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FENNEMA'S FOOD CHEMISTRY

FIFTH EDITION

edited by

Srinivasan Damodaran
Kirk L. Parkin

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

MARA-1023

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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Printed on acid-free paper
Version Date: 20170124

International Standard Book Number-13: 978-1-4822-0812-2 (Paperback) 978-1-4822-4361-1 (Hardback)

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Library of Congress Cataloging-in-Publication Data

Names: Fennema, Owen R. | Damodaran, Srinivasan. | Parkin, Kirk L. (Kirk Lindsay), 1955-
Title: Fennema's food chemistry.
Other titles: Food chemistry
Description: Fifth edition / [edited by] Srinivasan Damodaran & Kirk L. Parkin. | Boca Raton : CRC Press, 2017. | Includes bibliographical references and index.
Identifiers: LCCN 2016027062| ISBN 9781482243611 (hardback : alk. paper) | ISBN 9781482208122 (pbk. : alk. paper) | ISBN a9781482208139 (e-book) | ISBN 9781482243666 (e-book) | ISBN 9781482208146 (e-book)
Subjects: LCSH: Food--Analysis. | Food--Composition.
Classification: LCC TX541 .F65 2017 | DDC 664/.07--dc23
LC record available at https://lccn.loc.gov/2016027062

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4 Lipids

David Julian McClements and Eric Andrew Decker

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4.1 INTRODUCTION

Lipids comprise a broad group of chemically diverse compounds that are linked together by the fact that they are all soluble in organic solvents. Food lipids are generally referred to as fats (solid) or oils (liquid), indicating their physical state at ambient temperatures. Food lipids are also classified as nonpolar (e.g., triacylglycerol and cholesterol) and polar lipids (e.g., phospholipids) to indicate differences in their solubility and functional properties. The total lipid content and the lipid composition of foods can vary tremendously. Since food lipids play an important role in food quality by contributing to attributes such as texture, flavor, nutrition, and caloric density, the manipulation of these important food components has been a major emphasis in food product development since food research began. This research has focused on the alteration of lipid composition to change the texture, alter the fatty acid and cholesterol composition, decrease total fat, alter bioavailability, and make lipids more oxidatively stable. In addition, the physical stability of lipids is important in food quality since many lipids exist as dispersions/emulsions that are thermodynamically unstable. In order to make changes in lipid composition while ensuring the production of high-quality foods, a fundamental understanding of the chemical and physical properties of lipids is critical. This chapter focuses on the chemical composition of lipids, their physical properties and crystallization/solid phase behavior, methods to modify the fatty acid and triacylglycerol composition and thus the physicochemical properties of lipids and their propensity to undergo oxidative deterioration, and the role of lipids in health and disease.

4.2 MAJOR LIPID COMPONENTS

The following section is a brief description of the nomenclature of the major classes of food lipids. For more information of lipid nomenclature, see O'Keefe [63] or the web page of the International Union of Pure and Applied Chemistry (IUPAC) (http://www.chem.qmul.ac.uk/iupac/lipid/).

4.2.1 Fatty Acids

The major components of lipids are the fatty acids that contain an aliphatic chain with a carboxylic acid group. Most natural fatty acids have an even number of carbons due to the biological process of fatty acid elongation where two carbons are added at a time. The majority of fatty acids in nature contain 14–24 carbons. While some fats contain small amounts of fatty acids with <14 carbons, significant levels of short-chain fatty acids are mainly found in tropical oils and dairy fats. Fatty acids are generally classified as either saturated or unsaturated, with the latter containing double bonds. Fatty acids can be described by systematic, common, and abbreviated names.

4.2.1.1 Nomenclature of Saturated Fatty Acids

The IUPAC has standardized systematic descriptions of fatty acids. The IUPAC system names the parent hydrocarbon of the fatty acid based on the number of carbons (e.g., 10 carbons would

TABLE 4.1
Systematic, Common, and Numerical Names for Fatty Acids Found in Foods

Systematic Name	Common Name	Numerical Abbreviation
Saturated fatty acids		
Hexanoic	Caproic	6:0
Octanoic	Caprylic	8:0
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myristic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
Unsaturated fatty acids		
cis-9-Octadecenoic	Oleic	18:1 Δ9
cis-9, *cis*-12 Octadecadienoic	Linoleic	18:2 Δ9
cis-9, *cis*-12, *cis*-15 Octadecatrienoic	Linolenic	18:3 Δ9
cis-5, *cis*-8, *cis*-11, *cis*-14 Eicosatetraenoic	Arachidonic	20:4 Δ5
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 Eicosapentaenoic	EPA	20:5 Δ5
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 Docosahexaenoic	DHA	22:6 Δ4

be decane). Since fatty acids contain a carboxylic acid group, the terminal *e* in the name is replaced by *oic* (e.g., decanoic). Common names exist for most of the even number and many of the odd number fatty acids (Table 4.1). Many of the common names originate from the source that the fatty acid was commonly or traditionally isolated (e.g., palmitic acids and palm oil). A numerical system can be used for abbreviated names. The first number in this system designates the number of carbons in the fatty acids, while the second number designates the number of double bonds (e.g., hexadecanoic = palmitic = 16:0). Obviously, this second number will always be zero for the saturated fatty acids.

4.2.1.2 Nomenclature of Unsaturated Fatty Acids

Fatty acids that contain double bonds in their aliphatic chain are referred to as unsaturated fatty acids. In the IUPAC system, the *anoic* designation is changed to *enoic* to designate the presence of a double bond (Table 4.1). Based on the number of double bonds, the terms *di*, *tri*, *tetra*, and so on are added. Common names also exist for the unsaturated fatty acids (with the exception of some of the long-chain polyunsaturated fatty acids), and the numerical abbreviation system is similar to that of the saturated fatty acids with the second number indicating the number of double bonds (e.g., octadecadienoic = 18:2). The positions of the double bonds in the IUPAC system are numbered by the Δ method starting at the carboxylic acid carbon. For example, oleic acid which has 18 carbons and one double bond would be 9-octadecenoic acid, and linoleic acid which has 18 carbons and two double bonds would be 9,12-octadecadienoic acid. An alternative numbering system that indicates the position of the double bonds from the methyl end of the fatty acids is known as the omega (ω) system (sometimes given a shorthand notation of "n"). The omega system is sometimes useful because it can group fatty acids based on their biological activity and biosynthetic origin, since many enzymes recognize fatty acids from the free methyl end of the molecule when it is esterified to glycerol. For instance, the ω-3 fatty acids often have similar bioactivity in their ability to decrease blood triacylglycerol levels [11].

The natural configuration of double bonds in unsaturated fatty acids is the *cis* configuration. In this configuration, the carbons of the aliphatic chain extend on the same side of the double bond, while *trans* double bonds would have the carbons on opposite sides (Figure 4.1). Double bonds

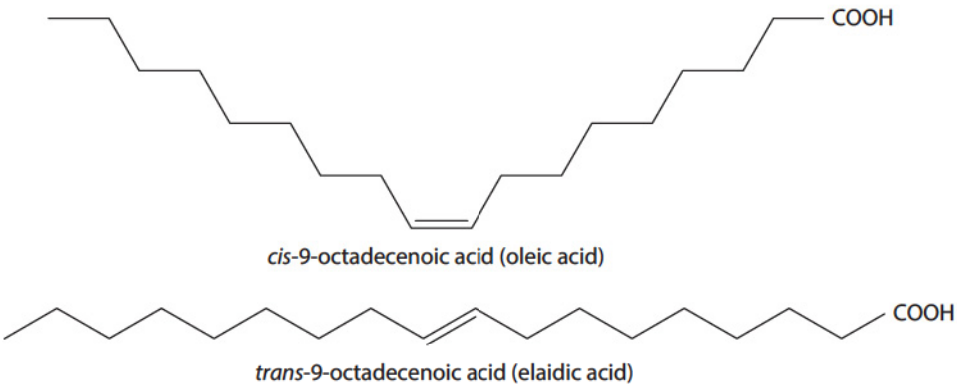


FIGURE 4.1 Differences between *cis* and *trans* double bonds in unsaturated fatty acids.

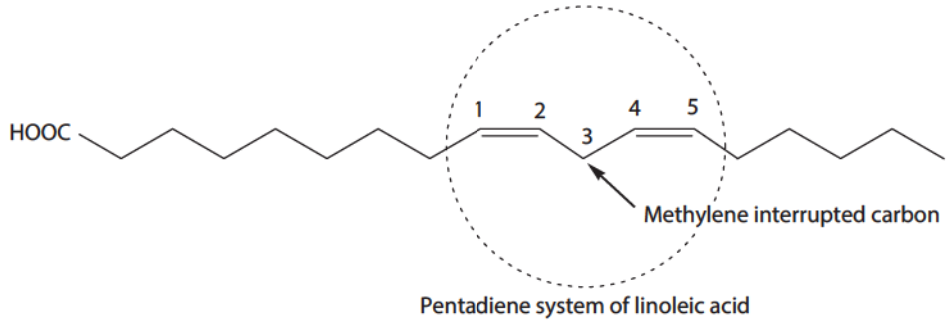


FIGURE 4.2 Pentadiene systems of the polyunsaturated fatty acid, linoleic acid.

in polyunsaturated fatty acids (≥2 double bonds) are most commonly in a methylene interrupted configuration, often termed the pentadiene system. In a pentadiene system, the two double bonds would be at carbons 1 and 4. In other words, the double bonds are not conjugated but, instead, are separated by a methylene carbon (Figure 4.2). This means that the double bonds of most unsaturated fatty acids are three carbons apart (e.g., 9, 12, 15 octadecatrienoic). It is therefore possible to predict the position of all the double bonds in most natural unsaturated fatty acids if the location of the first double bond is known. This is why the numerical abbreviation system will sometimes give only the number of double bonds and the position of the first double bond (e.g., 9, 12, 15 octadecatrienoic = 18:3Δ9 = 18:3ω3).

The presence of double bonds influences the melting point of the fatty acids. Double bonds in the *cis* configuration will cause the fatty acid to arrange in a bent configuration. Thus, unsaturated fatty acids are not linear, making it difficult for them to orient themselves into tight packing configurations. Since the unsaturated fatty acids do not pack tightly, van der Waals interactions between the molecules are relatively weak, and less energy is required to promote solid–liquid phase transitions, so that the melting point decreases. As more double bonds are added, the molecule becomes more bent, van der Waals interactions decrease further, and the melting point decreases. Fatty acids with double bonds in the *trans* configuration are more linear than unsaturated fatty acids in the *cis* configuration. This results in tighter packing of the molecules and higher melting points. For example, the approximate melting point of stearic acid (octadecanoic) is 70°C, oleic acid (*cis*-9-octadecenoic) is 5°C, and elaidic (*trans*-9-octadecenoic) is 44°C [66].

4.2.2 Acylglycerols

Over 99% of the fatty acids found in plants and animals are esterified to glycerol. Free fatty acids are not common in living tissues because they are cytotoxic due to their ability to disrupt cell membrane

organization. Once fatty acids are esterified onto glycerol, their surface activity decreases, as does their cytotoxicity.

Acylglycerols can exist as mono-, di-, and triesters, known as monoacylglycerols, diacylglycerols, and triacylglycerols, respectively. Triacylglycerols are the most common of the three in foods, although the mono- and diesters are sometimes used as food additives (e.g., emulsifiers). The central glycerol carbon of a triacylglycerol exhibits chirality if different fatty acids are present at the terminal carbons of the glycerol. Because of this, the three carbons on the glycerol portion of the triacylglycerol can be differentiated by stereospecific numbering (*sn*). If the triacylglycerol is shown in a planar Fischer projection, the carbons are numbered 1–3 from top to bottom.

Triacylglycerols can be named by several different systems. Triacylglycerols are often named using the common names of the fatty acids. If the triacylglycerol contains only one fatty acid (e.g., stearic acid, abbreviated as St) it could be named tristearin, tristearate, glycerol tristearate, tristearoyl glycerol, StStSt, or 18:0-18:0-18:0. Triacylglycerols that contain different fatty acids are named differently depending on whether the stereospecific location of each fatty acid is known. The nomenclature for these heterogeneous triacylglycerols replaces the *-ic* at the end of the fatty acid name with *-oyl*. If the stereospecific location is not known, a triacylglycerol containing palmitic acid, stearic acid, and oleic acid would be named palmitoyl-oleoyl-stearoyl-glycerol. Alternatively, this triacylglycerol could be named palmito-oleo-stearin or glycerol-palmito-oleo-stearate. If the stereospecific location of the fatty acids is known, *sn-* is added to the name such as in 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol, *sn*-1-palmito-2-oleo-3-stearin, or *sn*-glycerol-1-palmito-2-oleo-3-stearate. If two of the fatty acids are identical, the naming can be shortened as 1,2-dipalmitoyl-3-stearoyl-*sn*-glycerol, *sn*-1,2-dipalmito-3-stearin, or *sn*-glycerol-1,2-dipalmito-3-stearate. Heterogeneous triacylglycerols can also be named using fatty acid abbreviations such as in PStO or 16:0-18:0-18:1 (sterospecific location unknown) or *sn*-PStO, or *sn*-16:0-18:0-18:1 (sterospecific location known) for 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol.

4.2.3 Phospholipids

The phospholipids or phosphoglycerides are modifications of triacylglycerols where the phosphate groups are typically found in the *sn*-3 position (see Figure 4.3 for the structures of phospholipids). The simplest phospholipid is phosphatidic acid (PA), where the substitution group on the phosphate at *sn*-3 is an –OH. Other modifications of the substitution group on the phosphate at *sn*-3 result in phosphotidylcholine (PC), phosphotidylserine (PS), phosphotidylethanolamine (PE), and phosphotidylinositol (PI) (Figure 4.3). Nomenclature is similar to that of triacylglycerols, with the name and location of the phosphate group coming at the end of the name (e.g., 1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphoethanolamine). The term "lyso" signifies that a fatty acid has been removed from the phospholipid. In the food industry, lysophospholipids usually refer to a phospholipid where the fatty acid has been removed from the *sn*-2 position. Official nomenclature requires that the stereospecific location of the fatty acid removed should be named (e.g., 2-lysophospholipids, IUPAC). Phosphotidylcholine is commonly referred to as lecithin in the food industry; however, the lecithin sold as a food additive is not usually pure phosphotidylcholine.

The presence of the polar phosphate group on phospholipids makes these compounds surface active (see Chapter 7). This surface activity allows phospholipids to arrange in bilayers that are critical for the properties of biological cell membranes. Since cell membranes need to maintain fluidity, the fatty acids found in phospholipids are often unsaturated to prevent crystallization at environmental temperatures. The fatty acids at the *sn*-2 position are typically more unsaturated than those at the *sn*-1 position. The unsaturated fatty acids at the *sn*-1 and *sn*-2 positions can be released by phospholipases so they can be utilized as substrates for enzymes such as cyclooxygenase and lipoxygenase. The surface activity of phospholipids means that they can be used to modify the physical properties of lipids by acting as emulsifiers and by modifying lipid crystallization behavior (see Chapter 7).

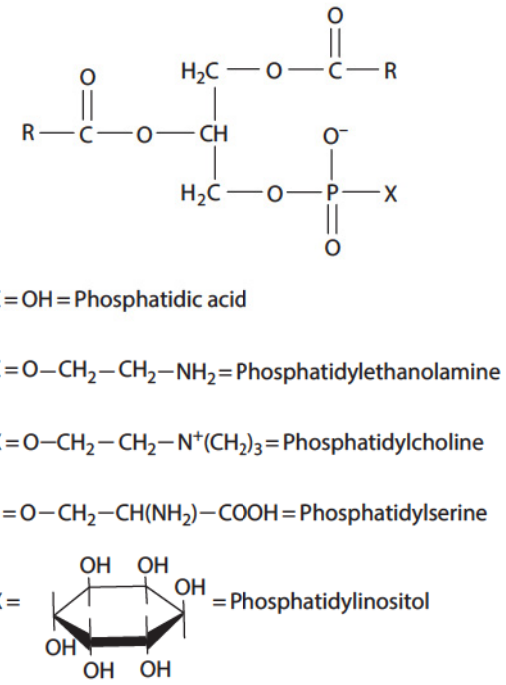


FIGURE 4.3 Structures of phospholipids commonly found in foods.

4.2.4 Sphingolipids

Sphingolipids are lipids that commonly contain a sphingosine base. Common sphingolipids include sphingomyelin (a sphingophospholipid), ceramides, cerebrosides, and gangliosides. These lipids are most commonly found associated with cell membranes, especially in the nervous tissue. They are generally not major components of food lipids.

