase. The genes encoding the ACC synthase have been cloned from tomato and squash,[22,23] and those encoding ACC oxidase have been cloned from tomato.[24] Transgenic tomato plants have been produced showing a highly reduced level of EFE. This was due to the expression of a chimeric gene which was under the control of the constitutive 35S CaMV promoter encoding the anti-sense RNA of ACC oxidase.[12] The anti-sense fruit never ripens naturally; they have no aroma and do not turn red or soft. But when exogenous ethylene is added to reverse the inhibition, the fruit becomes indistinguishable from naturally ripened fruits with respect to texture, color, aroma, and compressibility.[24] Another approach to inhibit the formation of ethylene was followed by Klee and co-workers from the Monsanto Company. They identified a bacterial gene encoding an enzyme able to

TABLE 10.3
Strategies to Obtain Transgenic Tomato Plants with Longer Shelf Life and Better Taste

Strategy	Description	Ref.
Inhibition of ethylene formation	Chimeric gene construction under the control of the constitutive 35S CaMV promoter which encoded the anti-sense RNA of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase	12
	A bacterial gene, the ACC deaminase (ACCD) which degrades ACC, precursor of ethylene	21
	Use of bacteriophage-T3-encoded S-adenosylmethionine hydrolase (SAM), which degrades SAM, precursor of ethylene	21
	Transwitch technology: the gene of interest is duplicated, inserting an exact copy of the gene back into the plant producing the inhibition of the translation of RNA into protein	21
Inhibition of polygalacturonase enzyme (softening enzyme)	Chimeric gene construction under the control of the constitutive 35S CaMV promoter which encoded the anti-sense RNA of polygalacturonase	12

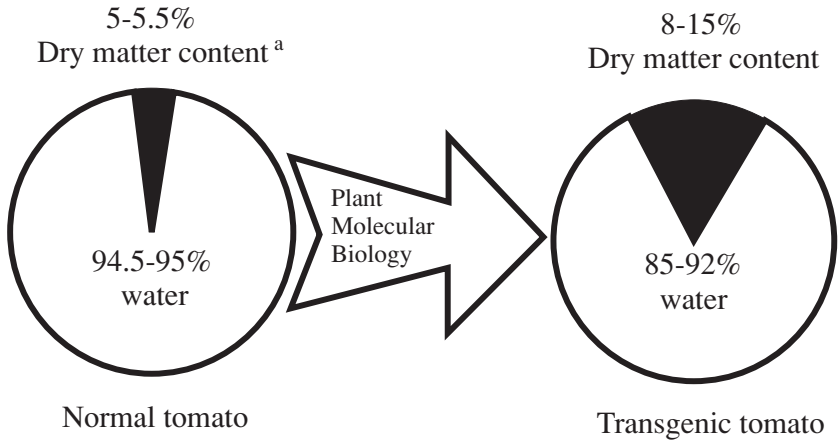
degrade ACC, the immediate precursor of ethylene synthesis, which also significantly decreased ethylene biosynthesis.[21,25] The enzyme able to degrade ACC is called ACC deamidase (ACCD).[21] The reduced level of ethylene was accompanied by a 2-week delay in fruit ripening, which clearly has commercial potential. The general appearance of the tomato fruit, apart from the delay in ripening, was completely normal when compared to control experiments.[12] On the other hand, researchers at Agritope (Beaverton, OR) have chosen to interfere with ethylene biosynthesis by focusing on SAM hydrolase expression. Their first experiments in tobacco used bacteriophage-T3-encoded SAM hydrolase to generate transgenic plants with a reduced capacity to synthesize ethylene. Afterwards, they switched to tomatoes. With the goal of obtaining stage-specific and tissue-specific SAM hydrolase gene expression, the scientists employed tomato's E8 promoter. The native E8 gene is only expressed in ripening fruit beginning at the mature green stage. It worked: SAM hydrolase expression was indeed restricted to ripening fruit. Then they examined the genetic construct's effect on post-harvest ripening. The goal was to slow down fruit ripening or even stop it at a certain stage until the fruit was ready for shipping.[21]

Approaching the ethylene pathway from a slightly different angle is DNA Plant Technology (DNAP, Cinnaminson, NJ), which has developed a technology it terms transwitch. In transwitch technology, the gene of interest is duplicated; inserting an exact copy of the gene back into the plant appears to inhibit the translation of RNA into protein, but that effect is not well characterized.[4,21] DNAP researchers have also been developing an improved tomato using somaclonal variation, a method based on culturing plant somatic cells to generate single gene mutations, chromosomal variants, and changes in multi-gene traits. The company has field tested a somaclonal tomato that can be harvested just as the first shades of pink start to appear. Ripening proceeds after harvest without ethylene.[21]

May et al.[26] have recently developed an *Agrobacterium*-based genetic transformation system for banana using shoot tip plant cultures. Their goal was to use this technology to the benefit of developing countries by modifying desirable agronomic and post-harvest characteristics of potential new varieties in order to increase their suitability for transport and storage in international trade, and therefore, stimulating the economies of export nations. The onset of fruit ripening entails the coordinated expression of an array of associated proteins. While much work has been performed on the analysis of gene expression in the ripening fruits of tomato, avocado, and apple, little is known about the specific changes in gene expression that occur during banana fruit ripening. May et al.[26] are investigating the regulation of, and the changes in, gene expression in ripening banana fruit. Banana differential cDNA and genomic libraries have been constructed and screened with a variety of heterologous ripening related cDNAs [i.e., EFE, polygalacturonase (PG)]. Several clones have been isolated and their sequences have been determined. Using a *Musa* transformation technology developed in their laboratory, these workers were aiming to determine the patterns of expression of such regulatory sequences controlling gene expression during banana fruit ripening. They have reported the relative abundance of a variety of mRNAs present in banana fruits during seven separate stages of ripening. Huang et al.[27] have also been working with the cloning and analysis of the mRNAs involved in the ripening of banana. They have cloned the ACC synthase and the ACC oxidase specific for fruit ripening. They have also found that the expression of the ACC oxidase gene occurs earlier than that of the ACC synthase gene during ripening. Moreover, there was a high level of mRNA accumulation for ACC oxidase in each ripening stage. The expression of both ACC synthase and ACC oxidase genes could be induced by 10 µl/l of exogenous ethylene. Hurst et al.[28] proved that the glutamine synthetase (GS) enzyme is very important in the shelf life of asparagus; if this enzyme is inhibited its shelf life is reduced. Although this inhibition results in a huge increase in ammonia (which is toxic) in spear tips, they concluded that glutamine depletion rather than ammonia toxicity could be the reason for the reduction of asparagus shelf life. Thus, an approach in order to extend the asparagus shelf life could be the over-expression of the GS gene (tissue specific) to reassimilate considerable amounts of ammonia in the tips.

Increasing Solids Content

Research being conducted on tomato nowadays is already beginning to make an impact on the food and processing industries. Because of the tomato crop's financial importance, several major projects are being conducted.[17] Consumers like a thick tomato sauce, but the thicker the sauce, the higher the production costs. Tomatoes are 95% water and the more water that has to be removed from tomatoes the higher the cost of processing.[4,17] Several projects are being directed in order to develop tomatoes with a higher ratio of solids to water; just a 1% increase in the solids content could result in substantial economic savings because less water would have to be removed during processing.[16,17] Tomatoes with a higher solids content more than just save time and energy during processing; they also result in higher quality products. One of the most important quality components in processing tomatoes is



a. - 80% Soluble solids content
20% Insoluble solids content

FIGURE 10.2 Use of biotechnology to increase the dry matter content in tomatoes. (Adapted from Waterman.[17])

the dry matter content, both total solids content and soluble solids content. The level and quality of insoluble solids is directly related to the consistency of tomato products.[17] The USDA Laboratory in Albany, CA, has already produced tomatoes with a solids content of 8 to 13%, and one over-achiever registered an astonishing 15% (Figure 10.2).[16,17]

Improving Flavor

One criticism of many research programs is that often they focus more on culture, disease resistance, and uniformity than on flavor. Tomatoes traditionally have been selected by breeders for yield, fruit size, lack of defects, and resistance to disease. Unfortunately, this has often resulted in cultivars lacking in flavor.[17]

A USDA laboratory in Winter Haven, FL, conducting tomato flavor volatile research has determined that tomato varieties differ in the level of flavor volatiles; these volatiles are influenced by ripening, harvest maturity, breeding, and genetics. Significant differences were found in sugar, including glucose and fructose, and citric acid content. It was concluded that good flavor may be due not only to higher volatile levels, but also to the proper balance of individual volatiles, sugar and acid levels.[17] Poor soil and growing conditions can generate losses of flavor as well as losses by steam processing and storage. Thus, there are studies focused on the recovery of aroma compounds from various waste products such as peels, culled tomatoes, and even the plant itself.[17,29]