4.2.5 Sterols

Sterols are derivatives of steroids. These nonpolar lipids all have three six-carbon rings and a five-carbon ring that is attached to an aliphatic chain (Figure 4.4). Sterols have a hydroxyl group

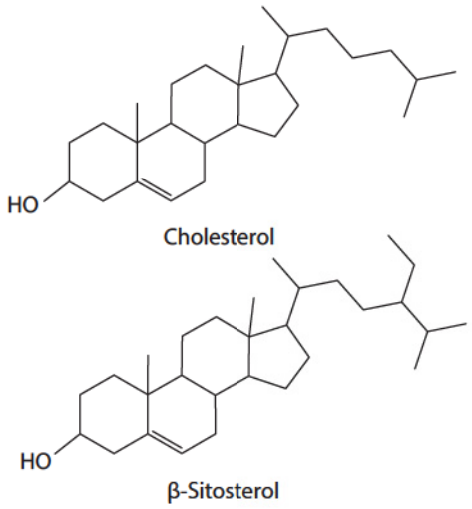


FIGURE 4.4 Structures of sterols commonly found in foods.

attached to carbon 3 of the A ring. Sterol esters are sterols with a fatty acid esterified onto the hydroxyl group at carbon 3. Sterols are found in both plants (phytosterols) and animals. Cholesterol is the major sterol found in animal lipids. Plant lipids contain numerous sterols, with sitosterol and stigmasterol predominating. Cholesterol can be found in plant lipids as a minor sterol component. The hydroxyl group at carbon 3 of sterols makes these compounds surface active. Cholesterol therefore can orient itself into cell membranes where it is important in stabilizing membrane structure. Cholesterol is also important because it is the precursor for the synthesis of bile acids, and 7-dehydrocholesterol is the precursor for the ultraviolet irradiation production of vitamin D in the skin [68]. High blood cholesterol, in particular high low-density lipoprotein cholesterol, has been attributed to increased risk for cardiovascular disease. For this reason, many attempts have been made to alter dietary cholesterol levels by the removal of animal fats from the diet and of cholesterol in animal foods by the reduction in total fat. Phytosterols have been found to decrease cholesterol absorption and synthesis, and thus have been added to foods to reduce blood cholesterol levels (see Section 4.12).

4.2.6 Waxes

The strict chemical definition of a wax is an ester of a long-chain acid and a long-chain alcohol. In reality, industrial and food waxes are a combination of chemical classes including wax esters, sterol esters, ketones, aldehydes, alcohols, and sterols [68]. Waxes can be classified according to their origin as animal (beeswax), plant (carnauba wax), and mineral (petroleum waxes). Waxes are found on the surface of plant and animal tissues to inhibit water loss or to repel water. Waxes are commonly added to the surface of fruits to slow transpirational moisture losses during storage.

4.2.7 Miscellaneous Lipids

Other food lipids, including the fat-soluble vitamins (A, D, E, and K) and carotenoids, are covered in other chapters of this book.

4.2.8 Composition of Fats

Food lipids contain a wide variety of fatty acid compositions, as shown in Table 4.2. Several general trends can be seen among lipids. Most vegetable oils, especially those from oilseeds, are highly unsaturated and contain primarily fatty acids in the 18-carbon series. Oils high in oleic acid include olive and canola, oils high in linoleic include soybean and corn, and oils high in linolenic include linseed. Triacylyglycerols from plant sources that contain high amounts of saturated fatty acids include cocoa butter and the tropical oils (e.g., coconut). The coconut and palm kernel are also unique in that they contain high amounts of the medium-chain fatty acids 8:0–14:0, with 12:0 predominating. The level of saturated fatty acids in fats and oils from animals is generally in the order of milk fat > sheep > beef > pig > chicken > turkey > marine fish, with palmitic and stearic being the major saturated fatty acids. The fatty acid composition of animal fats is dependent on the digestive system of the animal, with fat from nonruminants (e.g., poultry, pigs, and fish) being more dependent on the fatty acid compositions of their diets than ruminants. An example of this is pigs used to produce Iberian hams, where dietary regimes are manipulated to produce lard with a high oleic acid composition. Among the nonruminants, triacylglycerols from marine animals are unique because they contain high amounts of the ω-3 fatty acids, eicosapentaenoic and docosahexaenoic. In sheep and cows, dietary fatty acids are subject to biohydrogenation by microbial enzymes in the rumen. This results in the conversion of much of the dietary unsaturated fatty acids into saturated fatty acids and also the production of fatty acids with conjugated double bonds (including *trans* types) such as conjugated linoleic acid. Since ruminants consume primarily lipids of plant origin in which

TABLE 4.2
Fatty Acid Composition (%) of Common Foods

Food	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1 Δ9	18:0	18:1 Δ9	18:2 Δ9	18:3 Δ9	20:5 Δ5	22:6 Δ4	Total Sat'd
Olive							13.7	1.2	2.5	71.1	10.0	0.6			16.2
Canola							3.9	0.2	1.9	64.1	18.7	9.2			5.5
Corn							12.2	0.1	2.2	27.5	57.0	0.9			14.4
Soybean						0.1	11.0	0.1	4.0	23.4	53.2	7.8			15.0
Linseed							4.8		4.7	19.9	15.9	52.7			9.5
Coconut		0.5	8.0	6.4	48.5	17.6	8.4		2.5	6.5	1.5				91.9
Cocoa						0.1	25.8	0.3	34.5	35.3	2.9				60.4
Butterfat	3.8	2.3	1.1	2.0	3.1	11.7	26.2	1.9	12.5	28.2	2.9	0.5			62.7
Beef fat				0.1	0.1	3.3	25.5	3.4	21.6	38.7	2.2	0.6			50.6
Pork fat				0.1	0.1	1.5	24.8	3.1	12.3	45.1	9.9	0.1			38.8
Chicken					0.2	1.3	23.2	6.5	6.4	41.6	18.9	1.3			31.1
Atlantic Salmon						5.0	15.9	6.3	2.5	21.4	1.1	0.6	1.9	11.9	23.4

Only the major fatty acids in these products are listed. All fatty acid compositions are adapted from White [93] with the exception of Atlantic Salmon, which is adapted from Ackman [1].

the fatty acids are primarily in the 18-carbon series, the end product of an exhaustive biohydrogenation pathway is stearic acid. Thus, butter as well as beef and sheep fats contain higher amounts of stearic acid than fats from nonruminants. Ruminal bacteria are also unique in that they can ferment carbohydrates to acetate and β-hydroxybutyrate. In the mammary gland, these substrates are converted to fatty acids to give butter fat a high concentration of saturated, short-chain (4:0 and 6:0) fatty acids that are not found in other food triacylglycerols. Ruminal bacteria also promote the formation of keto-, hydroxyl-, and branched-chain fatty acids. Because of the impact of ruminal bacteria on fatty acids, butter fat contains hundreds of different fatty acids.

The stereospecific location of fatty acids can also vary in food triacylglycerols. Triacylglycerols in some fats such as tallow, olive oil, and peanut oil have most of their fatty acids evenly distributed among all three positions of glycerol. However, some fats can have very specific trends for the stereospecific location of fatty acids. Many triacylglycerols from plants sources have the unsaturated fatty acids concentrated at the *sn*-2 position. The best example of this is cocoa butter, where over 85% of its oleic acid is *sn*-2, with palmitic and stearic acids being evenly distributed at *sn*-1 and *sn*-3. Triacylglycerols from animal fats tend to have saturated fatty acids concentrated at *sn*-2. For instance, palmitic acid is primarily at the *sn*-2 position in milkfat and lard. The stereospecific location of a fatty acid can be an important determinant on its impact in nutrition. When triacylglycerols are digested in the intestine, fatty acids from *sn*-1 and *sn*-3 are released by pancreatic lipase, resulting in two free fatty acids and an *sn*-2 monoacylglycerol. If long-chain saturated fatty acids are at *sn*-1 and *sn*-3, their bioavailability is lower because the free fatty acids can form insoluble calcium salts. Thus, placement of long-chain saturated fatty acids at *sn*-2 in milk fats may be a mechanism to ensure that these fatty acids are absorbed by infants. Since long-chain saturated fatty acids at *sn*-1 and *sn*-3 are absorbed inefficiently, they provide less calories [16] and have less impact on blood lipid profiles. For example, when lard has its fatty acids randomly distributed and thus has less palmitic acid at *sn*-2, it does not increase plasma palmitic acid or total lipid concentrations as high as unmodified lard where 65% of palmitic is at *sn*-2. This principle has been used to produce low-calorie triacylglycerols such as Salatrim (see Section 4.12).

4.2.9 Summary

- Fatty acids are the major building block of most food lipids.
- Fatty acids can be saturated or unsaturated, which affects their physical and biological properties.
- Food lipids vary widely in fatty acid composition as a function of the source of plant or animal from which the lipid is obtained.
- The position of fatty acids on triacylglycerols is also a function of the source of plant or animal from which the lipid is obtained.

4.3 LIPID REFINING

Triacylglycerols are extracted from both plant and animal sources. "Rendering" is a thermal processing operation that breaks down cellular structures to release the triacylglycerols from animal byproducts and oil-laden underutilized fish species. Plant triacylglycerols can be isolated by pressing (olives), solvent extraction (oilseeds), or a combination of the two (for a detailed discussion of fat and oil extraction see Reference 44). The resulting crude oils and fats from these processes will contain not only triacylglycerols but also lipids such as free fatty acids, phospholipids, lipid-soluble off-flavors and carotenoids, as well as nonlipid materials such as proteins and carbohydrates. These components must be removed to produce oils and fats with the desired color, flavor, and shelf-life. The major refining steps are described below.

4.3.1 Degumming

The presence of phospholipids will cause the formation of water-in-oil (W/O) emulsions in fats and oils. These emulsions will make the oil cloudy, and the water can present a hazard when the oils are heated to temperatures above 100°C (spattering and foaming). Phospholipids also contain amines that can interact with carbonyls to form browning products during thermal processing and storage. Degumming to remove phospholipids is accomplished by the addition of 1%–3% water at 60°C–80°C for 30–60 min. Small amounts of acid are often added to the water to increase the phospholipids' solubility. This occurs because the citric acid can bind calcium and magnesium, thereby decreasing phospholipid aggregation and making them more hydratable. Settling, filtering, or centrifugation is then used to remove the coalesced gums. With oils such as soybean, the phospholipids are recovered and sold as lecithin.

4.3.2 Neutralization

Free fatty acids must be removed from crude oils because they can cause off-flavors, decrease smoke point, accelerate lipid oxidation, cause foaming, and interfere with hydrogenation and interesterification operations. Neutralization is accomplished by reacting the oil with a solution of caustic soda and then removing the water containing the soaps of the free fatty acids. The amount of caustic soda used is dependent on the free fatty acid concentrations in the crude oil. The resulting soap stock can be used as animal feed or to produce surfactants and detergents.

4.3.3 Bleaching

Crude oils will contain pigments that produce undesirable colors (carotenoids, gossypol, etc.) and can promote lipid oxidation (chlorophyll). Pigments are removed by mixing the hot oil (80°C–110°C) with absorbents such as neutral clays, synthetic silicates, activated carbon, or activated earths. The absorbent is then removed by filtration. This process is usually done under vacuum since absorbents can accelerate lipid oxidation. An added benefit of bleaching is the removal of residual free fatty acids and phospholipids and the breakdown of lipid hydroperoxides.

4.3.4 Deodorization

Crude lipids contain undesirable aroma compounds such as aldehydes, ketones, and alcohols that occur naturally in the oil or are produced from lipid oxidation reactions that occur during extraction and refining. These volatile compounds are removed by subjecting the oil to steam distillation at high temperatures (180°C–270°C) at low pressures. Deodorization processes can also breakdown lipid hydroperoxides to increase the oxidative stability of the oil but can also result in the formation of *trans* fatty acids. The latter is the reason why most lipid-containing foods are not free of *trans* fatty acids. Oils can also be physically refined to remove both free fatty acids and off-flavors, thus skipping the neutralization step. This process requires higher temperatures, increases yield, but increases *trans* fatty acid formation [85]. After deodorization is complete, citric acid (0.005%–0.01%) is added to inactivate prooxidant metals. The deodorizer distillate will contain tocopherols and sterols, which can be recovered and used as antioxidants and functional food ingredients (phytosterols).

4.4 MOLECULAR INTERACTIONS AND ORGANIZATION OF TRIACYLGLYCEROLS

In the next few sections, we are primarily concerned with the molecular properties of lipids and their influence on food properties. In particular, we focus on how the structure, organization, and interactions of lipid molecules determine their functional properties (e.g., melting and crystallization,

surface activity, and interactions with other food components), which in turn determine the bulk physicochemical and sensory properties of food products (e.g., appearance, texture, stability, and flavor).

As discussed in previous sections, there are a number of different categories of lipids present in food systems, each with its own molecular characteristics. In this section, we will concentrate primarily on triacylglycerols because of their high natural abundance and major importance in food products. As mentioned earlier, triacylglycerols are esters of a glycerol molecule and three fatty acid molecules, and each fatty acid may have different numbers of carbon atoms and degrees of unsaturation in the hydrocarbon chain. The fact that there are many different types of fatty acid molecules, and that these fatty acids can be located at different positions on the glycerol backbone, means that there are a large number of possible triacylglycerol molecules present in foods. Indeed, edible fats and oils always contain a great many different types of triacylglycerol molecules, with the precise type and concentration depending on their origin [3,32,33].

Triacylglycerol molecules are portrayed as having a "tuning-fork" structure, with the two fatty acids at the ends of the glycerol molecule pointing in one direction, and the fatty acid in the middle pointing in the opposite direction (Figure 4.5). In the liquid state, there is considerable rotational freedom along the acyl chain where double bonds do not exist. They are predominantly nonpolar molecules, and so the most important types of molecular interaction that are responsible for their structural organization are van der Waals attraction and steric repulsion [43]. At a certain molecular separation (s^*), there is a minimum in the intermolecular pair potential $w(s^*)$ whose depth is a measure of the strength of the attractive interactions that hold the molecules together in the solid and liquid states (Figure 4.6). In the case of triacylglycerol molecules, s^* will be close to the distance between neighboring hydrocarbon chains. The structural organization of the molecules in triacylglycerols is primarily determined by its physical state, which depends on the balance between the attractive molecular interactions and the disorganizing influence of the thermal energy. Lipids tend to exist as liquids above their melting point and as solids at temperatures sufficiently below their melting point (Section 4.7).

Lipid molecules may adopt a variety of different structural organizations in both the solid and liquid states depending on their precise molecular characteristics, for example, chain length, degree of unsaturation, and polarity [37,38]. In the solid state, the organization of the lipid molecules may vary in a number of ways. Triacylglycerol molecules may stack together in crystals so that the height of the layers formed is approximately two (e.g., α and β-L2) or three (e.g., β-L3) fatty acid chains in dimensions (Figure 4.7). In addition, triacylglycerol molecules may pack together at different tilt angles with respect to the plane of the layers, for example, compare α and β-L2 (Figure 4.7). The crystals formed by triglyceride molecules can also be described in terms of the arrangement of the molecules within a "point lattice," for example, as hexagonal, triclinic, or orthorhombic (Figure 4.8). These differences mean that fat crystals can exist in a number of different polymorphic crystal forms, which have different physical properties and melting behavior (Section 4.7.5). The type of crystalline form adopted depends on the molecular structure and composition of the lipids, as well as on the environmental conditions during crystallization (cooling rate, holding

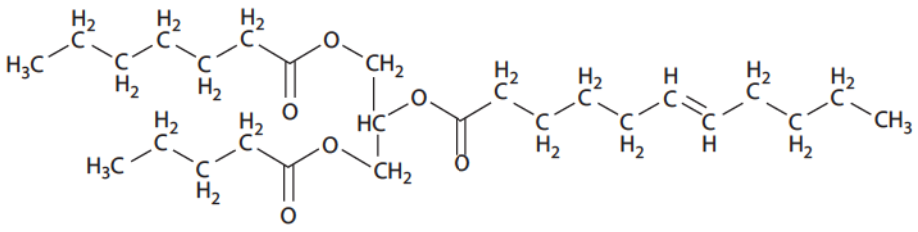


FIGURE 4.5 Chemical structure of a triacylglycerol molecule, which is assembled from three fatty acids and a glycerol molecule.

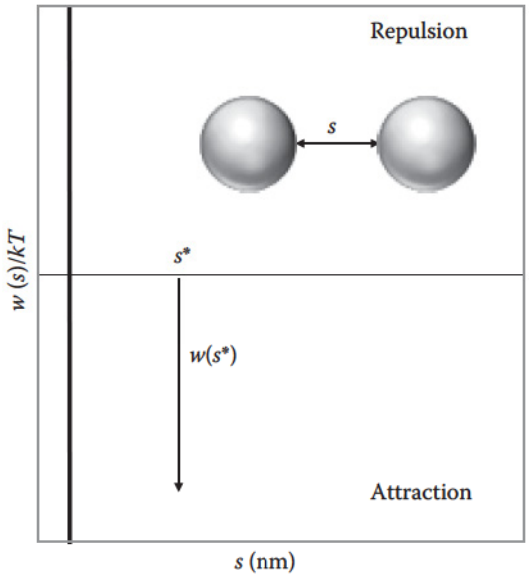


FIGURE 4.6 Strength of the attractive interactions between lipid molecules depends on the depth of the minimum in the overall molecular interaction potential.

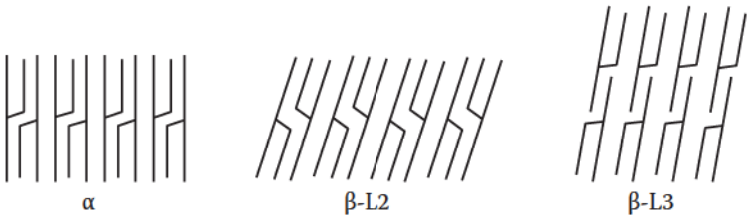


FIGURE 4.7 Common types of overall molecular organization of triacylglycerols within crystalline phases. (Adapted from Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

temperature, shearing). Even in the liquid state, triacylglycerols are not completely randomly orientated but have some order due to self-organization of the lipid molecules into structural entities, for example, lamellar structures [37,38]. The size and number of these structural entities are believed to decrease as the temperature is increased.

It should be noted that the term *fat* is conventionally used to refer to a lipid that is predominantly solid-like at room temperature (around 25°C), whereas the term *oil* is used to refer to a lipid that is liquid, although these terms are often used interchangeably [91,92].

4.5 PHYSICAL PROPERTIES OF TRIACYLGLYEROLS

The physical properties of edible fats and oils depend primarily on the molecular structure, interactions, and organization of the triacylglycerol molecules that they contain [32,56,91]. In particular, the strength of the attractive interactions between the molecules and the effectiveness of their packing within a condensed phase largely determine their thermal behavior, density, and rheological properties (Table 4.3).

4.5.1 Rheological Properties

Most liquid triacylglycerol oils are Newtonian liquids with viscosities between ~30 and 60 mPa·s at room temperature (around 25°C), for example, corn oil, sunflower oil, canola oils, and

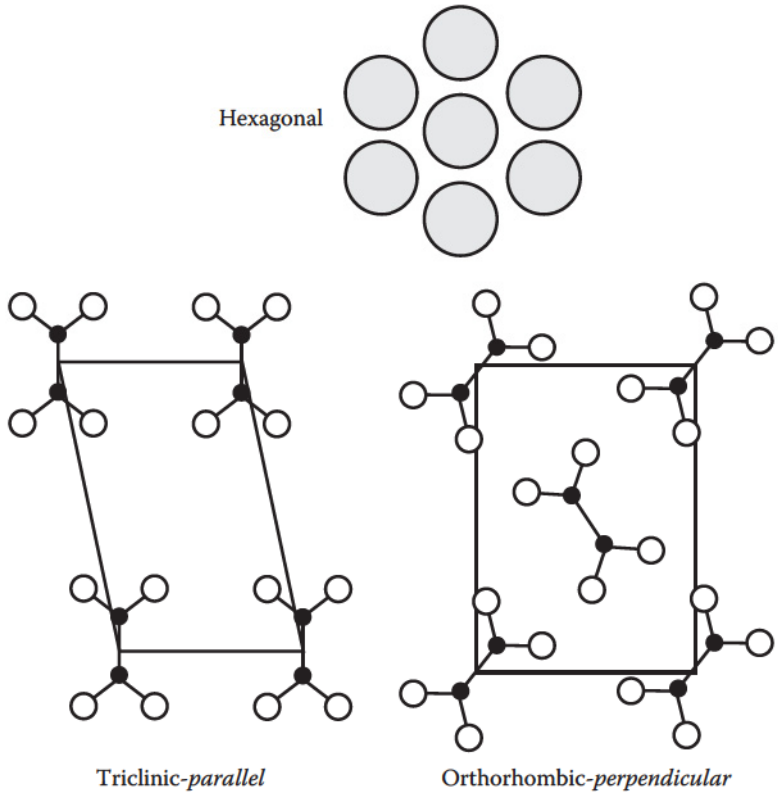


FIGURE 4.8 Three of the most common packing types of hydrocarbon chains: hexagonal, triclinic (*parallel*), and orthorhombic (*perpendicular*). For triclinic and orthorhombic packing, the black circles represent carbon atoms and the white circles represent hydrogen atoms. The hydrocarbon chains are viewed from the top. (Adapted from Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

TABLE 4.3
Comparison of Some Bulk Physicochemical Properties of a Liquid Oil (Triolein) and Water at 20°C

	Oil	Water
Molecular weight	885	18
Melting point (°C)	5	0
Density (kg m^{-3})	910	998
Compressibility	5.03×10^{-10}	4.55×10^{-10}
Viscosity (mPa·s)	≈50	1.002
Thermal conductivity (W m^{-1} K^{-1})	0.170	0.598
Specific heat capacity (J kg^{-1} K^{-1})	1980	4182
Thermal expansion coefficient ($°C^{-1}$)	7.1×10^{-4}	2.1×10^{-4}
Dielectric constant	3	80.2
Surface tension (mN m^{-1})	≈35	72.8
Refractive index	1.46	1.333

fish oil [15,21,78]. Nevertheless, castor oil tends to have a much higher viscosity than most other triacylglycerol oils because it contains an alcohol group along its hydrocarbon backbone, which is capable of forming hydrogen bonds with neighboring molecules. The viscosity of liquid oils tends to decrease steeply with increasing temperature and can be conveniently described by a logarithmic relationship.

Most "solid fats" actually consist of a mixture of fat crystals dispersed in a liquid oil matrix. The rheological properties of these solid fats are highly dependent on the concentration, morphology, interactions, and organization of the fat crystals present in the system [54,56,91]. Solid fats normally exhibit a type of rheological behavior known as "plasticity." A plastic material behaves like a solid below a critical applied stress, known as the yield stress (τ_0), but behaves like a liquid above this stress. The rheological behavior of an ideal plastic material, known as a *Bingham plastic,* is shown in Figure 4.9. For an applied *shear* stress, the rheological characteristics of this type of material can be described by the following equation:

$$\tau = G\gamma \quad (\text{for } \tau < \tau_0) \tag{4.1}$$

$$\tau - \tau_0 = \eta\dot{\gamma} \quad (\text{for } \tau \geq \tau_0) \tag{4.2}$$

where

τ is the applied shear stress
γ is the resultant shear strain
$\dot{\gamma}$ is the rate of shear strain
G is the shear modulus
η is the Bingham shear viscosity
τ_0 is the yield stress

In practice, solid fats tend to exhibit a nonideal plastic behavior. Above the yield stress, the fat may not flow like an ideal liquid and therefore exhibit non-Newtonian behavior, for example, shear thinning. Below the yield stress, the fat may also not behave like an ideal solid and exhibit some flow characteristics, for example, viscoelasticity. In addition, the yield stress may occur not at a well-defined value but over a range of stresses because there is a gradual breakdown of the fat crystal

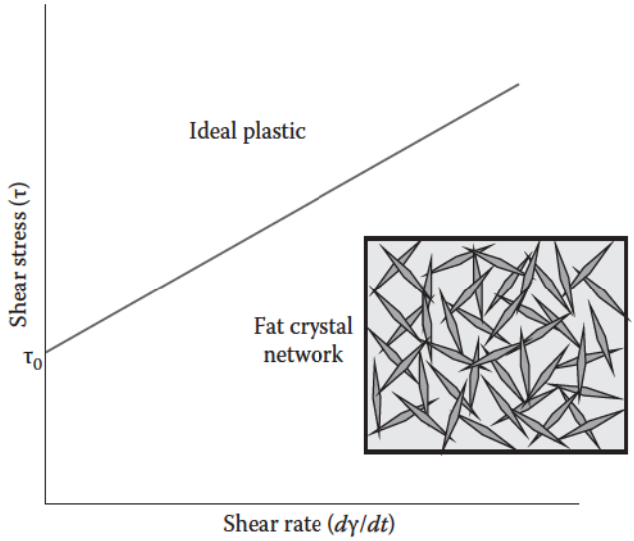


FIGURE 4.9 An ideal plastic material ("Bingham Plastic") behaves like a solid below a critical applied stress, known as the yield stress (τ_0), but behaves like a liquid above this stress.

network structure. The yield stress of a fat tends to increase with increasing solid fat content, and will be higher for crystal morphologies that are able to form three-dimensional networks that extend throughout the volume of the system more easily. Detailed discussions of the characteristics of plastic fats have recently been given elsewhere [54,56,91].

The structural origin of the plastic behavior of solid fats can be attributed to their ability to form a three-dimensional network of tiny fat crystals dispersed in a liquid oil matrix (Figure 4.9). Below a certain applied stress, there is a small deformation of the sample, but the weak bonds between the fat crystals are not disrupted. When the critical yield stress is exceeded, the weak bonds are broken and the fat crystals slide past one another, leading to flow of the sample. Once the force is removed, the flow stops, and the fat crystals begin to form new bonds with their neighbors. The rate at which this process occurs may have important implications for the functionality of the product, for example, the packing of margarines into containers after their production. In this case, it is important that the margarine flows into the container immediately after production and hardens later. The influence of the rheological characteristics of triacylglycerols on the physicochemical and sensory properties of foods is described later.

4.5.2 Density

The density of a lipid is the mass of material required to fill a given volume. This information is often important when designing food processing operations, since it determines the amount of material that can be stored in a tank or flow through a pipe of a given volume. The density of lipids is also important in certain food applications because it influences the overall properties of the system, for example, the creaming rate of oil droplets in oil-in-water (O/W) emulsions depends on the density difference between the oil and aqueous phases [59]. The densities of liquid oils tend to be ~910–930 kg m^{-3} at ambient temperature (around 25°C), and decrease with increasing temperature [15]. The densities of completely solidified fats tend to be ~1000–1060 kg m^{-3}, and also decrease with increasing temperature [78]. In many foods, the fat is partially crystalline and so the density depends on the solid fat content (SFC), that is, the fraction of the total fat phase that is solidified. The density of a partially crystalline fat tends to increase as the SFC increases, for example, after cooling below the crystallization temperature. Measurements of the density of a partially crystalline fat can therefore be used sometimes to determine its SFC.

The density of a particular lipid depends primarily on the efficiency of the packing of the triacylglycerol molecules within it: the more efficient the packing, the higher the density. Thus, triacylglycerols that contain linear saturated fatty acids are able to pack more efficiently that those that contain branched or unsaturated fatty acids, and so they tend to have higher densities [91,92]. The reason that solid fats tend to have higher densities than liquid oils is also because the molecules tend to be packed more efficiently. Nevertheless, this is not always the case in practice. For example, in lipid systems containing high concentrations of pure triacylglycerols that crystallize over a narrow temperature range, it has been shown that the density of the overall lipid system actually decreases upon crystallization because of void formation [39].

4.5.3 Thermal Properties

The most important thermal properties of lipids from a practical standpoint are the specific heat capacity (C_P), thermal conductivity (κ), melting point (T_{mp}), and enthalpy of fusion (ΔH_f) [21,32,78]. These thermal characteristics determine the total amount of heat that must be supplied (or removed) from a lipid system to change its temperature from one value to another, as well as the rate at which this process can be achieved. The specific heat capacities of most liquid oils and solid fats are ~2 J g^{-1}, and increase with increasing temperature [24]. Lipids are relatively poor conductors of heat and tend to have appreciably lower thermal conductivities (~0.165 W m^{-1} s^{-1}) than water

TABLE 4.4
Melting Points and Heats of Fusion of the Most Stable Polymorphic Forms of Selected Triacylglycerol Molecules

Triacylglycerol	Melting Point (°C)	ΔH_f (J g^{-1})
LLL	46	186
MMM	58	197
PPP	66	205
SSS	73	212
OOO	5	113
LiLiLi	–13	85
LnLnLn	–24	—
SOS	43	194
SOO	23	—

L = lauric acid (C12:0), M = myristic acid (C14:0), P = palmitic acid (C16:0), S = stearic acid (C16:0), O = oleic acid (C18:1), Li = linoleic (C18:2), Ln = linolenic (C18:3). The melting point also depends on polymorphic form, for example, for SSS it is 55°C, 63°C, and 73°C for the α, β′, and β forms, respectively. (Data from various sources.)

(~0.595 W m^{-1} s^{-1}). Detailed information about the thermal properties of different kinds of liquid and solid lipids has been provided elsewhere [15,24,34,78].

The melting point and heat of fusion of a lipid depend on the packing of the triacylglycerol molecules within the crystals formed: the more effective the packing, the higher the melting point and the enthalpy of fusion [43,91]. Thus, the melting points and heats of fusions of pure triacylglycerols tend to increase with increasing chain length, are higher for saturated than for unsaturated fatty acids, are higher for straight chained than for branched fatty acids, are higher for triacylglycerols with a more symmetrical distribution of fatty acids on the glycerol molecule, and are higher for more stable polymorphic forms (Table 4.4). The crystallization of lipids is one of the most important factors determining their influence on the bulk physicochemical and sensory properties of foods, and therefore it will be treated in some detail in Sections 4.7 and 4.9.

For some applications, knowledge of the temperature at which a lipid starts to breakdown due to thermal degradation is important, for example, frying or grilling. The thermal stability of lipids can be characterized by their smoke, flash, and fire points [64]. The *smoke point* is the temperature at which the sample begins to smoke when tested under specified conditions. The *flash point* is the temperature at which the volatile products generated by the lipid are produced at a rate where they can be temporarily ignited by the application of a flame but cannot sustain combustion. The *fire point* is the temperature at which the evolution of volatiles due to thermal decomposition occurs so quickly that continuous combustion can be sustained after the application of a flame. Measurements of these temperatures are particularly important when selecting lipids that are to be used at high temperatures, for example, during baking, grilling, or frying. The thermal stability of triacylglycerols is much better than that of free fatty acids; hence the propensity of lipids to breakdown during heating is largely determined by the amount of volatile organic material that they contain, such as free fatty acids.

4.5.4 Optical Properties

The optical properties of lipids are important practically because they influence the overall appearance of food materials (i.e., color and opacity), but also because they can be related to the molecular

characteristics of lipids so that their measurement can be used to assess oil quality [63]. The most important optical properties of lipids are their refractive index and absorption spectra. The refractive indices of liquid oils typically fall between 1.43 and 1.45 at room temperature (~25°C) [24]. The precise value of the refractive index of a particular liquid oil is mainly determined by the molecular structure of the triacylglycerols it contains. The refractive index tends to increase with the chain length, the number of double bonds, and the conjugation of double bonds. Empirical equations have been developed to relate the molecular structure of lipids to their refractive indices [24]. The refractive index of lipids is important in emulsified foods because the magnitude of the refractive index contrast determines the amount of light scattering and opacity [57].

The visible absorption spectra of an oil can have a pronounced influence on the final color of a food product. In addition, measurements of the absorption spectra may provide valuable information about the composition, quality, or properties of an oil, for example, the degree of unsaturation, extent of lipid oxidation, presence of impurities, and *cis–trans* isomerization [64]. Pure triacylglycerols tend to be colorless since they do not absorb any light in the visible region of the electromagnetic spectrum. Nevertheless, commercial oils tend to be colored because they contain appreciable amounts of pigmented impurities that absorb light, for example, carotenoids and chlorophyll. For this reason, oils often undergo a de-colorization step during their refinement.

4.5.5 Electrical Properties

Knowledge of the electrical properties of lipids is sometimes important because a number of analytical techniques used to analyze fatty foods are based on measurements of their electrical characteristics, for example, electrical conductivity measurements of fat concentration or electrical pulse counting of fat droplet size [59]. Lipids tend to have fairly low relative dielectric constants ($\varepsilon_R \approx 2–4$) because of the low polarity of triacylglycerol molecules (Table 4.3). The dielectric constant of pure triacylglycerols tends to increase with the chain length, polarity (e.g., due to the presence of –OH groups or to oxidation), and decreasing temperature [24]. Lipids also tend to be poor conductors of electricity, with relatively high electrical resistances.