The Food and Drug Administration has agreed that Calgene's (Davis, CA) Flavr Savr tomato is safe for consumers and the environment. This tomato is genetically

engineered to ripen on the vine longer and so be tastier than currently available fresh produce.[30,31] The Flavr Savr is the first genetically engineered whole food to receive premarket scrutiny from the FDA, which ordinarily evaluates food additives rather than whole foods. Calgene used resistance to the antibiotic, kanamycin, as a marker while selecting modified plant cells during the early stages of engineering the Flavr Savr tomato. The kanamycin-resistance gene (kan-r), though, plays no further role in the intact, engineered plant or in its tomatoes.[30,32-34] The business end of the two-part gene construct in the Flavr Savr tomato is an antisense gene targeting the gene encoding PG, an enzyme produced in ripening tomatoes. Since this antigene blocks production of PG, it prevents PG's catalytic activity. When active, PG helps to dissolve pectin, a polysaccharide that imparts firmness to tomatoes. Because the antisense gene halts PG production, Flavr Savr tomatoes remain firm instead of becoming soft, as they ripen. Since ordinary tomatoes produce plenty of PG, they are picked when still hard and long before they ripen naturally. In this hardened, but not so tasty state, ordinary tomatoes survive better transport and handling on the way to distant markets. By contrast, because the Flavr Savr tomato lacks PG and softens much less readily, it can stay on the vine until ripe and flavorful and still survive transport and handling. According to Calgene, the Flavr Savr is not so tasty as tomatoes produced by traditional farmers but is far more tasty than tomatoes currently available in most U.S. supermarkets.[18,30] The consensus view is that use of antisense gene to block PG production poses no conceivable risk to consumers. And although speculative concerns were raised regarding the kan-r gene's potential for spreading antibiotic resistance, which is a public health problem, FDA officials have not argued that putting the kan-r gene in the Flavr Savr poses a significant risk. Moreover, traditional toxicology experiments in which rats were administered high doses of Flavr Savr also indicated that the tomato is safe.[30,31,34] To allow the processing industries to benefit from these genetic improvements, field trials have been carried out with tomato hybrids whose viscosity parameters have been improved through the introduction of PG effect genes. It is anticipated that commercial products resulting from this work will most likely reach the market.[35,36] The full range of benefits of enhanced-viscosity tomatoes to the processing company lies in cost savings from increased product yield. However, a much broader range of benefits will be realized by the grower, the consumer, and the environment (Table 10.4).[36]

A number of investigators[37,38] have examined the effects of storage temperature on fruit and vegetable quality, especially chill damage. These studies have centered mainly on gross changes. Other studies[39,40] have focused on changes in volatile composition during ripening.[41] Another study[42] has been comprehensive in correlating various parameters of tomato flavor. There are also reports of some of the methodology used in the data gathering and analysis of tomato volatiles.[43] Changes in the most important volatile and non-volatile compounds have been quantified along with physical properties and attempts have been made to correlate these flavor chacteristics with each other and with treatments under conditions that closely simulate current commercial practices of storage and ripening.[44] Although more than 400 compounds have been identified as volatile constituents of tomatoes and tomato products,[45] only a limited number are essential to tomato flavor. Stern et al.[44] found that the generation of volatiles decreases significantly with storage and ripening

TABLE 10.4
Potential Benefits of Low-Polygalacturonase Tomatoes

Benefit	Recipient			
	Farmer	Processor	Consumer	Environment
Reduced chemical input	X			X
Reduced water usage on farm	X			X
Reduced production costs	X			
Reduced harvest costs	X			
Reduced harvest waste	X	X		
Reduced transport waste	X	X		
Reduced transport cost		X		
Reduced water usage in processing plant		X		X
Reduced waste water		X		X
Increased yield		X	X	
Reduced product cost			X	
Improved flavor		X	X	
Differentiated texture and flavor		X	X	
Improved range of products		X	X	
Better shelf life	X	X	X	X

Adapted from Schuch.[36]

temperatures below 10°C, but the final ripening temperature is the most significant factor in determining levels of volatiles produced. If final ripening temperatures are raised to 20°C for tomatoes initially stored at 10°C or less, volatiles are produced at a level comparable to tomatoes stored above 10°C. When final ripening takes place at temperatures below 10°C, volatile production is curtailed.[44]

Improving Color

Bachem et al.[46] investigated the inhibition of browning in potato tubers. The brown pigmentation by melanins is a ubiquitous phenomenon in living systems. They have isolated a number of potato tuber specific cDNA clones representing transcripts of genes coding for polyphenol oxidase (PPO), the enzyme catalyzing the first steps in melanin synthesis. Antisense inhibition of the expression of PPO genes significantly reduces browning of potato tubers under field conditions. Using tissue specific promoters to express the antisense RNA, inhibition of melanin formation can be restricted to the potato tuber. These authors found that in transformants the brown discoloration after bruising can be virtually eliminated. The lack of a bruising phenotype in transgenic potatoes expressing the antisense PPO gene opens up the possibility of preventing melanin generation.

Roukas and Kotzekidou[47] developed a methodology for the improvement of quality of canned okra; with their methodology they obtained slight changes in the natural color of okra. Okra (*Hibiscus esculentus*) is believed to have originated in Ethiopia. Now it is grown in many areas of the world including the Mediterranean region, the Middle East, Africa, and southern states of the U.S. In Greece, okra is

one of the most important canned vegetables. It is either canned fresh or soaked in 1.67% (w/v) brine for a period of about 18 h. This vegetable contains a mucilage which is a viscoelastic substance exhibiting both elastic and viscous properties.[47] Such mucilage is extracted into the brine of the canned product and results in a slimmy texture undesirable to the consumer. The normal pH range of okra is 5.8 to 6.4. Thus unfermented, canned okra requires a high thermal processing which causes squashing and changes of its natural color. Roukas and Kotzekidou[47] overcame these problems associated with the canning of okra. They used fermentation with starter cultures in order to decrease the pH value and eliminate thermal processing and also remove as much of the mucilage as possible and retain the natural color. The strains used were *Lactobacillus plantarum*, *L. brevis*, *L. cellobiosus*, and a commercially available starter culture (Vege-Start) (Chr. Hansen's Laboratorium A/S, Copenhagen, Denmark).

Design of a Special Composition

Plants represent the major renewable resource of complex carbohydrate polymers such as fiber and starches. Plants will find applications as bioreactors for producing new varieties of complex carbohydrate polymers because they have a great advantage over all other organisms, which is their mass potential. Only plants are able to produce millions of tons of, for example, carbohydrates which are needed for technical processes.[12] With respect to starch composition, one of the priorities is to produce plants that would only produce one sort of starch (i.e., either amylopectin or amylose[48,49]); this goal has been reached in transgenic potatoes. By expressing a gene under the control of the 35S RNA promoter, which encoded the anti-sense RNA of the granule-bound starch synthase (the enzyme responsible for amylose synthesis), transgenic potatoes were created devoid of amylose and containing only amylopectin.[50]

Van der Meer et al.[51] induced fructan accumulation in normally non-fructan-storing plants and analyzed the metabolic and physiological properties of such plants. Fructans are polyfructose molecules functioning as nonstructural storage carbohydrates in several plant species that are important crops. The normally non-fructan-storing potato plant was modified by introducing the microbial fructosyltransferase genes so that it could accumulate fructans. Constructs were created in order that the fructosyltransferase genes of either *Bacillus subtilus* (*sac B*) or *Streptococcus mutans* (*ftf*) were fused to the vacuolar targeting sequence of the yeast carboxypeptidase Y (*cpy*) gene. These constructs were placed under the control of the constitutive cauliflower mosaic virus 35S promoter and introduced into potato tissue. The regenerated plants accumulated high molecular mass ($>5 \times 10^6$ D) fructan molecules in which the degree of polymerization of fructose units exceeded 25,000. This modification affected photosynthate partitioning in microtubers and leaves and increased the nonstructural carbohydrate content in leaves. Unlike starch, which is insoluble, vacuolar fructans are soluble and can contribute to the osmotic potential of this compartment. Next to their role as a plant carbohydrate reserve, fructans may have other functions, including involvement in dry and cold tolerance.[52]

On the other hand, researchers at the University of California, Berkeley, have successfully transferred a gene into tomatoes and lettuce which manufactures a

sweet-tasting protein called monellin. The gene comes from a tropical shrub, and opens up the prospect for low-calorie, sweet-tasting vegetables.[10] Also researchers at the Institute of Genetics in Berlin have inserted an antisense gene that blocks the production of ADP-glucose pyrophosphorylase, an enzyme which converts sucrose into starch in tubers; sucrose just piles up instead of being converted into starch.[10]

Nutrition

A wild tomato from the Galapagos islands (*Lycopersicon chessmanii*) has been selected because it has 40 times more vitamin A than regular commercial tomatoes. The Galapagos tomatoes also contain an average of 58 mg per gram of beta-carotene, compared with approximately 1.5 mg per gram found in the common tomato.[17] The USDA has crossed the Galapagos tomatoes with Floradade, a commercially cultivated tomato grown for fresh market uses. The result has been orange tomatoes with about 30 mg of beta carotene per gram. The new hybrid also has a tomato taste reminiscent of Floradade. With consumers becoming more and more health-conscious, foods containing vitamin-packed tomatoes may be demanded from food processors in the future.[17]

OILSEEDS AND LEGUMES

Transgenic Oilseeds

Improving Shelf Life

When variation is limited or when plant breeders are forced to use unadapted germplasm, traditional plant breeding requires many generations and considerable effort to produce elite lines with the desired phenotype. Creating a large mutagenized population and screening the resultant plants for reduced expression is time-intensive and potentially costly. Plants selected also may need extensive backcrossing to remove random mutations not associated with the trait of interest. In addition, a trait may have a negative commercial value when expressed in seed, but may be required by roots or leaves for optimum growth and development.