4.6 SOLID FAT CONTENT OF FOOD TRIACYLGLYCEROLS

As mentioned earlier, edible triacylglycerols contain a variety of different fatty acids. If these fatty acids were randomly distributed on the glycerol backbone, the number of possible combinations of triacylglycerol molecules with different fatty acids at *sn*-1, *sn*-2, and *sn*-3 positions will depend on the number of different fatty acids in the lipid. The fatty acid combinations on triacylglycerols impact the liquid–solid phase transitions of the lipid since each triacylglycerol type has a different melting point. This means that food triacylglycerols do not typically have a sharp melting point, but, instead, they melt over a wide temperature range. This temperature range is often referred to as the "plastic range," since the existence of both liquid oil and solid fat usually gives the lipid rheological properties that are characterized as being plastic-like, that is, they act like a solid below a certain yield stress and as a liquid above this stress (Figure 4.9). While the term "plastic range" is commonly used, it is nevertheless possible that a fat can be partially crystalline and not have rheological properties that can be strictly classified as plastic. For example, a pourable lipid could contain nonaggregated fat crystals. The melting profile of triacylglycerols is commonly described in terms of the "solid fat content" (SFC), which specifies the fraction or percentage of lipid that is solid at a given temperature. Figure 4.10 shows the melting profile of a typical food triacylglycerol. At a sufficiently low temperature, the triacylglycerol is completely solid (SFC = 100%). As the temperature is increased, the fat enters the plastic range, with the shorter and more unsaturated triacylglycerols melting first, followed by the longer and more saturated ones until the lipid melts and is completely liquid (SFC = 0%). Due to the presence of different crystal types, the possibility of supercooling, and the solubility of high melting triacylglycerols in lower melting triacylglycerols, the melting

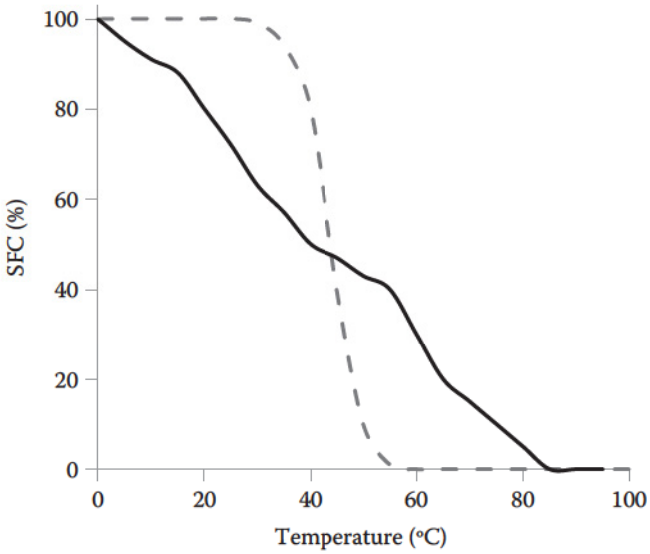


FIGURE 4.10 Comparison of the melting profile of a pure triacylglycerol and a typical edible fat. The edible fat melts over a much wider range of temperatures because it consists of a mixture of many different pure triacylglycerol molecules each with different melting points.

properties of lipids cannot be predicted directly from the triacylglycerol compositions [91]. The SFC of fatty foods is usually measured by calorimetry, changes in volume (dilatometry), or nuclear magnetic resonance (NMR) [64]. NMR is often the preferred method to measure SFC since it is rapid and non-destructive and requires little sample preparation.

Fatty acyl groups in natural fats are not usually randomly distributed. Some natural sources of fats have only a few combinations of different triacylglycerols, while others have numerous combinations of triacylglycerols. Fats that contain triacylglycerols with similar melting points tend to melt over a narrow temperature (plastic) range. These triacylglycerols will solidify into the most stable crystal states. On the other hand, fats that contain triacylglycerols and are more heterogeneous have a wide range of melting (plastic). Some lipids (butterfat) can have mixtures of high- and low-melting triacylglycerols, which produce a stepped rather than a smooth, continuous melting curve.

The solid fat content vs. temperature profile of an edible lipid is one of the most important factors determining its selection for a particular application since it affects many important functional attributes of food products. For example, it influences the appearance and stability of salad oils and dressings stored at refrigeration temperatures, the spreadability of margarines and butters under different conditions (e.g., refrigeration or ambient), the melting of chocolate in the mouth, and the texture of many baked products.

4.7 CRYSTALLIZATION OF TRIACYLGLYCEROLS

Solid–liquid phase transitions are an integral part of many processing operations used to produce food products, for example, margarine, butter, ice-cream, and whipped cream. The creation of food products with desirable properties therefore depends on an understanding of the major factors that influence the crystallization and melting of lipids in foods [37,38,56,91].

The contrasting arrangements of triacylglycerol molecules in the solid and liquid state are shown schematically in Figure 4.11. The physical state of a triacylglycerol at a particular temperature depends on its free energy, which is made up of contributions from both enthalpy and entropy terms: $\Delta G_{S\rightarrow L} = \Delta H_{S\rightarrow L} - T\Delta S_{S\rightarrow L}$ [5]. The enthalpy term ($\Delta H_{S\rightarrow L}$) represents the change in the overall strength of the molecular interactions between the triacylglycerols when they are converted from

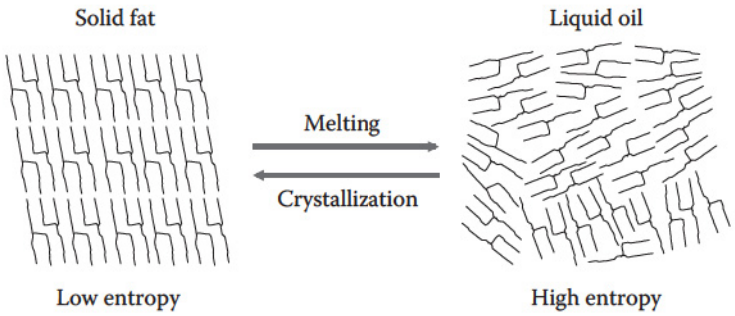


FIGURE 4.11 The arrangement of triacylglycerols in the solid and liquid states depends on a balance between the organizing influence of the attractive interactions between the molecules and the disorganizing influence of the thermal energy.

a solid to a liquid, whereas the entropy term ($\Delta S_{S\rightarrow L}$) represents the change in the randomness of the organization of the molecules brought about by the melting process. The strength of the bonds between the lipid molecules is greater in the solid state than in the liquid state because the molecules are able to pack more efficiently, and so $\Delta H_{S\rightarrow L}$ is positive (unfavorable), which favors the solid state. On the other hand, the entropy of the lipid molecules in the liquid state is greater than that in the solid state, and therefore $\Delta S_{S\rightarrow L}$ is positive (favorable), which favors the liquid state. At low temperatures, the enthalpy term dominates the entropy term ($\Delta H_{S\rightarrow L} > T\Delta S_{S\rightarrow L}$), and therefore the solid state has the lowest free energy [91]. As the temperature increases, the entropy contribution becomes increasingly important. Above a certain temperature, known as the *melting point*, the entropy term dominates the enthalpy term ($T\Delta S_{S\rightarrow L} > \Delta H_{S\rightarrow L}$) and so the liquid state has the lowest free energy. A material therefore changes from a solid to a liquid when its temperature is raised above the melting point. A solid-to-liquid transition (melting) is endothermic because energy must be supplied to the system to pull the molecules further apart. Conversely, a liquid-to-solid transition (crystallization) is exothermic because energy is released as the molecules come closer together. Even though the free energy of the solid state is lowest below the melting point, solid crystals may not appear until a liquid oil has been cooled well below the melting point because of a free energy penalty associated with nuclei formation.

Overall, the crystallization of fats can be conveniently divided into a number of stages: supercooling, nucleation, crystal growth, and post-crystallization events [37,38,55,91].

4.7.1 Supercooling

Although the solid form of a lipid is thermodynamically favorable at temperatures below its melting point, the lipid can persist in the liquid form below the melting point for a considerable time before any crystallization is observed. This is because of an activation energy (energy barrier) associated with nuclei formation that must be overcome before the liquid–solid phase transition can occur (Figure 4.12). If the magnitude of this activation energy is sufficiently high compared to the thermal energy, crystallization will not occur on an observable timescale, and the system will exist in a *metastable* state. The height of the activation energy barrier depends on the ability of crystal nuclei to be formed in the liquid oil that are stable enough to grow into crystals. The degree of supercooling of a liquid can be defined as $\Delta T = T_{mp} - T$, where T is the temperature and T_{mp} is the melting point. The value of ΔT at which crystallization is first observed depends on the chemical structure of the oil, the presence of any contaminating materials, the cooling rate, the microstructure of the lipid phase (e.g., bulk vs. emulsified oil), and the application of external forces [37,91]. Pure oils containing no impurities can often be supercooled by more than 10°C before any crystallization is observed.

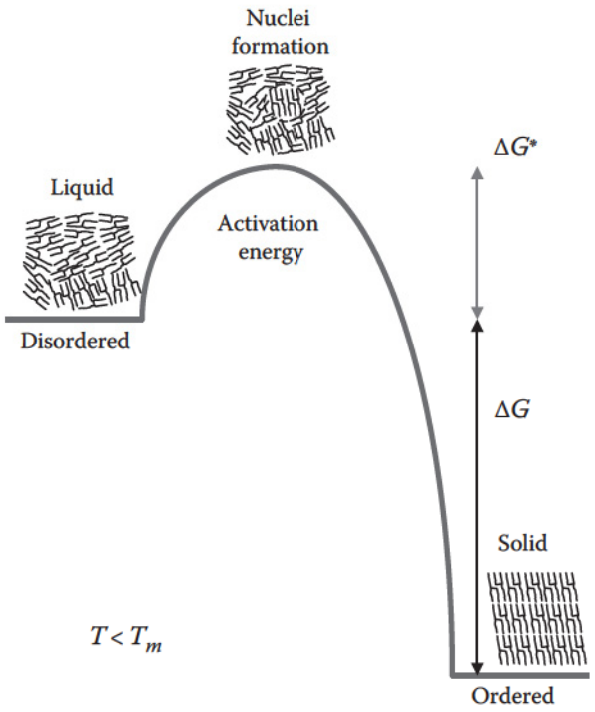


FIGURE 4.12 When the activation energy associated with nuclei formation is sufficiently high, a liquid oil can persist in a metastable state below the melting point of a fat.

4.7.2 Nucleation

Crystal growth can occur only after stable nuclei have been formed in a liquid. These nuclei are believed to be clusters of oil molecules that form small, ordered crystallites, and are formed when a number of oil molecules collide and become associated with each other [44,55]. There is a free energy change associated with the formation of one of these nuclei [37]. Below the melting point, the bulk crystalline state is thermodynamically favorable, and so there is a decrease in free energy when some of the oil molecules in the liquid cluster together to form a nucleus. This negative free energy (ΔG_V) change is proportional to the *volume* of the nucleus formed. On the other hand, the formation of a nucleus leads to the creation of a new interface between the solid and liquid phases, and this process involves an increase in free energy to overcome the interfacial tension. This positive free energy (ΔG_S) change is proportional to the *surface area* of the nucleus formed. The total free energy change associated with the formation of a nucleus is, therefore, a combination of a volume term and a surface term [91]:

$$\Delta G = \Delta G_V + \Delta G_S = \frac{4}{3}\pi r^3 \frac{\Delta H_{fus}\Delta T}{T_{mp}} + 4\pi r^2 \gamma_i \tag{4.3}$$

where

r is the radius of the nuclei

ΔH_{fus} is the enthalpy change per unit volume associated with the liquid-solid transition (which is negative)

γ_i is the solid–liquid interfacial tension

The volume contribution becomes increasingly negative as the size of the nuclei increases, whereas the surface contribution becomes increasingly positive (Figure 4.13). Hence, the surface contribution tends to dominate for small nuclei, while the volume contribution tends to dominate for

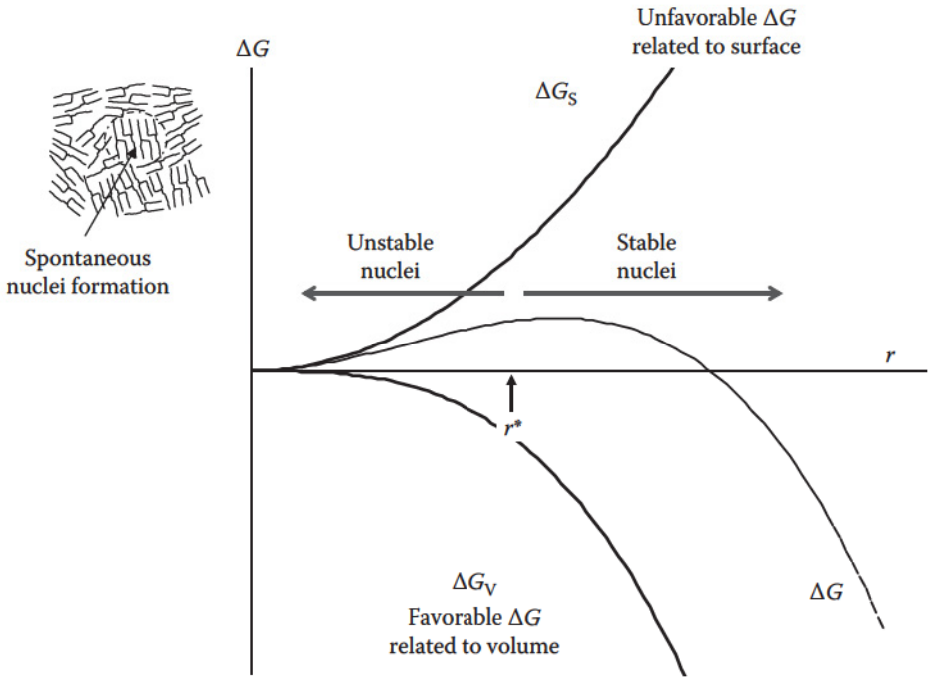


FIGURE 4.13 The critical size of a nucleus required for crystal growth depends on a balance between the volume and surface contributions to the free energy of nuclei formation. Nuclei that are spontaneously formed with radii below r^* grow, whereas those formed with radii below this value dissociate.

large nuclei. As a result, the overall free energy change associated with nuclei formation has a maximum value at a critical nucleus radius (r^*):

$$r^* = \frac{2\gamma_i T_{mp}}{\Delta H_{fus} \Delta T} \tag{4.4}$$

If a nucleus is spontaneously formed and has a radius that is below this critical size, then it will tend to dissociate so as to reduce the free energy of the system. On the other hand, if a nucleus is formed that has a radius above this critical value, then it will tend to grow into a crystal. This equation indicates that the critical size of nuclei required for crystal growth decreases as the degree of supercooling increases, which accounts for the increase in nucleation rate that is observed experimentally when the temperature is decreased.

The rate at which nucleation occurs can be mathematically related to the activation energy ΔG^* that must be overcome before stable nuclei are formed [37]:

$$J = A \exp\left(\frac{-\Delta G^*}{kT}\right) \tag{4.5}$$

where

J is the nucleation rate, which is equal to the number of stable nuclei formed per second per unit volume of material
A is a pre-exponential factor
k is Boltzmann's constant
T is the absolute temperature

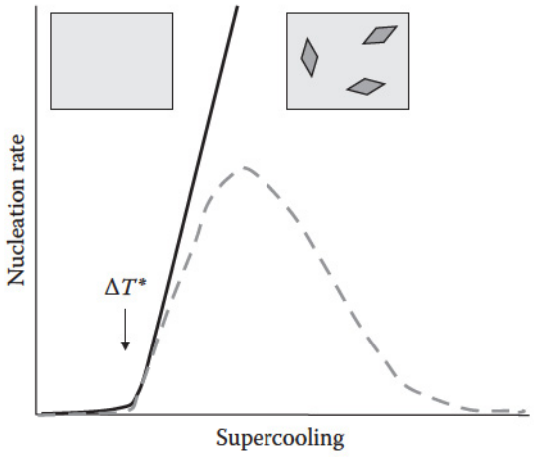


FIGURE 4.14 Theoretically, the rate of the formation of stable nuclei increases with supercooling (solid line), but in practice the nucleation rate decreases below a particular temperature because the diffusion of oil molecules is retarded by the increase in oil viscosity (broken line).

The value of ΔG^* is calculated by replacing r in Equation 4.3 with the critical radius given in Equation 4.4. The variation of the nucleation rate predicted by Equation 4.5 with the degree of supercooling (ΔT) is shown in Figure 4.14. The formation of stable nuclei is negligibly slow at temperatures just below the melting point, but increases dramatically when the liquid is cooled below a certain temperature T^*. In reality, the nucleation rate is observed to increase with the degree of cooling down to a certain temperature, after which it decreases upon further cooling. This is because the increase in viscosity of the oil that occurs as the temperature is decreased slows down the diffusion of oil molecules toward the liquid–nucleus interface [8,37]. Consequently, there is a maximum in the nucleation rate at a particular temperature (Figure 4.14).

The type of nucleation described above occurs when there are no impurities present in the oil, and is usually referred to as *homogeneous nucleation* [37,40]. If the liquid oil is in contact with foreign surfaces, such as the surfaces of dust particles, fat crystals, oil droplets, air bubbles, reverse micelles, or the vessel containing the oil, then nucleation can be induced at a higher temperature than expected for a pure system. Nucleation due to the presence of these foreign surfaces is referred to as *heterogeneous nucleation*, and can be divided into two types: primary and secondary [55]. Primary heterogeneous nucleation occurs when the foreign surfaces have a different chemical structure to that of the oil, whereas secondary heterogeneous nucleation occurs when the foreign surfaces are crystals with the same chemical structure as the liquid oil. Secondary heterogeneous nucleation is the basis for "seeding" nucleation in supercooled lipids. This process involves adding preformed triacylglycerol crystals to a supercooled liquid consisting of the same triacylglycerol so as to promote nucleation at a higher temperature than would otherwise be possible.