The advent of recombinant DNA technology has given plant breeders new tools in their quest to improve agronomically important crops. One of the uses of the recombinant DNA technology is the production of new lines of oilseeds with less polyunsaturated fatty acids which increases shelf life.[53,54] Figure 10.3 shows generated changes in the fatty acids of genetically engineered sunflower and soybean as compared to regular crops.[53] The improved oxidative stability over regular soybean oil can be translated into longer shelf life and longer fry life. Considering that soybean oil made up 75% of total U.S. consumption of edible fats and oils in 1992, this is a significant development.[53] The susceptibility to oxidation of polyunsaturated fatty acids is greater than that of monounsaturated and saturated fatty acids. In an attempt to increase the oxidative stability of oils for use in high-temperature applications, while maintaining the cholesterol-lowering properties, recombinant DNA technology has been used in soybean and canola oils.[54] Saturated fatty acid levels remained the same (Figure 10.3).[53] The presence of linolenic acid in soybean oil has

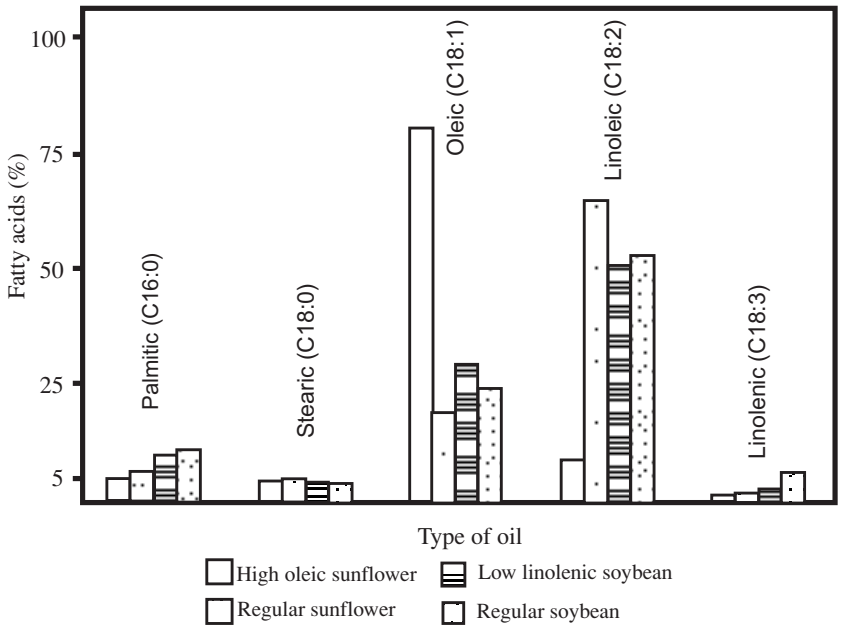


FIGURE 10.3 Comparison of fatty acids in regular sunflower and soybean vs. genetically engineered crops. (Adapted from Skillicorn.[53])

been blamed for its relatively poor oxidative stability compared with corn, cotton, and sunflower oils. This is particularly important in frying, where oil flavor, odor, and color deteriorate with prolonged exposure to air at high temperatures.[53]

To reduce the level of polyunsaturated fatty acids, the genes for the enzymes that desaturate oleic acid to make linoleic acid (Δ, 12-desaturase) and desaturate linoleic acid to make linolenic acid (Δ, 15-desaturase) have been cloned from both soybean and canola. Fader et al.[55] evaluated the co-suppression in soybean and canola. They used a DNA coding for the two desaturase enzymes (oriented in the sense direction), which were joined with seed-specific promoters and placed into vectors suitable for transformation. For soybean an embryonic axis tissue which was transformed using gene gun technology was used. For canola, an *Agrobacterium tumefaciens*-based system was used to transform hypocotyl tissue of the cultivar Westar. Soybean lines containing co-suppressed Δ, 12-desaturase were identified with linoleic acid levels less than 3% and oleic acid levels over 76%. In canola, co-suppressed Δ, 12-desaturase lines were selected that had oleic acid levels of 83% and linoleic acid levels near 6%.

Designing a Special Fatty Acids Composition

Control of the degree of unsaturation has been directed to change the oil composition of a crop like sunflower from high linoleic acid to oleic acid. This can change the value of the oil, both from the point of view of food usage and for industrial purposes, because the presence of high purity oleic acid increases the possiblities for derivation

at places other than the carboxyl group. Another case would be the change of a crop like linseed from high linolenic to high linoleic acid content in order to use the oil for culinary purposes.[56]

In the two specific cases for control of the degree of unsaturation mentioned above, sunflower and linseed, the desired goals have been reached by mutation breeding. One may ask the question whether genetic engineering could have done the job as well or better. A decade ago using genetic engineering to create oil crops with dramatically altered lipid composition was a target almost impossible to reach. Various fundamental biological observations have to be taken into account and were not so clear at that time. First, the percentage of plant seeds' three major storage products (oil, starch, and protein) differs considerably between and within species. Pea primarily stores carbohydrate with little oil reserve, whereas in rapeseed the opposite occurs.[54] Second, there are major differences in the types of fatty acids which can be stored as a major product in seeds. There is a striking difference in the oil palm fatty acid composition: the major stored triglycerides are lauric acid (C_{12}:0) in the kernel, and palmitic acid (C_{16}:0) in the mesocarp. Third, lipids are known to have a number of different roles in plants and the membrane lipid fatty acid composition generally is highly conserved. Because storage could be held to be a largely luxury function, it is more amenable to manipulation in levels and content. To alter fatty acid composition, a maximal metabolic alteration of storage lipid fatty acids combined with a minimal perturbation to membrane lipids should occur. The fourth, and last, of these basic biological observations is that the timing and tissue specific deposition of storage triglycerides is strictly controlled.[54]

It is estimated that three requirements are needed to successfully utilize recombinant DNA technology to generate crops with new oils: (1) isolation of genes of interest, (2) a transformation system, and (3) a regeneration system. For the isolation of genes of interest, basically two broad classes of sequences need to be identified and isolated: (a) the promoters, which will show highly selective tissue specific and temporal expression, and (b) the structural genes of the enzymes which control the production of the desired product. For the isolation of promoters it is important to consider that the deposition of storage lipids in plants occurs within a strictly defined time frame. The expression of the biosynthetic enzymes also is controlled in a temporal manner. Figure 10.4 shows the expression of a fatty acid synthetase constituent β-keto reductase during oilseed embryogenesis.[54] Step B should be selected to isolate the genes before the synthesis has stopped and the product has been stored. Promoters covering the time phase B are required. There are several lipid synthesis genes cloned to date (Table 10.5).[54] Another important drawback is that transformation systems do not exist for all oilseed crops, being only well established for rapeseed and soybean. Studies on more high-yielding crops, such as oil palm, are rapidly advancing. Regeneration systems are available for several species, including rapeseed, corn, and soybean. Again as with palm transformation, there is still much work to be done in this topic.[54]

High-laurate canola became the first transgenic oilseed crop whose DNA code has been altered by biotechnologists and the first one to be planted as a commercial crop. Regular canola does not produce lauric fatty acids, but the genetically engineered seed could yield about 40% lauric fatty acids.[54] This oil could be used in the

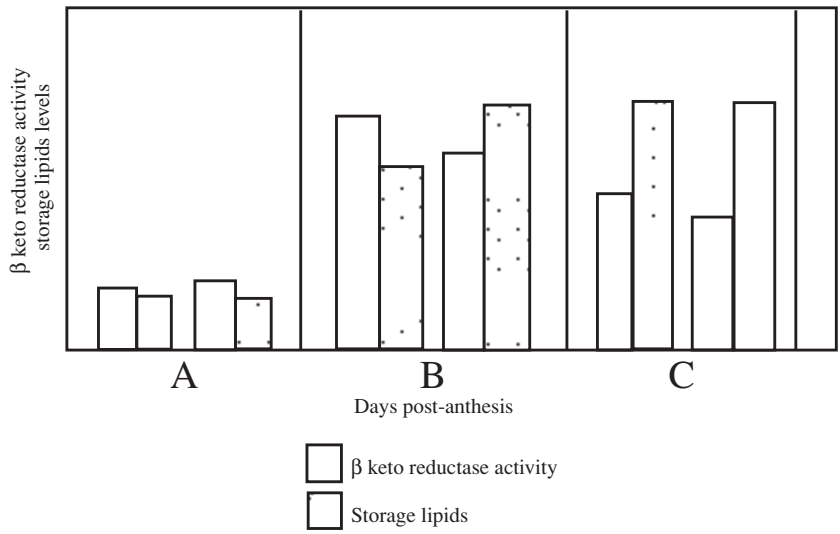


FIGURE 10.4 Relationship between deposition of lipids and β-keto reductase activity in a developing oil seed. (Adapted from Slabas et al.[54])