Heterogeneous nucleation occurs when the impurities provide a surface at which the formation of stable nuclei is more thermodynamically favorable than in the pure oil. As a result, the degree of supercooling required to initiate fat crystallization is reduced. On the other hand, certain types of impurities are capable of decreasing the nucleation rate of oils because they are incorporated into the surface of the growing nuclei and prevent any further oil molecules being incorporated [82]. Whether an impurity acts as a catalyst or an inhibitor of nucleation depends on its molecular structure and interactions with the nuclei. It should be noted that there is still considerable debate about the mathematical modeling of nucleation, since existing theories often give predictions of nucleation rates that are greatly different from experimental measurements [38,40,91]. Nevertheless, the general form of the dependence of nucleation rates on temperature is predicted fairly well by existing theories (see Figure 4.14).

4.7.3 Crystal Growth

Once stable nuclei have formed, they grow into crystals by incorporating molecules from the liquid oil at the solid–liquid interface [40]. Lipid crystals have a number of different faces, and each face may grow at an appreciably different rate. This partially accounts for the wide variety of different crystal morphologies that can be formed by lipids. The overall crystal growth rate depends on a number of factors, including mass transfer of the lipid molecules to the solid–liquid interface, mass transfer of noncrystallizing species away from the interface, incorporation of the liquid molecules into the crystal lattice, or removal of the heat generated by the crystallization process from the interface [37]. Any of these processes can be rate-limiting depending on molecular and physical properties of the lipids and the prevailing environmental conditions, for example, viscosity, thermal conductivity, crystal structure, temperature profile, and mechanical agitation. Consequently, a general theoretical model of crystal growth is difficult to construct. In crystallizing lipid systems, the incorporation of a molecule at the crystal surface is often rate-limiting at high temperatures, whereas the diffusion of a molecule to the solid–liquid interface is often rate-limiting at low temperatures. This is because the viscosity of the liquid oil increases as the temperature is lowered, and so the diffusion of a molecule is retarded. The crystal growth rate therefore tends to increase initially with increasing degree of supercooling until it reaches a maximum rate, after which it decreases [37]. The dependence of the growth rate on temperature therefore shows a similar trend to the nucleation rate; however, the temperature-dependence of the nucleation rate is usually different from that of the crystal growth rate (Figure 4.15). This difference accounts for the dependence of the number and size of crystals produced on the cooling rate and holding temperature. If a liquid oil is cooled to a temperature at which the nucleation rate is slower than the growth rate, then a small number of large crystals will be formed. On the other hand, if it is cooled to a temperature at which the growth rate is slower than the nucleation rate, then there will be a large number of small crystals formed. Experimentally, it has been observed

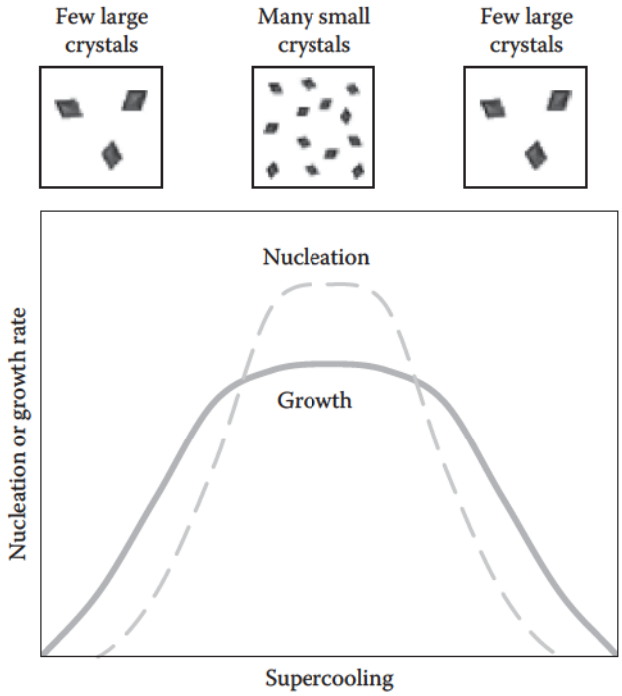


FIGURE 4.15 The nucleation and crystal grow rates have different temperature dependencies, which accounts for differences in the number and size of fat crystals produced under different cooling regimes.

that the rate of crystal growth is proportional to the degree of supercooling and inversely proportional to the viscosity of the melt [37,86].

A variety of mathematical theories have been developed to model the rate of crystal growth in crystallizing fats [37,38]. The most appropriate model for a specific situation depends on the rate-limiting step for that particular system under the prevailing environmental conditions, for example, mass transfer of the liquid molecules to the solid–liquid interface, mass transfer of the noncrystallizing species away from the interface, incorporation of the liquid molecules into the crystal lattice, or removal of the heat generated by the crystallization process from the interface. In practice, it is often difficult to develop fundamental models because of the complexity in mathematically describing the numerous physicochemical processes involved.

4.7.4 Post-Crystallization Events

Once the crystals have been formed in a lipid system, changes in their packing, size, composition, and interactions are likely to occur [37,91]. Post-crystallization events may involve a change from a less stable to a more stable polymorphic form due to rearrangement of the triacylglycerol molecules within the crystals. If a lipid forms mixed crystals (i.e., crystals that contain a mixture of different types of triacylglycerols), then there may be a change in the composition of the crystals during storage due to diffusion of triacylglycerol molecules between the crystals. There may also be a net growth in the average size of the crystals within a lipid with time due to Ostwald ripening, that is, growth of the large crystals at the expense of the smaller ones due to diffusion of oil molecules between the crystals. Finally, the bonds between fat crystals may strengthen over time during storage due to a sintering mechanism, that is, fusion of the crystals together. These post-crystallization changes can have pronounced influences on the bulk physicochemical and sensory properties of foods, and therefore it is important to understand and control them. For example, post-crystallization events often lead to an increase in the size of the crystals in a lipid, which is undesirable because it leads to a gritty perception during consumption.

4.7.5 Crystal Morphology

The morphology of the crystals depends on a number of internal (e.g., molecular structure, composition, packing, and interactions) and external factors (e.g., temperature–time profile, mechanical agitation, and impurities). In general, when a liquid oil is cooled rapidly to a temperature well below its melting point, a large number of small crystals are formed, but when it is cooled slowly to a temperature just below its melting point, a smaller number of larger crystals are formed [37,91]. This is because of the differences in the temperature dependences of the nucleation and crystallization rates (Figure 4.15). The nucleation rate tends to increase more rapidly with decreasing temperature than the crystallization rate up to a certain maximum value, and then it tends to decrease more rapidly with a further decrease in temperature. Thus, rapid cooling tends to produce many nuclei simultaneously, which subsequently grow into small crystals, whereas slow cooling tends to produce a smaller number of nuclei that have time to grow into larger crystals before more nuclei are formed (Figure 4.15). Crystal size has important implications for the rheology and organoleptic properties of many types of foods. When the crystals are too large, they are preceived as being "grainy" or "sandy" in the mouth. The efficiency of molecular packing in crystals also depends on the cooling rate. If a fat is cooled slowly, or the degree of supercooling is small, then the molecules have sufficient time to be efficiently incorporated into a crystal. At faster cooling rates, or higher degrees of the molecules have insufficient time to pack efficiently before another molecule is incorporated. Thus, rapid cooling tends to produce crystals that contain more dislocations, and in which the molecules are less densely packed [86]. The cooling rate therefore has an important impact on the morphology and functional properties of crystalline lipids in foods.

4.7.6 Polymorphism

Triacylglycerols exhibit a phenomenon known as *polymorphism*, which is the ability of a material to exist in a number of crystalline structures with different molecular packing [50,55,76]. Triacylglycerol molecules may pack into fat crystals in a number of different ways, leading to different polymorphic forms (Figures 4.7 and 4.8) having different physicochemical properties. The three most commonly occurring types of packing in triacylglycerols are hexagonal, orthorhombic, and triclinic, which are usually designated as α, β′, and β polymorphic forms, respectively [37,91]. The thermodynamic stability of the three forms decreases in the order β > β′ > α. Even though the β-form is the most thermodynamically stable, triacylglycerols often crystallize in the α-form initially because it has the lowest activation energy for nuclei formation (Figure 4.16). With time, the crystals transform to the most stable polymorphic form at a rate that depends on environmental condition such as temperature, pressure, and the presence of impurities. The conversion from one polymorphic form to another can be monitored using methods such as differential scanning calorimetry (DSC), which measures changes in the heat released (exothermic) or absorbed (endothermic) by a material when a phase transition occurs (Figure 4.17). In this example, when a fat initially in the α-form is heated, it undergoes a series of transitions: α–β′ transformation; β′ melting; β crystallization; and β melting. The time taken for these types of crystal transformation to occur is strongly influenced by the homogeneity of the triacylglycerol composition. The transition from the α-form tends to occur fairly rapidly for relatively homogeneous compositions where the triacylglycerols all have fairly similar molecular structures. On the other hand, the transition is relatively slow for multicomponent fats where the triacylglycerols have diverse molecular structures. The different types of polymorphic forms of lipids can be distinguished from each other using a variety of methods, including x-ray diffraction, DSC, as well as infrared (IR), NMR, and Raman spectroscopy [37,55,91]. These methods are largely based on the fact that different polymorphic forms have different unit cells, which can be categorized by their height, width, and angles of tilt (Figure 4.18). Knowledge of the polymorphic form of the crystals in lipids is often important because it can have a large impact on the thermal behavior and morphology of the crystals formed, and therefore on the physicochemical and sensory properties of foods. For example, the desirable textural characteristics

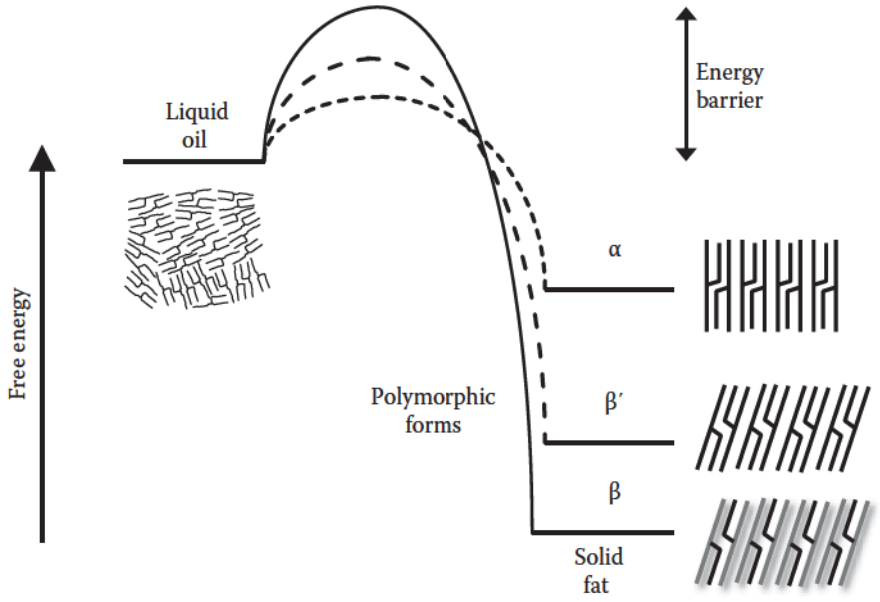


FIGURE 4.16 The polymorphic state that is initially formed when an oil crystallizes depends on the relative magnitude of the activation energies associated with nuclei formation.

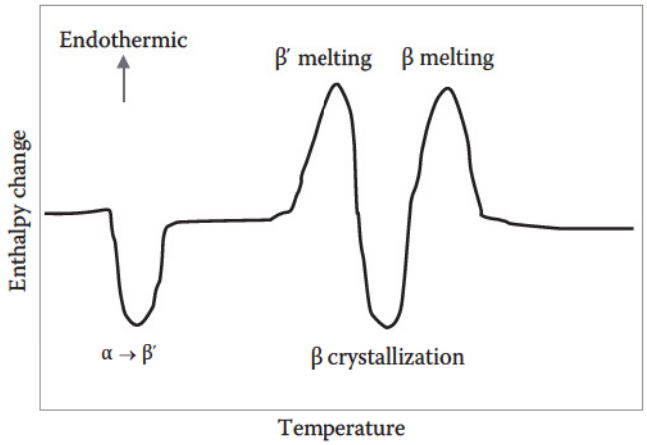


FIGURE 4.17 Polymorphic changes can be monitored using differential scanning calorimetry. This DSC thermogram shows transitions from one polymorph to another (α–β′), as well as melting and crystallization of different polymorphs (β or β′).

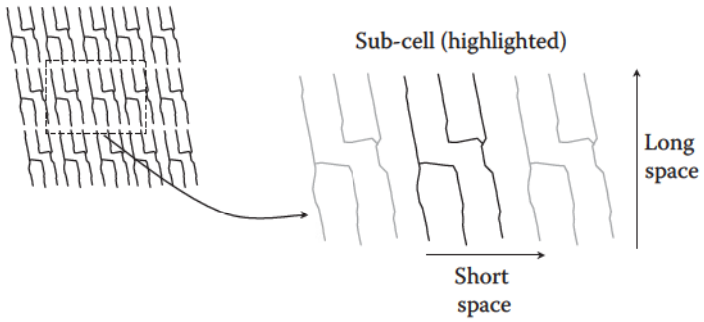


FIGURE 4.18 The unit cells in crystalline lipids can be characterized by their dimensions.

and appearance of chocolate depends on ensuring that the fat crystals are produced and maintained in the appropriate polymorphic form [2]. Edible lipids from different biological sources or that have been processed differently (e.g., fractionation, interesterification, or hydrogenation) often tend to adopt preferred polymorphic forms, typically β, or β′, since the α form is usually unstable [3]. Lipids that adopt a β′ form (such as palm oil and many hydrogenated oils) tend to give smaller crystals with a smoother texture, which is desirable in spreads and shortenings. On the other hand, lipids that adopt a β form often give larger crystals with a more gritty texture, which is undesirable for these applications.

4.7.7 Crystallization of Edible Fats and Oils

The melting point of a triacylglycerol depends on the chain length and the degree of unsaturation of its constituent fatty acids, as well as their relative positions along the glycerol molecule (Table 4.4). Edible fats and oils contain a complex mixture of numerous types of triacylglycerol molecules, each with a different melting point, and so they usually melt/solidify over a wide range of temperatures, rather than at a distinct temperature as would be the case for a pure triacylglycerol (Figure 4.10).

The melting profile of a fat is not simply the weighted sum of the melting profiles of its constituent triacylglycerols, because high-melting-point triacylglycerols are soluble in lower melting point ones [91]. For example, in a 50:50 mixture of tristearin and triolein it is possible to dissolve 10% of

solid tristearin in liquid triolein at 60°C. The solubility of a solid component in a liquid component can be predicted by assuming that they have widely differing melting points (>20°C):

$$\ln x = \frac{\Delta H_{fus}}{R}\left[\frac{1}{T_{mp}} - \frac{1}{T}\right] \tag{4.6}$$

where

x is the solubility, expressed as a mole fraction, of the higher melting point component in the lower melting point component

ΔH_{fus} is the molar heat of fusion

The structure and physical properties of crystals produced by cooling a complex mixture of triacylglycerols are strongly influenced by the cooling rate and temperature [37,55,91]. If an oil is cooled rapidly, all the triacylglycerols crystallize at nearly the same time and a *solid solution* is formed, which consists of homogeneous crystals in which the triacylglycerols are intimately mixed with each other. On the other hand, if the oil is cooled slowly, the higher melting point triacylglycerols crystallize first, while the low melting point triacylglycerols crystallize later, and so *mixed crystals* are formed. These crystals are heterogeneous and consist of some regions that are rich in high-melting-point triacylglycerols and other regions that are depleted in these triacylglycerols. Whether a crystalline fat forms mixed crystals or a solid solution influences many of its physicochemical properties, such as density, rheology, and melting profile, which could have an important influence on the properties of a food product. The type of crystal formed is influenced by the molecular compatibility of the various triacylglycerol molecules in the system, which depends on the chain length, unsaturation, and position of the fatty acids. A detailed review of the thermodynamic and kinetic aspects of fat crystallization and the type of crystal structures formed in mixed systems is given elsewhere [40]. Typically, a lipid may exhibit four different types of phase behavior depending on the nature of the triacylglycerol molecules present: (1) monotectic continuous solid solutions, (2) eutectic systems, (3) monotectic partial solid solutions, and (4) peritectic systems. A discussion of these different systems and the characteristics of lipid mixtures that typically lead to them is given in the article by Himawan et al. [40].

Once a fat has crystallized, the individual crystals may aggregate to form a three-dimensional network that traps liquid oil through capillary forces [55]. The interactions responsible for crystal aggregation in pure fats are primarily van der Waals interactions between the solid fat crystals, although "water bridges" may also play an important role in some products. Once aggregation has occurred, the fat crystals may partially fuse together, which strengthens the crystal network.

4.7.8 Fat Crystallization in Emulsions

The influence of fat crystallization on the bulk physicochemical properties of food emulsions depends on whether the fat forms the continuous phase or the dispersed phase. The characteristic stability and rheological properties of W/O emulsions, such as butter and margarine, are determined by the presence of a network of aggregated fat crystals within the continuous (oil) phase. The fat crystal network is responsible for preventing water droplets from sedimenting under the influence of gravity, as well as determining the textural attributes of the product. If there are too many fat crystals present, the product is firm and difficult to spread, but when there are too few crystals present, the product is soft and collapses under its own weight [91]. Selection of a fat with the appropriate melting characteristics is therefore one of the most important aspects of margarine and spread production. The melting profile of natural fats can be optimized for specific applications by various physical or chemical methods, including blending, interesterification, fractionation, and hydrogenation [32–34].

Fat crystallization also has a pronounced influence on the physicochemical properties of many O/W emulsions, such as milk or salad dressings. When the fat droplets are partially crystalline, a crystal from one droplet can penetrate into another droplet during a collision, which causes the two droplets to stick together. This phenomenon is known as *partial coalescence*, and leads to a dramatic increase in the viscosity of an emulsion, as well as a decrease in the stability to creaming [27]. Extensive partial coalescence can eventually lead to phase inversion, that is, conversion of an O/W emulsion to a W/O emulsion. This process is one of the most important steps in the production of butters, margarines, and spreads. Partial coalescence is also important in the production of ice cream and whipped creams, where an O/W emulsion is cooled to a temperature at which fat in the droplets partially crystallizes and is mechanically agitated to promote droplet collisions and aggregation [30]. The aggregated droplets form a two-dimensional network around the air bubbles and a three-dimensional network in the continuous phase, which contributes to the stability and texture of the product.

4.8 ALTERING THE SOLID-FAT CONTENT OF FOOD LIPIDS

Natural fats with desirable plastic ranges are not always available and are sometimes expensive. In addition, alteration of fatty acid profiles is often desirable to make the fat less susceptible to oxidation (decrease unsaturation) or more nutritionally desirable (increased unsaturation). Therefore, several technologies have been developed to alter the chemical structure and solid fat content of food lipids.

4.8.1 Blending

The simplest method to alter fatty acid composition and melting profile is by blending fats with different triacylglycerol compositions. This practice is performed in products such as frying oils and margarines.

4.8.2 Dietary Interventions

The fatty acid composition of animal fats can be altered by manipulation of the type of fats in the animal's diet. This practice is effective in nonruminants such as pigs, poultry, and fish. Increasing the levels of unsaturated fatty acids in fats from ruminants (cows and sheep) is not very efficient because bacteria in the rumen biohydrogenate the fatty acids before they reach the small intestine where they can be absorbed into the blood.

4.8.3 Genetic Manipulation

The fatty acid composition of fats can be manipulated genetically by altering the enzyme pathways that produce unsaturated fatty acids. Genetic manipulation has been done successfully by both traditional breeding programs and by genetic modification technologies. Several oils that have been obtained from genetically altered plants are commercially available. Most of these oils contain elevated levels of oleic acid.

4.8.4 Fractionation

The fatty acid and triacylglycerol composition of fats can also be altered by holding the fat at a temperature where the most saturated or long chain triacylglycerols will crystallize and then collecting either the solid (more saturated or long-chain) or liquid (more unsaturated or short-chain) phases. This is commonly done to vegetable oils in a process called *winterization*. Winterization is

necessary for oils used in products that are refrigerated to prevent the triacylglycerols from crystallizing and becoming cloudy. Winterization is also necessary for oils used in mayonnaise or salad dressings where crystallization would destabilize the emulsion. Palm, palm kernel (olein/stearin), butterfat, and fish oils are commonly fractionated to change their fatty acid composition.

4.8.5 Hydrogenation

Hydrogenation is a chemical process that adds hydrogen to double bonds. The process is used to alter lipids so they become more solid at room temperature, exhibit different crystallization behavior (by making them more compositionally homogeneous), and/or are more oxidatively stable. These goals are accomplished by the removal of double bonds to make the fatty acids more saturated. An additional use of hydrogenation is to bleach oils since the destruction of double bonds in compounds such as carotenoids will cause them to lose color. Products produced by hydrogenation include shortenings and partially hydrogenated oils that have improved oxidative stability.

The hydrogenation reaction requires a catalyst to speed up the reaction, hydrogen gas to provide the substrate, temperature control to initially heat up the oil to make it liquid and then cool the oil once the exothermic reaction is started, and agitation to mix the catalyst and substrates [45]. The oil used in hydrogenation must first be refined since contaminants will reduce the effectiveness of the catalysts. Hydrogenation is done as a batch or continuous process at temperatures ranging from 250°C to 300°C. Reduced nickel is the most common catalyst that is added at 0.01%–0.02%. The nickel is incorporated onto a porous support to provide a catalyst with high surface area that can be recovered by filtration. Mixing is a critical parameter since mass transfer of the reactants limits the reaction. The reaction takes 40–60 min, during which progress is monitored by change in the refractive index. Upon completion, catalysts are recovered by filtration so that they can be used in another reaction.

The mechanism of hydrogenation involves the unsaturated fatty acid associating with the catalyst, with metal–carbon complexes being formed at each end of the double bond (Figure 4.19, step 1). Hydrogen that is absorbed to the catalysts can then break one of the carbon–metal complexes to form a half-hydrogenated state, with the other carbon remaining linked to the catalysts (step 2). To complete hydrogenation, the half-hydrogenated state interacts with another hydrogen to break the remaining carbon–catalyst bond to produce a hydrogenated fatty acid (step 3). However, if hydrogen is not available, the reverse reaction can occur and the fatty acid is released from the catalyst and the double bond reforms (step 4). The double bond that reforms can be in the *cis* or *trans* configuration and can be at the same carbon number, or it can migrate to the adjacent carbon (e.g., a fatty acid with a double bond originally between carbons 9 and 10 can migrate to carbons 8 and 9 or 10 and 11). The propensity of the double bond to re-form is related to the concentration of hydrogen associated with the catalyst, with low hydrogen concentrations leading to the re-formation of the double bond and thus production of geometric and positional isomers. Thus, conditions such as low hydrogen pressure, low agitation, high temperature (reaction is faster than rate of hydrogen diffusion to the catalyst), and high catalyst concentrations (difficult to saturate catalyst with hydrogen) result in high levels of geometric and positional isomers. This can be problematic since *trans* fatty acids are negatively associated with cardiovascular disease.

The selectively of hydrogenation refers to the tendency of the hydrogenation process to hydrogenate one fatty acid faster than another (compared to random hydrogenation where all unsaturated fatty acids would be hydrogenated at similar rates). Hydrogenation of the most unsaturated fatty acids first is often desirable since this increases the oxidative stability of the oil with minimal formation of high-temperature-melting saturated triacylglycerols that cause problems with crystallization and texture. Selectivity occurs because the hydrogenation rate of polyunsaturated fatty acids is faster than monounsaturated fatty acids (partially due to the higher catalyst affinity

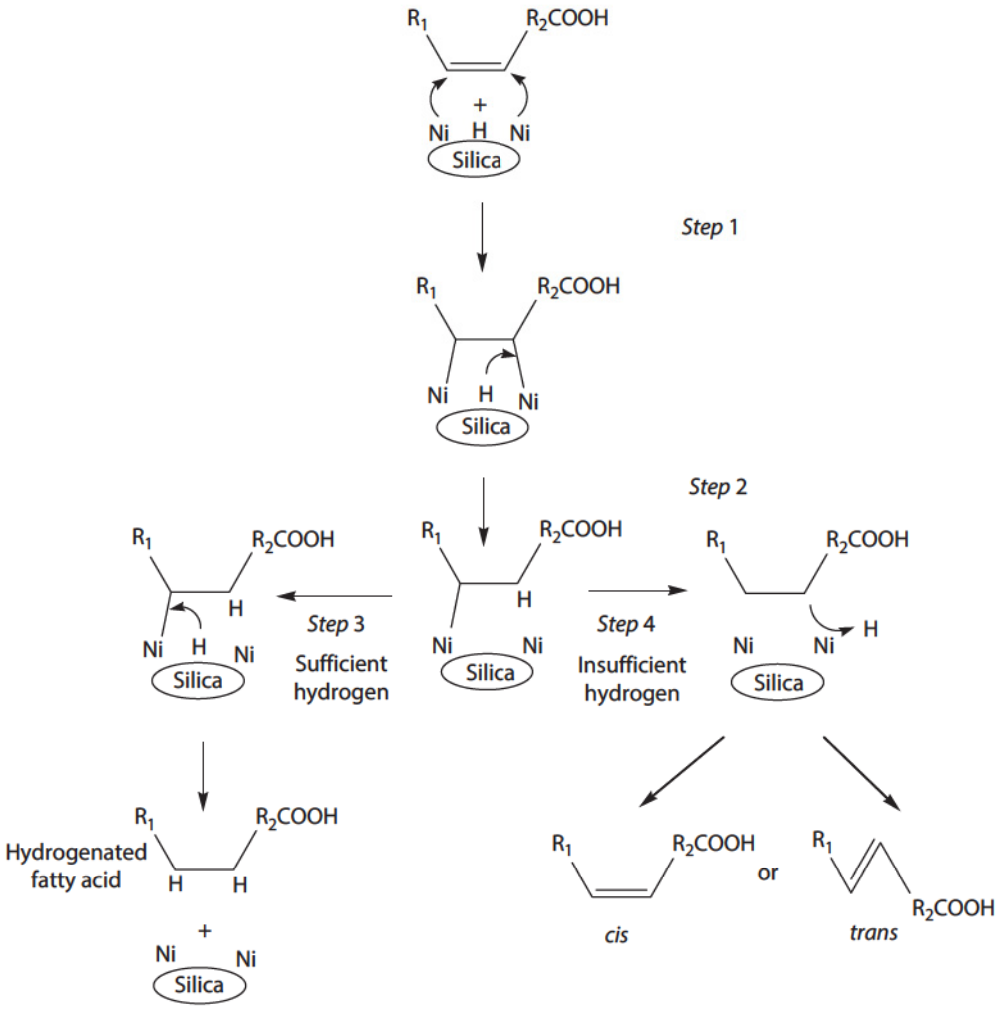


FIGURE 4.19 The pathways involved in hydrogenation that lead to formation of saturated fatty acids and *cis* and *trans* unsaturated fatty acids.

for pentadiene double bond systems than monounsaturated fatty acids). When hydrogen concentrations at the catalyst are low, hydrogenation is selective since polyunsaturated fatty acids are more rapidly hydrogenated than monounsaturated fatty acids. However, low hydrogen conditions also lead to high production of geometric and positional isomers, meaning that the lipid can contain high amounts of *trans* fatty acids.

4.8.6 Interesterification

Interesterification is a process that can change the melting profile of lipids without changing fatty acid composition [32,33]. Random interesterification works by rearranging the fatty acids to increase the number of different triacylglycerol types. Upon completion of random interesterification, all possible triacylglycerol combinations will be produced. This results in a change in the melting profile as new triacylglycerols are produced. In addition to alteration in melting profile, interesterification will also alter the crystallization behavior by making it more difficult for the lipid to form the most stable crystal type (e.g., β) since the triacylglycerol composition becomes more heterogeneous. Interesterification can be performed on mixtures of lipids such as a fat with a high-temperature melting range and an oil with a low-temperature melting range. If these two

lipid sources were simply blended, their melting profile could have a stair step appearance as the oil would melt first followed by the fat. Interesterification of these two lipids would create new triacylglycerols, with combinations of saturated and unsaturated fatty acids producing gradual melting throughout the plastic range. Another application would be to interesterify a lipid with a very homogeneous triacylglycerol composition to increase the heterogeneity of the triacylglycerols, a process that would widen the plastic range and make it more difficult for the lipid to form the most stable crystal types.

Interesterification does not always have to be random [32]. In directed interesterification, the reaction temperature is held low enough so that when highly saturated triacylglycerols are produced, they crystallize and are removed from participating in the reaction. This process would produce a liquid phase that is more unsaturated and a solid phase that is more saturated than the parent lipid. Interesterification can also be catalyzed by lipases. The advantage of lipases is that they can have specificity for different stereospecific locations on the triacylglycerol or for different fatty acids. This means that structured triacylglycerols can be produced with changes in fatty acid composition or triacylglycerol type (e.g., changes at *sn*-2 position). By altering fatty acid and/or triacylglycerol composition, these fats may have superior nutritional or physical properties. Interesterification can be performed by acidolysis, alcoholysis, glycerolysis, and transinteresterification [32]. Transinteresterification is the most common method used to alter the properties of food lipids. Alkylates of sodium (e.g., sodium ethylate) are commonly used to accelerate interesterification since they are inexpensive and active at low temperatures. The real catalyst for the reaction is thought to be a carbonyl anion of a diacylglycerol (Figure 4.20). The negative diacylglycerol can attack the slightly positive carbonyl group of a fatty acid on a triacylglycerol to form a transition-state complex. When interesterification takes place, the transition-state complex decomposes to transfer the fatty acid to the negative diacylglycerols plus the transfer of the anion to the site of the transferred fatty acid. This process can occur within the same (intraesterification) or a different (interesterification) triacylglycerol. To interesterify triacylglycerols, they must be very low in water, free fatty acids, and peroxides, which deactivate the catalyst. Random interesterification is performed at 100°C–150°C and is complete in 30–60 min. The reaction is stopped by the addition of water to inactivate the catalyst.

Interesterification was initially limited by its high cost and the low value of the resulting products such as shortenings and margarines. However, since the *trans* fatty acid labeling requirements prompted the removal of partially hydrogenated fats in foods, the utilization of interesterified oils has grown since their *trans* fatty acid composition is low because it is similar to their parent fats and oils.

4.8.7 Summary

Alteration of fatty acid profiles can alter the melting properties, oxidative stability, and nutritional quality of lipids.

- Alteration of fatty acids profiles can be accomplished by blending different sources or lipids or chemically modifying the fatty acid structure or triacylglycerol composition.
- Hydrogenation removes double bonds from fatty acids, which increases the melting ranges and oxidative stability.
- Hydrogenation can form nutritionally undesirable *trans* fatty acids.
- Interesterification rearranges fatty acids on triacylglycerol, which can alter the melting ranges.
- Interesterification can be used to produce solid fats with minimal *trans* fatty acid concentrations.

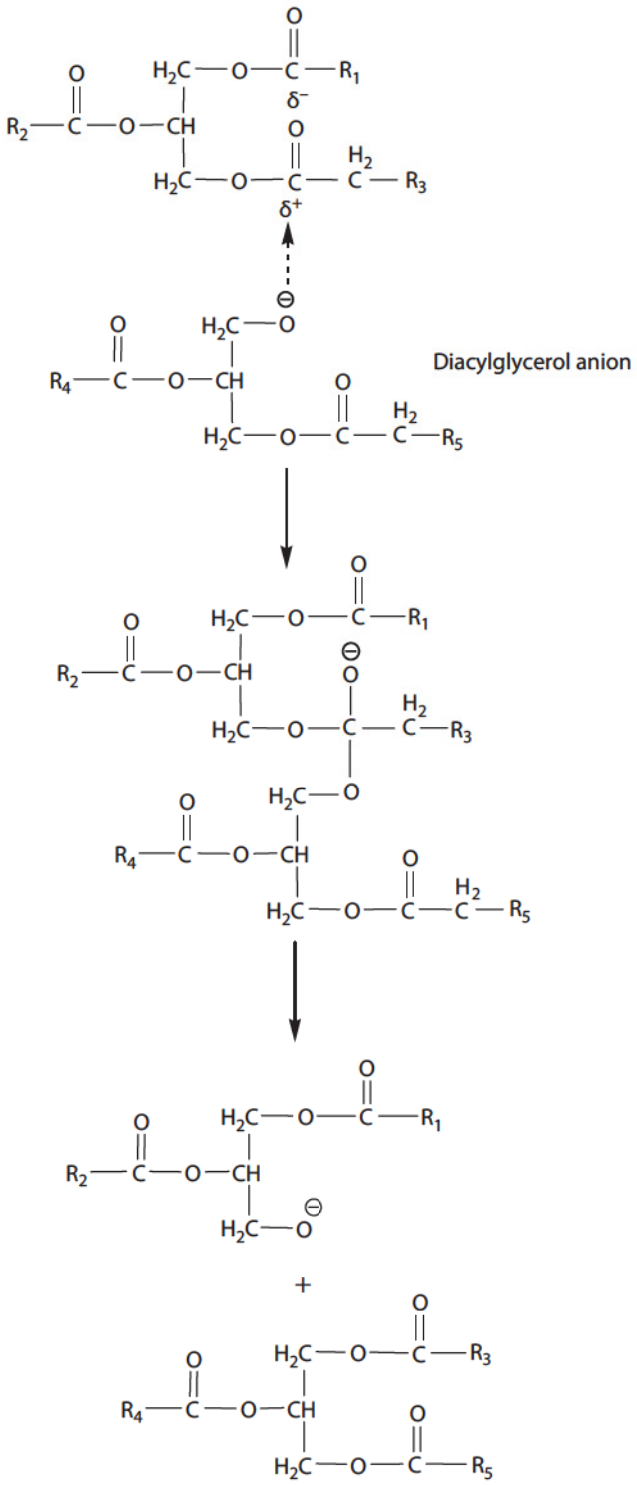


FIGURE 4.20 Proposed mechanism of the interesterification reaction involving catalysis by the carbonyl anion of a diacylglycerol. (Adapted from Shahidi, F. and Wanasundara, J.P.K., *Crit. Rev. Food Sci. Nutr.*, 32, 67, 1992.)