TABLE 10.5
cDNA/Genes Cloned to Date Related to Lipid Synthesis

Lipid Synthesis Gene	Source of cDNA and/or Gene Cloned
Acyl carrier protein (ACP)	Spinach leaf ACP I, barley leaf ACP I, barley leaf ACP II, barley leaf ACP III, *Arabidopsis* leaf, *Brassica campestris* seed, *B. napus* seed
Acetyl-CoA carboxylase	Carrot, wheat, maize, rapeseed, *Arabidopsis*
3-Ketoacyl-ACP synthase (KAS)	Barley leaf KAS 1, castor seed KAS 1
Enoyl-ACP reductase (NADPH)	*B. napus* seed, tobacco, petunia, *Arabidopsis* leaf
3-Ketoacyl-ACP reductase (NADPH)	*Arabidopsis* leaf, *Cupheas lanceolata*
Δ9-Desaturase (soluble)	Castor seed, cucumber, safflower seed, *B. rapa,* jojoba, *B. napus* seed
Δ6-Desaturase (membrane-bound)	Coriander
Δ15-Desaturase (membrane-bound)	*Arabidopsis*
Acyl-ACP thioesterase	Safflower, rapeseed
Acyl-ACP thioesterase (medium-chain specific)	California bay laurel
Glycerol-3-P acyltransferase	Pea leaf, squash leaf, *Arabidopsis* leaf, cucumber

Adapted from Slabas et al.[54]

soap and detergent industry and for various edible products as confectionery fats, or as feedstock for oleochemicals. Table 10.6 lists the status of other transgenic oilseeds nearing release to farmers. At the University of Saskatchewan, Canada, genetic engineering techniques are being applied to modify flax fatty acids. DuPont researchers have produced soybean lines with seed oil compositions of between

TABLE 10.6
Transgenic Oilseeds That Have Been Modified in Order to Improve Quality Traits or Shelf Life

Crop	Altered Trait	Target Year for Commercialization	Organization
Canola	Laurate	1996	Calgene
	High-stearate	Latter part of 1990s	Calgene
	Medium-chain fatty acids	Latter part of 1990s	Calgene
	Cocoa butter alternative	Latter part of 1990s	Calgene
	High-oleic	1996–1997	Inter Mountain Canola[a]
	High-oleic, low-saturate	1998	Inter Mountain Canola[a]
	High-stearate, low-polystearate	1997–1998	Inter Mountain Canola[a]
Soybean	High-oleic, low saturate	1998–1999	Du Pont
	High-lysine	1999–2000	Du Pont
	High-stearate, low-polystearate	1999–2000	Du Pont
Corn	High-oil, high-oleic	1999–2000	Du Pont

[a] Inter Mountain Canola is commercially developing transgenic canola produced by Du Pont.

Adapted from Slabas et al.[54]

70 and 80% oleic acid. They are trying to reduce the linolenic acid levels as well as increase saturates to produce feedstocks for spreads and margarines. The changes in fatty acid composition are seed-specific and do not have any effect on the rest of the plant.[54] Bacteria and fungi are also able to produce a wide array of fatty acids, some of which are commercially attractive. Their genes could be inserted in some oilseed crops to fulfill present and future industrial needs.

Use of Biotechnology to Reduce Unwanted Traits

Acyltransferases in plants are responsible for decorating the glycerol backbone with specific acyl groups. It seems that the 1- and 3-acyltransferases are fairly nonspecific in a wide variety of plants.[55] In rapeseed the 2-acyltransferase positively discriminates against the incorporation of erucic acid (C_{22}:1) and lauric acid (C_{12}:0). This barrier limits the maximal level of either lauric acid or erucic acid that can be incorporated into oilseed rape manipulated to produce more of these fatty acids.[54] However, erucic acid is an unwanted trait because it has been characterized as responsible for some intestinal disorders.[4,57] The Plant Biotechnology Institute in Saskatchewan, Canada is attempting to use molecular biology to produce seed without erucic acid.

A textured, plastic fat that enables shortening to be creamed and margarine to be spread requires a certain hardness from the fat, which includes considerable amounts of high-melting saturated fatty acids. Until recently, the only industrially and economically viable way to make oils hard, without blending them, was through hydrogenation. To produce a textured fat, hydrogenation is commonly used to convert some of the unsaturated oleic, linoleic, and linolenic fatty acids to stearic acid. Plastic

fats with the best properties have a high ratio of palmitic to stearic acid. During hydrogenation, the unsaturated fats are changed by the addition of hydrogen to the triple and double bonds of the oil. This results in the *trans*-isomerization of the fat, which causes the formation of *trans* fatty acids. The *trans* fatty acids do not normally occur in nature and have been shown in some studies to increase the body's level of serum cholesterol.[53] Selective hybridization of oil seeds can produce a high stearic acid hybrid oil that would reduce the need to hydrogenate for some solid fat applications. By using oil produced from selective hybridization, the problem of *trans*-isomerization would be eliminated and the labeling of "hydrogenated" avoided.

Transgenic Legumes

Quality of Seed Storage Proteins

After cereals, legume seeds are the most important source of food protein at the world level, and consequently are of remarkable nutritional and economic significance. The Leguminosae is an immense botanical family, whose seeds exhibit wide variations in protein content and quality. Seeds of wild and cultivated crops display a range of 12 to 55% protein; up to 80% of these proteins can be represented by storage proteins, reflecting their central role in seed viability. The remaining seed proteins, over 1000 different types, are involved in metabolic processes, cell structure, and antinutritional properties, e.g., lectins and protease inhibitors.[58]

Legume seed storage proteins are commonly referred to as globulins because they are salt-soluble at neutral pH. Seed globulins can be mostly divided, for simplicity, into two distinct classes termed 7S and 11S on the basis of their sedimentation coefficients. The ability to improve the quality of these food proteins would be of enormous benefit to the agriculture sector, the food industry, and finally the consumer. From a nutritional viewpoint, target areas are: increase of digestibility, removal of intolerance factors, inducing higher levels of methionine and tryptophan, much better palatability, and decrease of nutrient binding to improve bioavailability. From a functional perspective the main objectives are: increase of foaming and emulsifying properties, increase of solubility at or near the proteins' isoelectric point, better gelling and texturizing capabilities, lower heat stability, and higher flavor/water/fat binding.[59] An approach for improvement would be to "engineer" the proteins at the crop level. By defining the ideal combination and composition of seed globulins, enhancement in quality can be achieved. Recent advances in molecular technology offer a possible solution to the above-mentioned traits. It is now clear that the production of recombinant seed globulins in bioreactors will never compete economically with the existing low cost bulk production of protein concentrates and isolates. Thus, the generation of transgenic legumes with improved seed globulins should be considered; it could be achieved by introducing either totally foreign genes (e.g., globulins from other species) or modified endogenous globulin genes. The major obstacles to engineering legume seed globulins are related to the transformation and regeneration procedures of legumes species. Fortunately, some legumes have now proved to be more amenable to *Agrobacterium* and biobalistic transformations and subsequent regeneration.[59,60]

Improving Protein Composition

One molecular approach for altering the amino acid composition of seed proteins involves the transfer of genes that encode proteins containing large amounts of the limiting amino acid. For improving the quality of leguminous seed proteins, commonly deficient in the sulfur-containing amino acids, Altenbach et al.[56] have focused on a small family of proteins found in the seeds of the Brazil nut (*Bertholletia excelsa*) which are unusually rich in methionine and cysteine.[61] The sulfur-rich protein from the Brazil nut is a member of a family of small, water-soluble seed proteins that contain approximately 8% cysteine and 18% methionine. The mature protein consists of 9-kD and 3-kD subunits linked through disulfide bridges.[62] Like storage proteins from other seeds, the synthesis of the sulfur-rich protein is developmentally regulated; the protein is synthesized at a mid-maturation stage and accumulates in the mature seeds. The sulfur-rich protein is synthesized initially as a 17-kD precursor polypeptide that undergoes three proteolytic processing steps before it attains its mature form. First, a signal peptide of about 2-kD is cleaved from the precursor, leaving a 15-kD polypeptide precursor that is then trimmed to a 12-kD polypeptide and finally to the 9-kD and 3-kD subunits of the sulfur-rich protein.[62] cDNA clones representing several members of the sulfur-rich protein gene family have been isolated and sequenced by Altenbach et al.[56] One of these cDNAs was used to construct a chimeric gene in which the promoter region and 3′-flanking region of the phaseolin gene from French beans were linked to cDNA sequences encoding the 17-kD precursor from the sulfur-rich protein. They used a binary vector system of *Agrobacterium tumefaciens*, and transferred the chimeric gene to tobacco and regenerated transformed plants.[56] These authors[56] analyzed Southern blots from leaves of the transgenic tobacco plants, and analysis of RNA isolated from developing seeds, which indicated that the chimeric gene was transcribed. They detected that the sulfur-rich protein was expressed in the seeds at levels that approach 5% of the total seed protein. They used tobacco as a model plant system, but the transfer of the chimeric gene to several legumes was the next planned step.