4.9 FUNCTIONALITY OF TRIACYLGLYCEROLS IN FOODS

The ability of food scientists to improve the quality of food products depends on an improved understanding of the multiple roles that fats and oils play in determining their properties.

4.9.1 Texture

The influence of lipids on the texture of foods is largely determined by the nature of the lipid (e.g., solid vs. liquid) and the nature of the food matrix (e.g., bulk fat, emulsified fat, or structural fat). For liquid oils, such as cooking or salad oils, the texture is determined primarily by the viscosity of the oil over the temperature range of utilization. For partially crystalline fats, such as in chocolate, baked products, shortenings, butter, and margarine, the texture is mainly determined by the concentration, morphology, and interactions of the fat crystals [55]. In particular, the melting profile of the fat crystals plays a major role in determining properties such as texture, stability, spreadability, and mouthfeel. In O/W emulsions, the viscosity of the overall system is determined mainly by the concentration of oil droplets present rather than by the viscosity of the oil within the droplets [58]. The characteristic creamy texture of many food emulsions is determined by the presence of fat droplets, for example, creams, desserts, dressings, and mayonnaise. In W/O emulsions, the overall rheology of the system is largely determined by the rheology of the oil phase. In most food W/O emulsions, such as margarine, butter, and spreads, the oil phase is partially crystalline and has plastic-like properties. The rheology of these products is therefore determined by the solid fat content as well as the morphology and interactions of the fat crystals present, which in turn are governed by the crystallization and storage conditions [55]. For example, the "spreadability" of W/O emulsions such as margarines and butters is determined by the formation of a three-dimensional network of aggregated fat crystals in the continuous phase, which provides the product with mechanical rigidity. In many foods, the lipids form an integral part of a solid matrix that also contains various other components, for example, chocolate, cakes, cookies, crackers, biscuits, and cheese. The physical state of the lipids in these systems often plays an important role in determining their rheological properties, for example, firmness and snap.

4.9.2 Appearance

The characteristic appearance of many food products is strongly influenced by the presence of lipids. The appearance of pure lipids, such as cooking or salad oils, is mainly determined by the presence of pigmented impurities that absorb light, such as chlorophyll and carotenoids. Solid fats are often optically opaque because of the scattering of light by the fat crystals. The opacity of the fat depends on the concentration and size of the fat crystals present. The turbid, cloudy, or opaque appearance of food emulsions is a direct result of the immiscibility of oil and water, since this leads to a system where the droplets of one phase are dispersed in the other phase. Food emulsions usually appear optically opaque because the light passing through them is scattered by the droplets [57]. The intensity of the scattering depends on the concentration, size, and refractive index of the droplets present, so that both the color and opacity of food emulsions are strongly influenced by the presence of the lipid phase.

4.9.3 Flavor

The perceived flavor of a food is strongly influenced by the type and concentration of the lipids present. Triacylglycerols are relatively large molecules that have a low volatility and hence little inherent flavor. Nevertheless, different natural sources of edible fats and oils do have distinctive flavor profiles because of the characteristic volatile breakdown products and impurities that they contain. The flavor of many food products is also indirectly influenced by the presence of lipids because flavor

compounds can partition between oil, water, and gaseous phases according to their polarities and volatilities [57]. For this reason, the perceived aroma and taste of foods are often strongly influenced by the type and concentration of the lipids present.

Lipids also influence the mouthfeel of many food products [91,92]. Liquid oils may coat the tongue during mastication, which provides a characteristic oily mouthfeel. If a lipid phase contains fat crystals that are above a certain size, then an undesirable "gritty" mouthfeel is detected. The melting of fat crystals in the mouth causes a cooling sensation, which is an important sensory attribute of many fatty foods [88,89].

4.10 CHEMICAL DETERIORATION OF LIPIDS, HYDROLYTIC REACTIONS

Free fatty acids cause problems in foods because they can produce off-flavor, reduce oxidative stability, cause foaming, and reduce the smoke point (the temperature at which an oil begins to smoke). If the formation of free fatty acids results in the development of off-flavors (e.g., formation of short-chain free fatty acids in dairy products), this is known as *hydrolytic rancidity*. However, free fatty acids are sometimes desirable in products such as cheeses where they contribute to the flavor profiles.

Free fatty acids can be produced by enzymes called lipases. In living tissues, the activity of lipases is strictly controlled, since fatty acids can be cytotoxic by disrupting cellular membrane integrity. During the processing and storage of the biological tissues used as raw materials for foods, cellular structure and biochemical control mechanisms can be destroyed, and lipases can become active. A good example of this is seen in the production of olive oil, where the oil from the first pressing is low in free fatty acid concentrations. Oils from subsequent pressing and extraction of the pomace have higher free fatty acid concentrations as the cellular matrix is further disrupted and the activated lipases have time to hydrolyze the triacylglycerols. Triacylglycerol hydrolysis also occurs in frying oils due to the high processing temperatures and the introduction of water from the fried food. As the free fatty acid content of the frying oil increases, smoke point and oxidative stability decrease, and the tendency for foaming increases. Commercial frying oils are filtered on a regular basis with absorbents that are capable of binding free fatty acids to increase the useable life of the oil. Triacylglycerol hydrolysis will also occur at extreme pH values.

4.11 CHEMICAL DETERIORATION OF LIPIDS, OXIDATIVE REACTIONS

"Lipid oxidation" is a general term that is used to describe a complex sequence of chemical changes that result from the interaction of lipids with oxygen [25,61]. Triacylglycerols and phospholipids have low volatility and thus do not directly contribute to the aroma of foods. During lipid oxidation reactions, the fatty acids esterified to triacylglycerols and phospholipids will decompose to form small, volatile molecules that produce the off-aromas known as *oxidative rancidity*. In general, these volatile compounds are detrimental to food quality, although there are some food products such as fried foods, dried cereal, and cheeses where small amounts of lipid oxidation products are important positive components of their flavor profile.

4.11.1 Chemical Pathway

The centerpiece of these reactions is the molecular species known as *free radicals*. Free radicals are molecules or atoms that have unpaired electrons. Free-radical species can vary greatly in their energy. Radicals such as the hydroxyl radical ($^{\bullet}OH$) have very high energy and can oxidize virtually any molecule by causing hydrogen abstraction. Other molecules, such as the antioxidant α-tocopherol, can form free radicals with low energy. These antioxidants can slow down oxidation reactions by forming low-energy radicals that cannot attack molecules such as unsaturated fatty acids.

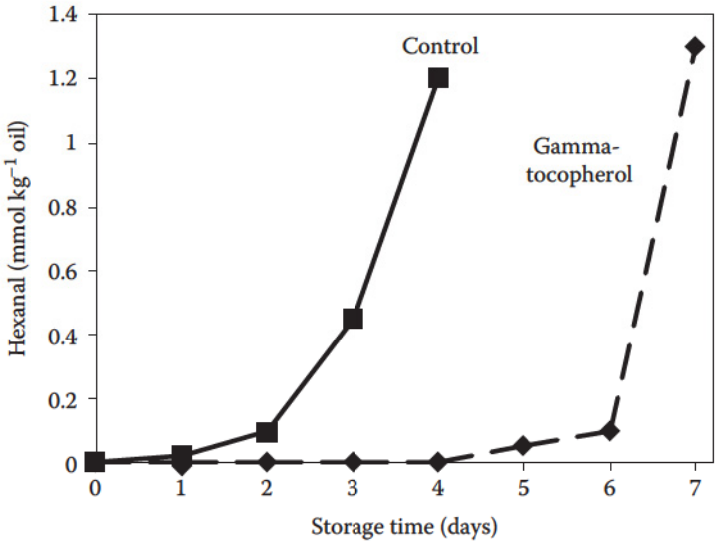


FIGURE 4.21 Impact of gamma tocopherol on the lag phase of the oxidation of corn oil-in-water emulsion. (Adapted from Huang, S.W. et al., *J. Agric. Food Chem.*, 42, 2108, 1994.)

The kinetics of lipid oxidation in foods often has a lag phase followed by an exponential increase in oxidation rate. The length of the lag phase is very important to food processors since this is the period where rancidity is not detected and the quality of the food is high. Once the exponential phase is reach, lipid oxidation proceeds rapidly and off-aroma development quickly follows. The length of the lag phase of oxidation will increase with decreasing temperature, oxygen concentrations, fatty acid unsaturation, activity of prooxidants, and increasing concentrations of antioxidants. Figure 4.21 shows how delta-tocopherol can increase the lag phase of the oxidation of a corn O/W emulsion [42].

The pathway of lipid oxidation has been simplified by dividing the reaction into three steps: initiation, propagation, and termination.

4.11.1.1 Initiation

This step describes the abstraction of a hydrogen from a fatty acid to form a fatty acid radical known as the alkyl radical (L•). Once the alkyl radical forms, the free radical is stabilized by its delocalization over the double bonds, resulting in double bond shifting and, in the case of polyunsaturated fatty acids, the formation of conjugated double bonds. This shift in location can produce double bonds in either the *cis* or *trans* configuration, with the latter predominating due to its greater stability. Figure 4.22 shows the initiation steps for hydrogen abstraction from the methylene-interrupted carbon of linoleic acid with double bond rearrangement, producing two isomers. When hydrogen is abstracted from oleic acid, the alkyl radical can exist at four different locations (Figure 4.23).

The ease of formation of fatty acid radicals increases with increasing unsaturation. The bond dissociation energy for the carbon–hydrogen covalent bond in an aliphatic chain is 98 kcal mol^{-1}. If a carbon atom is adjacent to an electron-rich double bond, the carbon–hydrogen covalent bond becomes weaker with the bond dissociation energy deceasing to 89 kcal mol^{-1}. In polyunsaturated fatty acids, the double bonds are in a pentadiene configuration with a methylene-interrupted carbon (Figure 4.24). Since the carbon–hydrogen covalent bond of the methylene-interrupted carbon is weakened by two double bonds, its bond dissociation energy is even lower at 80 cal mol^{-1}. As the bond dissociation energy of the carbon–hydrogen bond decreases, hydrogen abstraction becomes easier and lipid oxidation is faster. Linoleic acid (18:2) has been estimated to be 10–40 times more

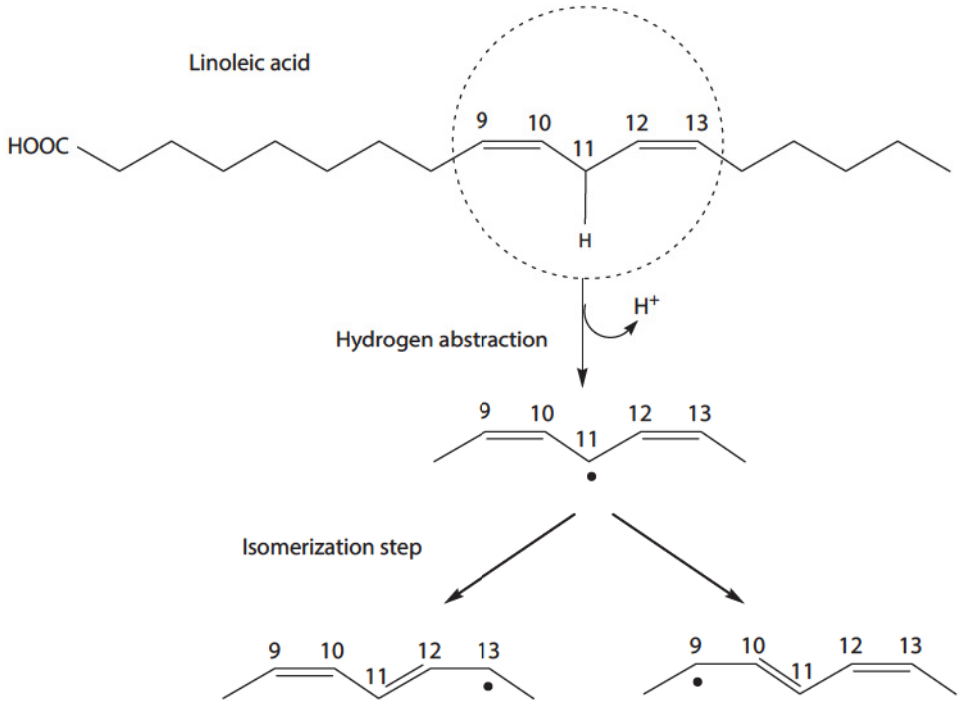


FIGURE 4.22 Initiation step of lipid oxidation for linoleic acid.

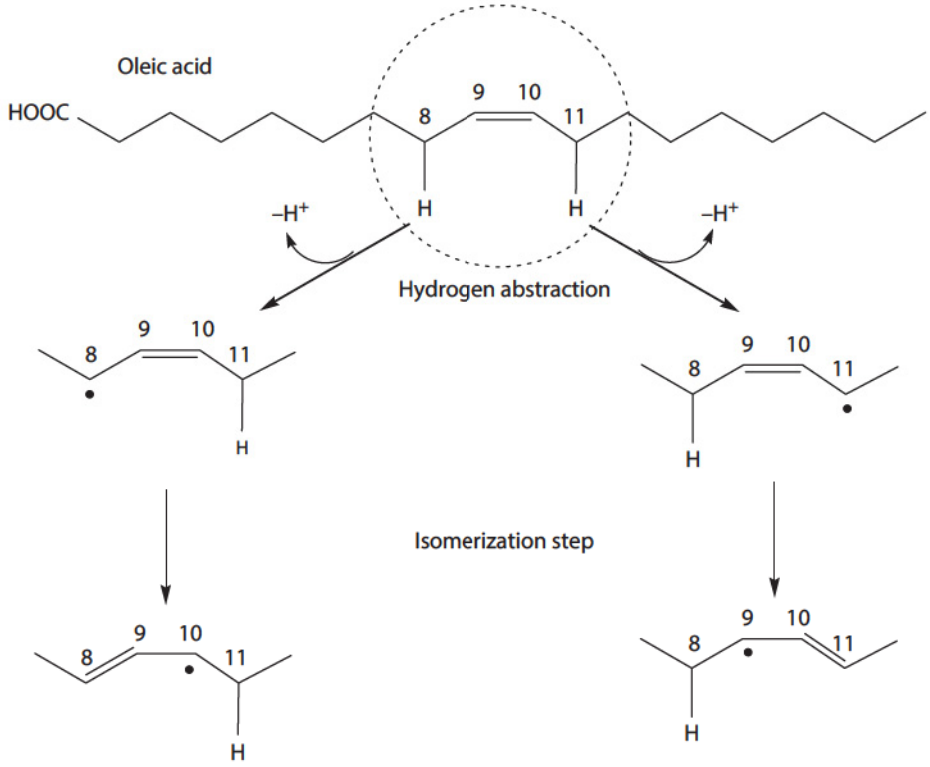


FIGURE 4.23 Initiation step of lipid oxidation for oleic acid.