Researchers at DuPont are modifying the amino acid content of soybeans. They have taken a gene from *Corynebacterium* and expressed it in soybeans to increase the lysine content.[54] The first transgenic material is high-lysine soybeans; commercial plantings are still several years away. Similar technology is being used to increase the levels of sulfur amino acids and threonine and tryptophan. Kim et al.[65] improved the nutritional value (methionine content) and functional properties (heat-induced gelation and emulsification) of soybean glycinin by protein engineering. Technology is now available to use the soybean crop as a factory, using sunlight and nitrogen to produce the desired amino acid levels, instead of production by fermentation.[54,63,64]

GRAINS

Shelf Life of Grains

Lipids and lipid-associated components are key factors in the quality of several grains such as maize and oats. The oat and oat products have a relatively high lipid

content, a large proportion of unsaturated fatty acids, and a significantly more active lipase than that of either barley or wheat.[66] Oats contain antioxidants and oat lipids are stable in mature, undamaged grains[67] and in sufficiently heat-treated oat products.[68] However, under unfavorable storage conditions or in untreated oat products lipolytic activity will cause rapid release of free fatty acids (FFA), which may then be oxidized and cause rancidity. Oxidative rancidity may also be caused by overprocessing.[69] Thus, adequate storage conditions[70] and appropriate heat treatment for inactivation of lipolytic enzymes before milling of oats[68] are essential in achieving stable oat products.[71] Because unsaturated FFA in oats are susceptible to oxidation and many form components with undesirable aroma and taste,[68] analysis of the amount of FFA may be useful for predicting lipid stability. For oats to be processed into food products, a maximun FFA level of 5% hexane extractable lipids has been suggested.[72] FFA in oats enhance formation of bitter compounds.[73] An approach to overcome the problems produced by the unsaturated FFA is the genetic engineering of oat crops where the levels of unsaturated FFA are reduced.[53,54]

Coffee is one of the most commercially important grains in the food industry. Coffee comprises a number of different forms, ranging from coffee cherries (berries); green coffee, which is trade beans that are removed by one of a number of different process sequences after harvesting; roast coffee, which is the green coffee beans that are roasted by a heat process, either domestically or commercially, and which may also be pre-ground; and the coffee beverage, which is the form in which it is actually consumed. It should also be understood that while coffee cherries/beans exist in a number of different botanical species within the corresponding genus, only two are used commercially, *Coffea arabica* and *C. canephora* (robusta in the trade), and can reflect different characteristics in storage behavior and in their subsequent coffee products.[74]

The greatest interest is in the shelf life of roasted (and ground) coffee, since this is the form in which coffee is most familiar, together increasingly with instant coffee to the consumer. Green coffee, roasted coffee, and instant coffee all have two main divisions of their chemical composition; first, the non-volatile matter, some contributing to basic taste sensations of acidity, bitterness, and astringency and the remainder, composed mostly of carbohydrates and proteins of generally neutral flavor characteristics; and second, the volatile substances, present in a very small amounts (ppm levels) but of great significance to overall flavor in the prepared cup of coffee beverages. In all these coffees, environmental factors of temperature, humidity, and oxygen exposure strongly determine storage behavior and therefore shelf life, together with the initial moisture content of the coffee, and its precise composition. The previously mentioned factors determine the condition or quality of roasted coffee after given periods of time. The terms "condition" and "quality", however, are very much subjective and are not amenable to scientific assessment.[74] Like all other foodstuffs, roast coffee, and even more rapidly, roast and subsequently ground coffee, deteriorate with time from their initial state of "freshness" (i.e., after roasting in roasted coffee), but the actual deterioration has to be assessed on the cupped beverage, prepared under standardized conditions for all samples being compared, by human senses. Panels of judges are asked to assess changes from "fresh" flavor quality on numerical scales, and at what point the coffee is no longer "acceptable"

(i.e., the end of its shelf life). Such assessment is therefore on the overall flavor quality of the prepared beverage. A separate assessment may be made on the headspace aroma impact from the dry roasted and ground coffee using only the external nostrils of the nose, i.e., by sniffing. In practice an early deterioration may not be so readily marked in actual flavor quality of the beverage. Appearance changes in the dry product are not generally evident, except moisture uptake during storage. Shelf life data are of special relevance for trade purposes.

A number of investigations have also been performed to determine differences in volatile compounds, as between robusta and arabica; such differences are clearly evident in their respective beverage flavor characteristics. Grosch et al. (cited by Reference 74) used the technique for assessing the important aroma impact compounds by serial dilution techniques to obtain flavor dilution (FD) values in each of arabica and robusta roasted coffee, both from brews and the dry product. Among the differences, they found that 4-vinyl guiacol is especially characteristic in brewed robusta coffee and furaneol in arabica; in the dry product, 3,5-dimethyl-2-ethyl pyrazine appeared with the highest FD-factor in both coffee species. The use of genetic engineering techniques may well be a useful tool to enhance volatile compound production responsible for flavor;[3] another approach is the reduction of caffeine content because of consumer demand for decaffeinated coffee.[3]

Remodeling Storage Proteins for Food Processing

The functional role of vegetable and seed proteins in food processing is to provide the required physical properties to the food material either during processing or in the final product. The physical properties of both the starting materials (protein extracts, isolates, concentrates, or flours) and products are determined by the level of protein present, the proportions of different protein types, and the presence of nonprotein components, and such properties are likely to manifest themselves in different ways, depending on the processing procedures used.[63] The types of functional properties sought in proteins are many and include those responsible for emulsification, foam formation, and stabilization, and also for texturing.[63,65] Whereas many functional properties rely on maintenance of the native configuration of the proteins, several others arise through complete or partial denaturation of the proteins, followed by rearrangements of the polypeptide chains and formation of new intramolecular and intermolecular bonds. In all cases, however, the behavior of a particular protein type depends ultimately on its intrinsic primary structure (amino acid sequence) encoded by the genes. Thus, the proportions and functional properties of certain seed proteins could be manipulated by genetic engineering to suit particular applications in food processing.[63-65]

Baking Quality

One example of the importance of protein functional properties is in baking quality. Payne and Rhodes (cited by Reference 63) have described a number of different baked products and the different qualities of wheat grain and wheat protein required for each. An important part of the basis of baking quality lies in the composition of

the gluten protein fraction in wheat flour. Gluten is the water-insoluble viscoelastic protein mass left after soluble proteins, starch, and other nonprotein materials have been washed out from the flour. Gluten is composed mainly of hydrated forms of the two major wheat protein fractions, namely, gliadins (wheat prolamins) and glutenins (wheat glutelins).[63,75] These proteins contribute in different ways to the properties of the flour during processing and in the final product. Thus, gliadins provide viscosity and extensibility to bread dough, whereas glutenins provide the elasticity that is all-important in dough stability and in the structure and texture of bread. Differences in proportions and properties of these two protein fractions determine whether a particular wheat variety has good bread-making properties or is more suitable for the production of other products, such as pasta or biscuits. A molecular basis for these properties has been proposed, based on the characteristics of the purified components, their amino acid composition and the primary structures of certain wheat proteins.[75] The components responsible for good bread-making quality have been tentatively identified and ascribed to the glutenin fraction, notably to the high molecular weight (HMW) glutenin proteins (95,000 to 150,000 apparent MW).[75,76] Payne et al. (cited by Reference 63) have shown a positive correlation between the molecular weight of native glutenin and the amount of HMW glutenin subunits, and have suggested that interactions of these subunits with other polypeptides are important in stabilizing the glutenin structure. Furthermore, it has been concluded that allelic variation does correlate with good or poor baking quality, although other factors, possibly other wheat proteins, may be involved.[63] Certain of the gliadin polypeptides have also been implicated in baking quality and dough strength. The positive identification and cloning of the genes encoding polypeptides that contribute to good bread-making quality could potentially allow the transfer of this trait to poorer-quality wheats carrying other desirable attributes.[63] Additionally, it might be possible to manipulate the functional properties of the gluten for purposes other than bread making by transferring multiple copies of certain genes, thereby altering the proportion of the HMW glutenins.[77]

There are 12 genes for HMW glutenin proteins in hexaploid bread wheat, four coming from each of the three progenitor species although two genes are inactive in all varieties.[75] The genes are of two types, *Glu-1-1* and *Glu-1-2*, which give rise to X and Y HMW glutenin subunits, respectively. *Glu-1-1* and *Glu-1-2* loci are very closely linked on the long arms of the chromosomes of the homologous group 1. Thus, there are six pairs of loci each of which carries an X and Y gene.[78] Series of genotypes have been assayed, possessing new combinations of X and Y subunit genes at the *Glu-1D-1* and *Glu-1D-2* loci.[79] The comparisons have enabled the separate contributions of subunits associated with poor bread-making quality [2(X) and 12(Y)] and subunits associated with good bread-making quality [5(X) and 10(Y)]. Flavell et al.[75] have showed that in these seeds the major variation was contributed by the Y subunits, with subunit 12 conferring poorer dough quality than subunit 10. They compared the amino acid sequences of these closely related proteins and found that differ in their central regions, which consist of an array of repeating hexamers and nonamer amino acid units; HMW glutenin 10 has a higher proportion of repeats of the consensus type than glutenin 12 and they postulated that this produces a more regular pattern of repetitive β turns in the protein, contributing to

dough elasticity. A cysteine residue, likely to become involved in intra- or intermolecular linkages, may also be in a different configuration in the two subunits. There are also other protein components, such as low molecular weight glutenin subunits[80,81] and gliadin proteins that have been found to be associated with quality.[82,83] Branlard and Dardevet[84] used the French wheat cultivar Darius, which has very good bread-making quality, even though it possesses the HMW glutenin subunit combination 2, 7 and 12, associated with poor quality, and a null allele at the *Gli-D1* locus. The absence of the *Gli-D1* encoded ω-gliadins was associated significantly with higher dough tenacity and strength. These results demonstrated that using only one locus breeders can improve particular quality traits.[84] In this way, genetic engineering could be used to aid in the construction of wheat and other cereal varieties with predetermined functional properties designed for a precise processing purpose, such as bread-making, breakfast foods, meat analogs, and hydrolyzed products, as well as for nonfood applications.[63]

Malting and Brewing

The technology of producing beer involves the processes of malting and brewing. In malting, the barley grain is germinated under conditions leading to enzymatic hydrolysis or modification of starch and protein reserves and the production of flavor compounds, whereas brewing involves the fermentation of sugars to produce alcohol by yeast.[85] Different varieties differ in regard to their suitability for malting, and in general barley suitable for malting should have a low protein content.[86] It is thought that storage proteins released from protein bodies during germination surround starch grains, and also reduce access to amylolytic enzymes and delay sugar release, and this leads to poor fermentation.[63] Malting quality may be affected by the hordein fractions,[63,87] particularly B hordeins and especially disulfide-linked aggregates that may be less easily degraded when adhering to starch. However, malting quality is a complex character, and hordeins may affect other stages of brewing, such as filterability, foaming, and haze formation,[88] but with further knowledge it should be possible to identify specific proteins (e.g., disulfide-linked components) that could be manipulated or their expression reduced such that malting quality will be improved. Other aspects that could be manipulated include identification and removal of genes involved in polyphenol (proanthyocyanidins) production to prevent the haze formed by interaction of polyphenols and protein.[86,89] The copy numbers of α-amylase genes and those of other hydrolytic enzymes (e.g., β-glucanases) can be increased to speed up breakdown of seed reserves during malting. The wheat α-amylase gene, the major enzyme of starch degradation, has already been cloned, and it has been expressed in modified yeast cells. The α-amylase has also been used for production of low-calorie beer.[90]

Improving Nutritional Properties

Seed Storage Proteins

The nutritional quality of grains very much depends on the amino acid composition of the storage proteins, and being macromolecules directly coded by specific genes,

unlike storage carbohydrates, they should be easier to manipulate. In terms of total storage protein production for cereals, wheat, maize, rice, and barley, it is much more significant than that produced by legumes, except for soybean.[63,64] Lysine is the first limiting amino acid in wheat, barley, maize, sorghum, and triticale; threonine (barley, sorghum) or tryptophan (maize) is the second. Thus, in a pure cereal diet in which lysine is limiting, the quality will be poor because the grain protein will not be metabolized efficiently by humans and animals.[91]

The discovery that the maize *opaque2* (*o2*) mutation dramatically increases the lysine content of the grain[92] led to the development of high lysine corn.[93] However, the soft, starchy endosperm of this mutant, which causes the kernel to be susceptible to pests and mechanical damage,[94] prevented significant utilization of the mutation. After the initial characterization of *o2*, genes that alter the mutant phenotype were identified, giving it a normal appearance. These genes designated *o2* modifiers[95] were subsequently used by plant breeders at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT)[96] to develop *o2* varieties with normal kernel hardness and protein content, as well as an enhanced percentage of lysine. These modified *o2* mutants are called quality protein maize (QPM).[97-99]

The major storage fraction of most cereals are prolamins, which are given trivial names such as gliadin (wheat), zein (maize), hordein (barley), and secalin (rye). In order to increase the limiting amino acids in cereals, two approaches have been suggested:[100] (1) insertion of extra codons for lysine, threonine, or tryptophan into cloned storage protein genomic DNA, followed by reintroduction of the gene into the plant; and (2) modification of the expression of existing genes so that proteins rich in limiting amino acids are preferentially synthesized. A specific problem is that prolamins are coded by multigene families (e.g., for zein, possibly up to 150 closely related genes), so replacement of a single modified copy would have little effect. Some likely approaches to circumvent this problem may include the following: (1) introduction of a modified gene into a recipient that has a deletion lacking part of the gene family; (2) inactivation of normal gene expression (without deletion), with expression of introduced modified genes; (3) insertion of a modified gene with a strong promoter such that it is transcribed more frequently than natural genes; and (4) insertion of multiple copies of the modified gene, perhaps combined with approaches 1 through 3. Eggum et al.[101] have developed several rice mutants for prolamin and glutelin in order to improve the nutritional properties of rice protein. They obtained mutants with a higher lysine content and a higher net protein utilization (NPU).

Soluble Amino Acids

It is also possible to improve the nutritional quality of cereals by increasing specific soluble amino acid levels; there has been some success in producing mutants with feedback-insensitive regulatory pathways, particularly those of lysine biosynthesis.[91] The amino acids lysine, threonine, methionine, and isoleucine are derived from aspartic acid, and it is known for barley that there is a negative feedback to three isozymes of aspartate kinase, the first enzyme in the pathway, by the end products lysine, threonine, and S-adenosyl methionine. Thus, cloning of the genes that encode for these isozymes is being developed in order to increase the production of such amino acids.[63]

MUSHROOMS

General Aspects

Fresh mushrooms are highly perishable and their quality declines rapidly after harvest. However, due to consumer demand for fresh produce and foreign competition with processed products, the percentage of U.S.-produced *Agaricus* mushrooms sold on the fresh market has increased dramatically in recent years and represents about 68% of all mushrooms grown in that country. A key factor in increasing demand and subsequent sale of fresh mushrooms is the improvement of the quality available to the consumer.[102]

Mushrooms, the edible species of a large group of fungi, have been consumed for several thousand years and can be divided into five groups: (1) Oomycetes, (2) Zygomycetes, (3) Ascomycetes, (4) Basidiomycetes, and (5) Deuteromycetes (Fungi imperfecti). Some mushrooms, such as truffles and morels, are Ascomycetes, while most, including species of *Agaricus*, *Lentinula*, *Pleurotus*, and *Volvariella*, are Basidiomycetes. Basidiomycete is also known as huitlacoche, which is the name the Aztecs applied to the young fruiting bodies (galls growing on the maize ears) of *Ustilago maydis*, which is the causal agent of common smut of maize. Huitlacoche is consumed as the main component of a dish or as a condiment and offers a very attractive chemical composition and desirable nutritional attributes.[103] Usually, the reproductive portion or fruiting body of the mushroom lies aboveground and is the part most commonly eaten, while the vegetative portion or mycelium is hidden beneath the soil. The fruiting body is comprised of three distinct parts: the pileus or umbrella-like cap; the lamella or delicate spore-forming gills; and the stipe, the stalk on which the cap is held (Figure 10.5). Because of their unique aroma and delicious taste, hundreds of wild mushroom species have been used as food, but only a few have been extensively cultivated on a commercial scale.[102,104-106]

America leads the world in production of *Agaricus* mushrooms accounting for about 24% of the total, which means about 1.4 million tons. *Pleurotus* spp., the

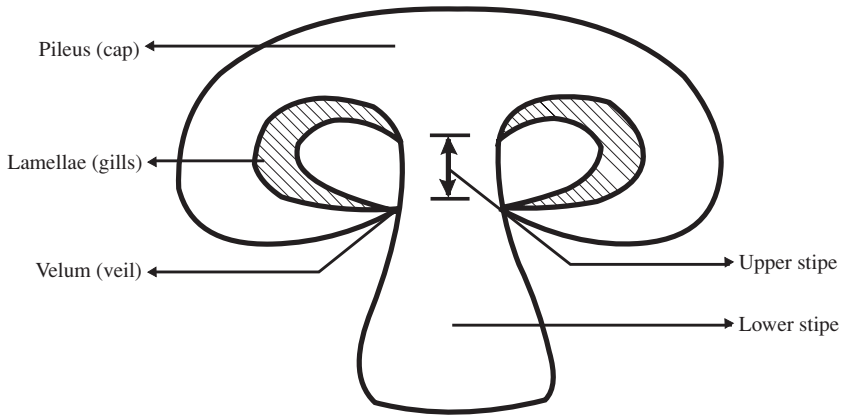


FIGURE 10.5 Cross-section of a mushroom fruiting body.

oyster mushroom, is the second most popular mushroom, accounting for about 24% of the world mushroom production. The third most important mushroom (11%) is *Lentinula edode*s, also called the shiitake mushroom. Duggar used the term mushroom in its widest sense to include all edible fungi, while Alkins suggested that cultivated mushroom of commerce should be referred to as *Agaricus bisporus*.[102] Off-white hybrid and white hybrid strains are two commercially grown hybrid types of high quality developed in the Netherlands through a cross-breeding of the white and off-white strains.

The agricultural wastes and by-products are under utilized. Those from gramineous are rich in cellulose, hemicellulose, and lignin, while those from horticultural origin contain higher concentrations of organic nitrogen. A mixture of these residues has a high potential to produce mushrooms, which are highly priced due to their sensory and nutritional attributes.[107,108]

Shelf Life of Mushrooms

Mushrooms contain large quantities of water and therefore suffer considerable weight loss during transportation and storage, which in turn causes serious economic losses. When the supplies of water, photosynthates, and minerals are cut off at harvest, the fresh mushroom enters a deteriorative or perishable phase. Cap opening and stem elongation are the usual symptoms of senescence constituting visible evidence of deterioration. At room temperature, the shelf life of mushrooms is restricted to only a few days. During this period there are considerable changes in color, texture, and taste, while water is lost continuously by transpiration and respiration. Additional losses occur from surface cracks and bruises that develop during growth and handling.[110] Fresh mushrooms are white or light buff, with no dark marks on either the cap or stem. The veil is closed and the gills are not visible. The upper surface of the caps should be strongly convex and stems should be plump rather than elongated.

Mushrooms are grown on compost, usually in mushroom houses where the temperature and humidity can be controlled. They are harvested daily, cooled immediately, and processed the same day. For mushrooms kept for more than a few hours before processing, refrigeration will delay veil opening and reduce weight loss.[111] Braaksma et al.[112] have studied the aging of the mushroom (*Agaricus bisporus*) under post-harvest conditions and found that in contrast to higher plants, mushroom senescence appears to be independent of degradation of specific membrane lipid contents. Several techniques exist for the commercial processing of mushrooms including canning and pasteurization, dehydration, and freeze-drying.[113] Many methods have been proposed to extend the shelf life of fresh mushrooms; some are cited in the section entitled "Methods of Improving Shelf Life and Quality".

Quality of Mushrooms

Chemical Composition and Nutritive Value

The consumption of *Agaricus* mushroom has increased in recent years and so has interest in its nutritional value. The solids content (dry matter) of fresh mushrooms

ranges from 6 to 11%. The values vary not only from study to study, but also between flushes within a given crop. Environmental conditions are important factors affecting the solids content of fresh mushroom.[102]

Apart from the high water content (89 to 94%), fresh mushrooms also contain 2.5 to 5.8% of carbohydrates, 2.6 to 4.0% of proteins, 0.2 to 0.7% of crude fat, 0.6 to 1.1% of fiber, and more than 1% of ash. The protein content of fresh mushroom is about twice as high as that of most vegetables. Based on its content and relative proportion of amino acids, mushrooms protein appears to be intermediate in nutritional quality between meat and vegetable proteins.[102] Mushroom carbohydrates are present in lower proportions than in other vegetables and provide a very small fraction of the energy requirement. Due to their low energy value (20 to 30 kcal/100 g), mushrooms can be used in low calorie diets. Since the mushroom contains more than 1% of ash, its mineral content is generally higher than in many fruits and vegetables. However, mushrooms are found to be low in sodium which is recommended in special diets.

It is well established that compost is a prime factor for increasing yield. Mau et al.[114] proved that nutrient supplementation at spawing increased the yield of mushrooms and supplementation at casting resulted in better color for the first two flushes of the crop cycle.

Quality Parameters of Fresh Mushrooms

There are several parameters that should be consider in order to diminish the quality deterioration of mushrooms after harvesting; among them the following can be mentioned:

Respiration rate: Fresh mushrooms are living organisms and after harvest they continue to develop. They take in oxygen, and through the process of respiration convert previously stored substances (primarily sugars) into energy, carbon dioxide, and water. Mushrooms have a higher rate of respiration than fruits and vegetables and much of the energy yielded by respiration is used in the ongoing processes of maturation. This leads to senescence giving a general deterioration of cells with a concurrent loss of moisture and increase in browning, all factors that most consumers find unacceptable.[102] Refrigeration, overwrapping of mushrooms packages with a permeable film, controlled or modified atmosphere storage, and irradiation will all slow, but not stop, these processes.

Postharvest development: Harvested mushrooms undergo a course of postharvest development similar to those allowed to remain growing on the bed. The development of harvested mushrooms goes from veil intact (tight) to veil open, gill surface flat. Ryall and Lipton reported that mushrooms of high quality should have closed veils, should not have elongated stipes, and gills should not be visible.[102] Mushrooms harvested at the bottom stage have a longer shelf life than mushrooms in the cap or open stage of development.

Fresh water loss: A major factor in fresh mushroom deterioration has been water loss and subsequent loss of weight. Loss of water is determined by the environment and can be reduced significantly by use of film-wrapped packaging.[102]

Browning: Whiteness is one of the most important aspects of fresh *Agaricus* mushroom quality. However, mushrooms are very sensitive to browning. Mushroom deterioration is marked by brown discoloration of the mushroom surface. Browning is caused mainly by the oxidation of phenolic substances, which is catalyzed by polyphenol oxidase (PPO); controlled or modified atmosphere storage can be helpful to slow this process. Sapers et al.[115] used browning inhibitor solutions and found that the most effective treatment was a combination of sodium erythorbate, cysteine, and EDTA at pH 5.5.

Texture: At harvest, mushrooms grown properly are firm, crisp (resist deformation), and tender (easy to shear or chew), but subsequently in postharvest deterioration they soften and toughen.[116] This is apparently caused by chitin synthesis in cell walls (toughening) and loss of cell turgency due to changes in membrane permeability (softening) after harvest.

Flavor and aroma: Flavor is one of the most important quality attributes contributing to the widespread consumption of mushrooms. The flavor substances of mushrooms can be classified into (1) non-volatile components such as amino acids and nucleotides, and (2) volatile compounds such as 1-octen-3-ol and 3-octanone. The amino acid fraction of mushrooms, consisting of alanine, arginine, aspartic acid, glutamic acid, glycine, and lysine, is probably responsible for some aspects of flavor, but all nitrogenous components contribute to some extent to the typical mushroom flavor. In addition, mushrooms contain relatively high levels of free glutamic acid, which is known to be a flavor enhancer. Several aroma compounds are produced by the Maillard reaction such as furans, pyrones, cyclopentenes, carbonyls, acids, aldehydes, sulfur compounds, pyrroles, and pyridines.[29] Charpentier et al.[117] quantified the flavor volatile and aroma compounds of shiitake mushrooms (*Lentinus edodes*).

Methods of Improving Shelf Life and Quality

Due to the high perishability of fresh mushrooms, several methods have been investigated to extend shelf life and to retard the deterioration processes in harvested mushrooms.

Irrigation Treatment with Calcium Chloride and Stabilized Chlorine Dioxide (Oxine)

A successful reduction of the initial bacterial population and an increased shelf life of mushrooms is accomplished by the addition of stabilized chlorine dioxide (oxine) or calcium chloride, to irrigation water during cropping.[102] However, a significant

problem in early studies using calcium chloride was the reduced yield at the concentration (0.5%) required for improved quality and shelf life. Oxine and calcium both reduced bacterial counts when added individually to irrigation water. The combination treatment (50 ppm oxine + 0.25% $CaCl_2$) gave the best overall results for improving shelf life. These two chemicals are thought to act synergistically as a result of the bactericidal activity of chlorine dioxide and the bacteriostatic effect resulting from the reduced water activity of the mushroom surface caused by crystallization of calcium chloride. Bacterial counts, browning, and rate of senescence were all reduced by the combination treatment. Although yield is reduced with this combination treatment, the mushrooms are significantly larger. Oxine is not currently approved for use in mushroom irrigation water and hence recent emphasis has been placed on determining the optimum concentration of calcium chloride in irrigation water. The use of $CaCl_2$ (0.3%) reduces the bacterial blotch without reducing the yield. Food grade gypsum (calcium sulfate) is an alternative source of calcium because of its lower price, but the results have indicated that it has no advantage over calcium chloride.[102]

Irrigation Treatment with Biological Control

Pseudomonas tolaasii is the major pathogenic bacterium that causes bacterial blotch on mushrooms. This bacterium is responsible for an estimated crop loss of 5 to 10% of mushrooms produced.[102] Chemical control of this pathogen has limitations, while biological control was successfully used in Australia. The use of antagonistic bacteria such as *Pseudomonas fluorescens* to control bacterial blotch was first studied in Australia. However, the mechanism by which biological control of blotch is achieved is still unclear.

Modified Atmosphere Packaging (MAP)

Modified atmosphere packaging (MAP) by definition is the packaging of the perishable products in an atmosphere that has been modified to contain significantly higher carbon dioxide and lower oxygen concentrations. MAP reduces the respiration rate of mushrooms and their deterioration. Kyuper et al.[118] have used MAP combined with $CaCl_2$ and obtained lower coliform and total plate counts compared with MAP alone. In order to achieve better results, the MAP should be developed at 0°C.[111] It is important that the O_2 concentration never falls below 2%, because according to Sugiyama and Yang[119] storage of mushrooms in such low concentrations could create a favorable micro-atmosphere at the center of mushrooms for growth and toxin production of the anaerobic spore former (*Clostridium botulinum*) and therefore is not recommended.[120]

Coating with Edible Films

Many papers have been written about coating mushrooms with edible films such as a biodegradable hydrocolloid film.[121-124] The film is intended to reduce transpiration and evaporation, to control respiration, and to maintain a modulated atmosphere around each individual piece of produce from the initial stages after harvest, through

transport, storage, and marketing.[111] Nussinovitch and Kampf[111] used 1 to 2% alginate-coated mushrooms in order to extend the shelf life and conserve the texture of these mushrooms.[111] The treated mushrooms showed better color and appearance than uncoated controls. The strength and integrity of coated mushrooms were maintained for longer periods of time than those of the uncoated controls, and the former therefore more closely resembled fresh, intact mushrooms.

Irradiation

Low-dose gamma-irradiation has been reported to be a very effective method of controlling deterioriation and improving quality and shelf life of fresh mushrooms. The irradiation, usually from a cobalt-60 source, is most effective when applied to the mushrooms shortly after harvest. Mushrooms exposed to 100 krad, a USFDA approved dose, showed reduced bacterial counts and a slower rate of senescence. Sensory data comparing irradiated mushrooms with unirradiated controls showed equal or superior flavor and texture scores for both raw and cooked samples.[102]

TECHNOLOGICAL PROCEDURES RELATED TO SHELF LIFE

Traditional Approaches

Table 10.7 shows the traditional methodologies that have been used by humans in order to extend the shelf life of foods and their basic preservation principles.[2,125-127] Freezing, canning, and drying are the three principal food preservation techniques used nowadays. The baking of bread, manufacture of ice cream, production of fruit jams, fermentation of yogurt, smoking of sausage, and many other processes result in foods with prolonged shelf life. These techniques, however, are more properly classified as manufacturing since their principal goal is the creation of a new food product. Freezing, drying, and canning are used to protect all foods (raw agricultural produce as well as manufactured food items) from microbial, chemical, or physical spoilage for many months.[125]

Novel Technologies

Biotechnology has been defined as a collection of technologies that employ living systems, or compounds derived from these systems, for the production of industrial goods and services.[128] Biotechnology is not new to the agricultural and food sector, as people have been utilizing living systems for the production, processing, and preservation of food for centuries. Classical breeding and selection techniques have been applied to develop superior varieties and species of plants and animals. Mutation and selection techniques have been applied to improve strains of bacteria and yeast used to produce fermented foods, such as cheese, sausage, bread, and wine. What distinguishes "modern" biotechnology from the more traditional examples cited above is the emergence within the last 20 years of recombinant DNA technology or genetic engineering. DNA, the universal code of life, is structurally and

TABLE 10.7
Basic Preservation Principles of Main Food Processing Techniques

Process	Type/Product	Principle	Ref.
Drying	Air, drum, vacuum drum, spray, puff, freeze, fluidized bed, osmotic	Removal of water necessary for growth of microorganisms.	125, 126
Thermal processing	Still retorts, agitating retorts, rotomats, hydrostatic sterilization, flame sterilization, flash "18", aseptic canning	Inactivation of all viable forms of microbial life in the food; hermetic packaging prevents recontamination.	126
Biological preservation	Fermentation	Lowering of pH by acid-producing microorganisms used as culture.	125, 126
	Antibiotics (nisin)	Antimicrobial action.	127
	Natural antioxidants	Avoiding free radicals formation.	
Chemical preservation	Controlled or modified atmosphere	Lack of O_2 for microbial growth.	2, 126
	Nitrites, NaCl, ethylene oxide, benzoates, methylene bromide, sorbic acid	Different antimicrobial actions.	
Pasteurization		Inactivation of many viable microorganisms.	125, 126
Refrigeration	Meats, dairy, produce, fish, miscellaneous	Microbial growth stopped or slowed down (psychotrophs) by low temperatures	125, 126
Freezing	Sharp, blast, plate, fluidized bed, freon, carbon dioxide, liquid nitrogen	Solid water (ice) in food unavailable to microorganisms; low temperature of storage inhibits microbial growth and many enzymatic reactions.	125, 126
Intermediate moisture foods	Pickling, salting, sugaring	Lowering of pH and/or a_w below microbial growth tolerance limits by added acidulants, salts, or sugars	125, 126
Physical methods	Ionizing energy; nonionizing radiations: macrowave dielectric heating, microwave heating	Killing of some microorganisms present in foods and inactivation of many viable microorganisms	125, 126
Other heating process	Baking, smoking, frying	Inactivation of many viable microorganisms.	125, 126

functionally identical in all living organisms. Thus, it can be transferred between related and unrelated living organisms, and specific vectors and gene transfer systems have been developed for microbial, plant, and animal applications. Genetic engineering has the potential to be more predictable, controllable, and precise than classical breeding and selection. In addition, genetic improvements can proceed at

TABLE 10.8
Genetic Improvement of Food-Grade Microorganisms

Type of Fermentation	Microorganism	Nature of Improvement	Implications
Dairy			
Cheese		Bacteriophase (virus) resistance	Eliminate economic losses due to destruction of culture by viruses
Yogurt		Accelerated ripening	Decreased storage costs
		Higher levels of beta-galactosidase	More digestible product for lactose-intolerant individuals
Meat			
Sausage	*Bacillus subtilis*	Bacteriocin production	Inhibition of pathogens and spoilage organisms
Cereal			
Beer	*Bacillus subtilis*	Alpha-amylase production	Production of "lite" or low-calorie beer
Bread	*Bacillus subtilis*	Higher levels of maltose permease and maltase	More consistent and improved leavening

Adapted from Harlender[90] and Jelen.[125]

a much faster pace, and the ability to cross species barriers greatly expands the available gene pool. Some of the genetic improvements that have been achieved in microorganisms are shown in Table 10.8.[90,127] This table shows microorganisms used to produce fermented dairy, meat, and cereal products. Current research is focused on the construction of food-grade cloning vectors (multifunctional plasmids derived solely from the DNA of food-approved organisms), the development of high frequency gene transfer systems, and the identification and characterization of desirable traits.

Another novel technology currently being used to select foods with longer shelf life is based on molecular markers. In contrast to morphological characters, the genetic basis, and thus the expression of molecular characters, is well worked out and predictable; nonetheless, there are certain complications to using different classes of molecular markers due to variation in inheritance, recombination, and linkages. Thus, the expression of molecular characters must be interpreted carefully in terms of what is known about the genetic basis of the markers used. If employed correctly, however, these types of markers can provide detailed information regarding the genealogy of hybrids and thus can be used to verify morphological and chemical predictions. The advantages of molecular vs. morphological markers for studies of hybridization have been reviewed by Rieseberg and Wendel[129] and include: (1) the large number of independent molecular markers available for analysis; (2) the generally low levels of nonheritable molecular variation; and (3) the apparent selective neutrality of many molecular markers.

Most studies of hybridization to date have been limited by the numbers of independent molecular markers differentiating hybridization taxa. This problem is most severe for the cytoplasmatic genomes and the nuclear ribosomal RNA genes,

which generally contribute a single independent marker to the study of hybridization. Isozymes are also limited in terms of the total number of loci available for analysis (30 to 50). In contrast, nuclear restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs) can theoretically yield an almost unlimited number of independent molecular markers for the analysis of hybrids.[130] It is important to take into consideration the patterns of inheritance. Markers with biparental codominant inheritance (e.g., isozymes or nuclear RFLPs) provide twice the information of either uniparentally inherited cytoplasmic markers or markers with predominantly dominant inheritance patterns, such as RAPDs or secondary compounds. The use of nuclear RFLP markers requires the construction of a genomic library for the hybridizing taxa or a close relative prior to the development of markers. In contrast, RAPD primers tend to be consistent among different flowering plant genera in terms of amplification strength.[131] A final consideration regarding the use of molecular markers concerns the heritability of the molecular variation scored in the parental populations and their hybrids. For example, intragenic recombination, concerted evolution, and sometimes non-Mendelian inheritance patterns make rDNA patterns in hybrids difficult to interpret at times and sometimes misleading.[132,133]

FUTURE TRENDS

Plant Foods for the Future

The most important impact of biotechnology on the food chain will be in the agriculture sector. The efficiency and profitability of producing raw agricultural commodities could be dramatically improved by increasing crop yields and decreasing agricultural inputs such as fertilizer, herbicides, pesticides, and fuel. Table 10.9

TABLE 10.9
Prospects in Biotechnology to Improve Shelf Life and Quality Traits of Foods

Product	Prospect	Future Benefit	Ref.
Strawberries	The gene cloned for ellagic acid is planned for insertion	An increased level of ellagic acid, a cancer protective agent that inhibits polycyclic aromatic hydrocarbons, nitrosamines, aflatoxins, and aromatic amines	4
Wheat	Working toward insertion of antisense ale-bound starch synthetase gene	A high content of amylopectin (waxy) starch	134
Wheat flour	Modification of gene that encodes the high molecular weight glutenin protein subunit 10	Improvement of dough elasticity	75
Oilseeds	Incorporation of three genes from a bacteria into *Arabidopsis* to produce polyhydroxybutyrate (PHB)	Reducing the lipid content of seed crops and replacing it by biodegradable polymers	54

shows some of the investigations being carried out with plants in order to improve shelf life and quality of their products.[4,54,75,134] Nowadays the recombinant DNA technology is being used and the genetically engineered plant food products are being approved as GRAS (generally recognized as safe). In a few years, with the improvement of plant transformation and regeneration technologies, one will find in the market fruits and vegetables with better color and flavor and extended shelf life, and legumes and grains with a nutritious message and functional properties.

Nutraceutical Foods

A nutraceutical is any substance that is a food or part of a food that provides medical or health benefits, including the prevention and treatment of diseases.[135] Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products, and processed foods such as cereal, soups, and beverages. It is important to note that this definition applies to all categories of foods and parts of foods, ranging from dietary supplements such as folic acid, used for prevention of spina bifida, to chicken soup, taken to lessen the discomfort of the common cold.[136] This definition also includes a bioengineered designer vegetable food, rich in antioxidant ingredients, and a stimulant functional food or pharmafood. The idea of health-filled foods is, of course, not new. The modern message probably took root in the soil of the nutrition evaluations of the 1950s and the "back to nature" revolution of the 1960s. The nutraceutical revolution may lead us into a new era of medicine and health, in which the food industry might become a research-oriented one similar to the pharmaceutical industry.[15,137-139] Genetic engineering holds the promise of a brave new world in which foods can be tailored into nutraceuticals that provide not only a better overall nutritional profile, but address specific medical conditions in both preventive and therapeutic capacities.

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