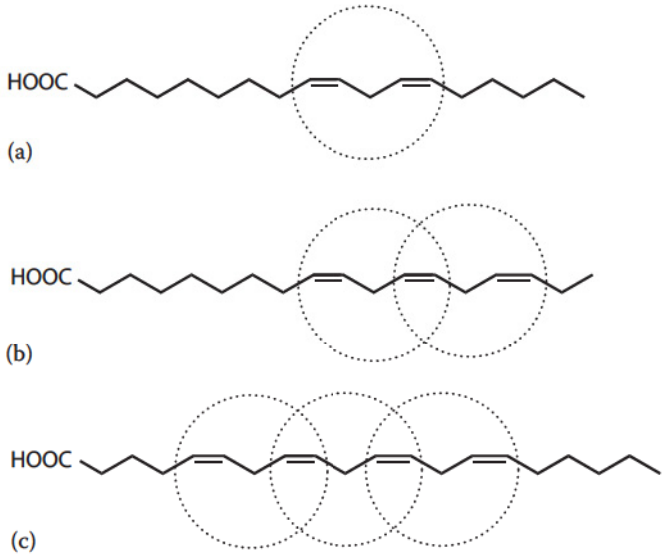


FIGURE 4.24 Pentadienes of (a) linoleic, (b) linolenic, and (c) arachadonic acids.

susceptible to oxidation than oleic acid (18:1). As additional double bonds are added onto polyunsaturated fatty acids, an additional methylene-interrupted carbon is added, resulting in the addition of another site for hydrogen abstraction. For example, linoleic (18:2) has one methylene-interrupted carbon, while linolenic (18:3) has two and arachadonic (20:4) has three (Figure 4.24). In most cases, oxidation rates double with the addition of a methylene-interrupted carbon. Thus, linolenic oxidizes twice as fast as linoleic and arachidonic oxidizes twice as fast as linolenic (four times faster than linoleic).

4.11.1.2 Propagation

The first step of propagation involves the addition of oxygen to the alkyl radical. Atmospheric or triplet oxygen is a biradical because it contains two electrons with the same spin direction that cannot exist in the same spin orbital. The free radicals on triplet oxygen are of low energy and rarely cause hydrogen abstraction. However, the free radicals on oxygen can react with the alkyl radical at a diffusion-limited rate. The combination of the alkyl radical with one of the radicals on triplet oxygen results in the formation of a covalent bond. The other radical on the oxygen remains free. The resulting radical is known as a *peroxyl radical* (LOO•). The high energy of peroxyl radicals allows them to promote the abstraction of a hydrogen from another molecule. Since the carbon–hydrogen covalent bond of unsaturated fatty acids is weak, they are susceptible to attack from peroxyl radicals. Hydrogen addition to the peroxyl radical results in the formation of a fatty acid hydroperoxide (LOOH) and the formation of a new alkyl radical on another fatty acid. Thus the reaction is propagated from one fatty acid to another. A schematic of this pathway for two linoleic molecules is shown in Figure 4.25. The location of the lipid hydroperoxide will correspond to the location of the original alkyl radicals, and thus linoleate will produce four hydroperoxides, and oleate will form two.

4.11.1.3 Termination

This reaction describes the combination of two radicals to form nonradical species. In the presence of oxygen, the predominant fatty acid is the peroxyl radical, since oxygen will be added onto alkyl radicals at diffusion-limited rates. Thus, under atmospheric conditions, termination reactions will occur between two peroxyl radicals. In low oxygen environments (e.g., frying oils), termination reactions can occur between alkyl radicals to form fatty acid dimers (Figure 4.26).

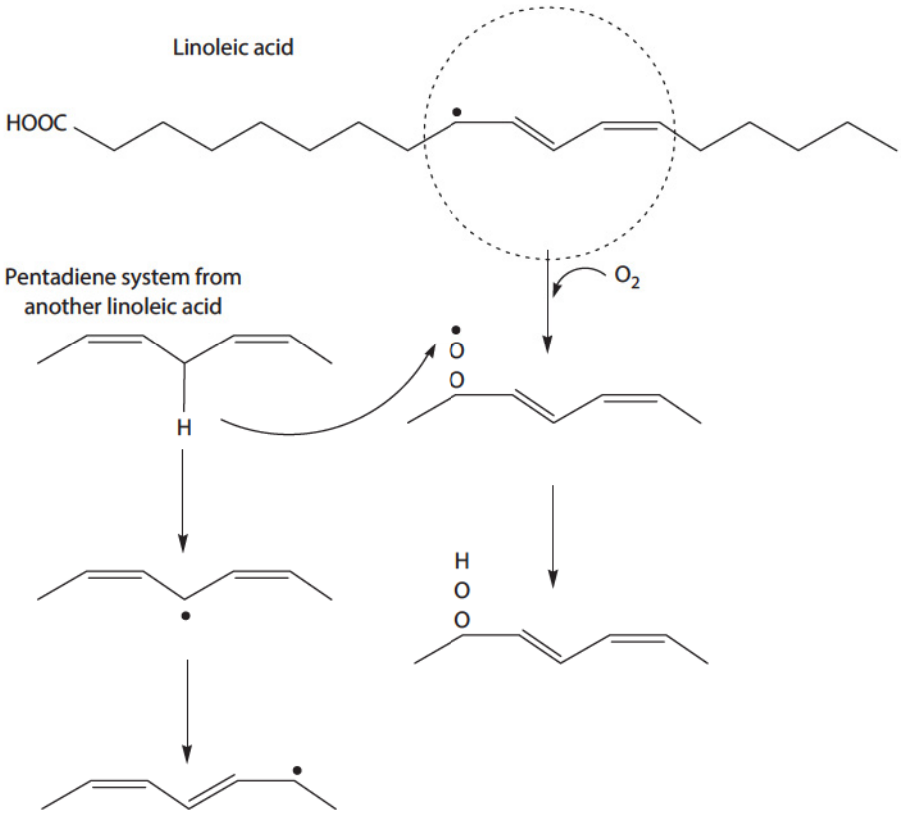


FIGURE 4.25 Propagation step of lipid oxidation for linoleic acid.

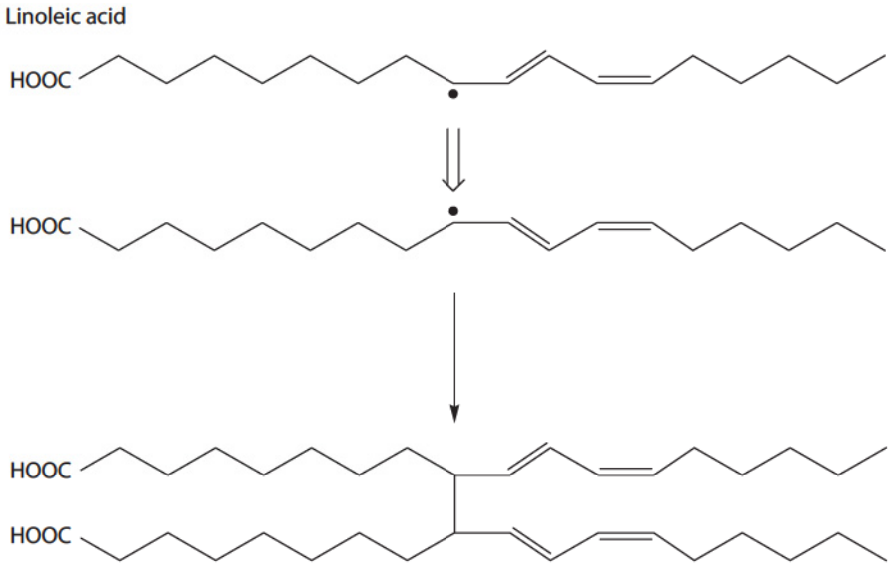


FIGURE 4.26 Example of a termination step of lipid oxidation under conditions of low oxygen concentrations.

4.11.2 Prooxidants

Lipid oxidation is often referred to as autoxidation. The prefix "auto" means "self-acting," and thus the term "autoxidation" has been used to describe the self-perpetuating generation of free radicals from unsaturated fatty acids in the presence of oxygen that occurs in lipid oxidation. In the initiation step, abstraction of hydrogen from unsaturated fatty acids results in the production of a single

free radical. The addition of oxygen to the alkyl radical to form a peroxyl radical and subsequent abstraction of hydrogen from another fatty acid or antioxidant to form a lipid hydroperoxide in the propagation step do not result in the formation of additional free radicals. Thus if "autoxidation" was the only reaction in lipid oxidation, the formation of oxidation products would increase linearly from time zero. However, in most foods the lag phase is followed by a rapid exponential increase in oxidation. This indicates that there are other reactions in lipid oxidation that produce additional free radicals.

Prooxidants, which are found in virtually all food systems, are compounds or factors that cause or accelerate lipid oxidation. Many prooxidants are not true catalysts, since they are altered during the reaction (e.g., singlet oxygen is converted to a hydroperoxide, and ferrous iron is converted to ferric). Prooxidants can accelerate lipid oxidation by direct interactions with unsaturated fatty acids to form lipid hydroperoxides (e.g., lipoxygenases and singlet oxygen) or by promoting the formation of free radicals (e.g., transition-metal- or ultraviolet-light-promoted hydroperoxide decomposition). It should be noted that lipid hydroperoxides do not contribute to off-aromas and thus do not directly cause rancidity. However, hydroperoxides are important substrates for rancidity since their decomposition results in the scission of the fatty acid to produce the low-molecular-weight, volatile compounds that produce off-aromas. The major prooxidants in foods are discussed.

4.11.2.1 Prooxidant That Promote Formation of Lipid Hydroperoxides

4.11.2.1.1 Singlet Oxygen

As mentioned earlier, triplet oxygen ($^3O_2$) is a biradical because its two electrons in the antibonding 2p orbital have the same (parallel or antiparallel) spin direction (Figure 4.27). The Pauli exclusion principle states that two electrons with the same spin direction cannot exist in the same electron orbital. Triplet oxygen cannot react directly with the electrons in the orbital of another molecule unless its electrons have matching parallel spin directions (two electrons in the orbital of a nonradical molecule would have opposite spin directions). If the electrons in the antibonding 2p orbital have opposite spin directions, oxygen is referred to as *singlet oxygen* ($^1O_2$). Singlet oxygen can exist in five different configurations, with the most common in foods being the $^1\Delta$ state where the electrons exist in the same orbital (for detailed description see Ref. [61]). Because singlet oxygen is more electrophilic than triplet oxygen, it can react with high-electron-density double bonds. Since the electrons in singlet oxygen match the spin direction of the electron in double bonds, it can react with an unsaturated fatty acid to directly form lipid hydroperoxides 1500 times faster than triplet oxygen. Singlet oxygen can react with either carbon at the end of a double bond, with the double bond then shifting to form a *trans* double bond. This means that oxidation of linoleate by singlet oxygen can produce four different hydroperoxides (Figure 4.27) compared to the typical two hydroperoxides produced in the propagation step of lipid oxidation (Figure 4.22). These different hydroperoxide locations will lead to the formation of several unique fatty acid decomposition products as will be discussed later.

Singlet oxygen is most commonly produced by photosensitization. Chlorophyll, riboflavin, and myoglobin are photosensitizers that can absorb energy from light to form an excited singlet state. The excited singlet state of the photosensitizer can then undergo intersystem crossing to produce an excited triplet state. The excited triplet state can react directly with substrates such as unsaturated fatty acids and abstract a hydrogen to cause initiation of lipid oxidation. This pathway is known as type 1 and will produce the same lipid hydroperoxides seen in the propagation step described previously for autoxidation. The excited triplet state of the photosensitizer can also react with triplet oxygen to form singlet oxygen and singlet state of the photosensitizer in the type 2 pathway. Type 1 and type 2 pathways are dependent on oxygen concentrations, with type 2 predominating in high oxygen environments. Singlet oxygen can also be formed chemically or enzymatically, or by the decomposition of hydroperoxides. However, production by photosensitization is believed to be the major pathway in foods.

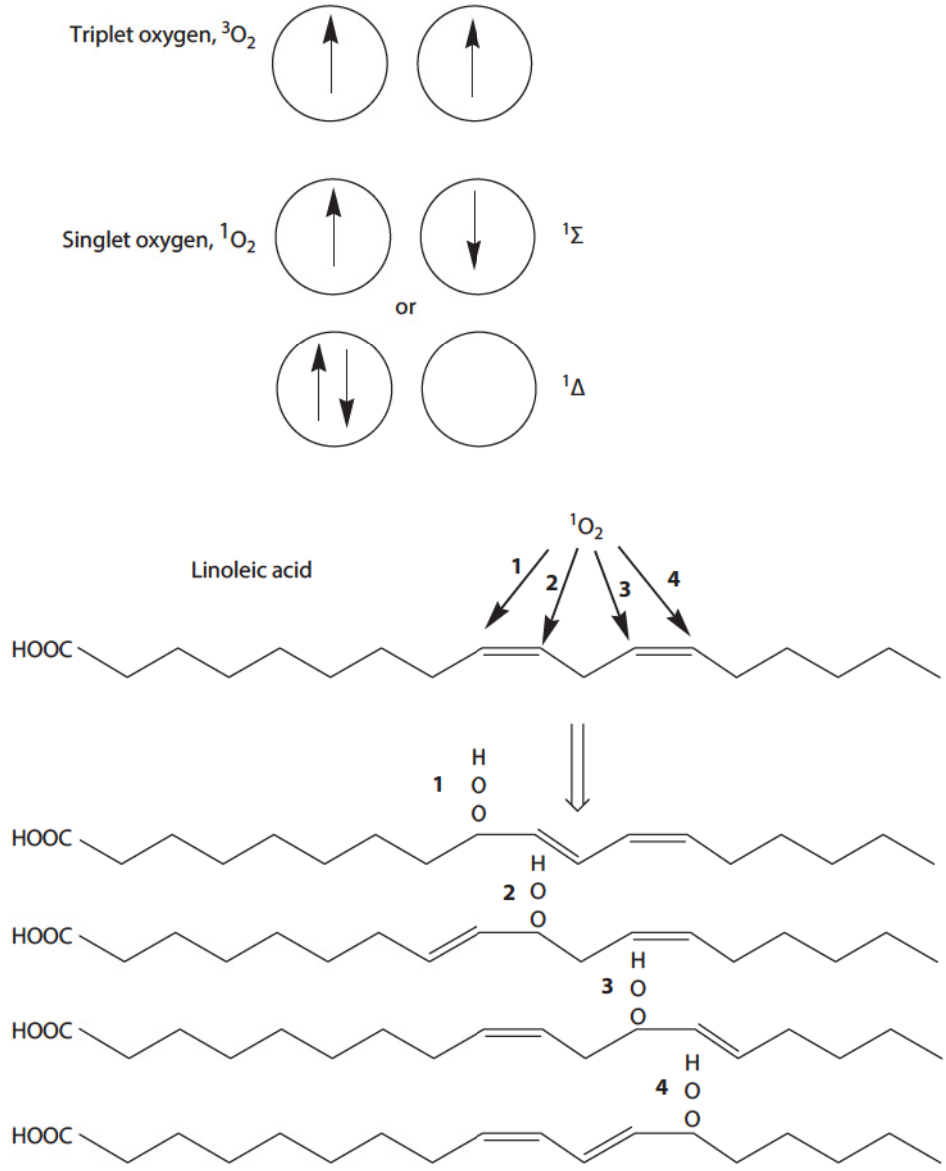


FIGURE 4.27 Singlet oxygen and singlet oxygen–promoted hydroperoxide formation on linoleic acid. (From Min, D.B. and Boff, J.M., Lipid oxidation in edible oil, in: *Food Lipid, Chemistry, Nutrition and Biotechnology*, eds. C.C. Akoh and D.B. Min, Marcel Dekker, New York, 2002, pp. 335–364.)

4.11.2.1.2 Lipoxygenase

Numerous plant tissues and selective animal tissues contain enzymes known as lipoxygenases, which produce lipid hydroperoxides. Lipoxygenases (LOX) from plant seed such as soybeans and peas exist as several isoforms (for review see Ref. [95]). In soybeans, isoform L-1 primarily reacts with free fatty acids and produces hydroperoxides at carbon 13 in both linoleic and linolenic acid. Isoform L-2 produces hydroperoxides at positions 9 and 13 and is active on both free and esterified linoleic and linolenic acid. Plant LOXs are cytoplasmic enzymes that contain a nonheme iron. The iron in inactive LOX is in the ferrous state. Activation occurs by the oxidation of the iron to the ferric state, a process that is usually promoted by a hydroperoxide. LOX catalyzes the abstraction of hydrogen from the methylene-interrupted carbon to form the alkyl radical and the conversion of the LOX iron back to the ferrous state. The enzyme can then control the stereospecific location where oxygen adds on to the alkyl radical to the peroxyl radical. An electron from the ferrous iron is then

donated to the peroxyl radical to form a peroxyl anion. When the peroxyl anion reacts with hydrogen to form the hydroperoxide, the fatty acid is released from the enzyme. Once oxygen is depleted from the system, the enzyme abstracts a hydrogen from a fatty acid and the iron is converted to ferrous. Since no oxygen is present, the alkyl radical is released, and LOX is returned to its inactive form. Lipoxygenases have also been reported in animal tissues especially those highly associated with the circulatory system (e.g., fish gills [28]).

4.11.2.2 Prooxidants That Promote Formation of Free Radicals

4.11.2.2.1 Ionizing Radiation

Foods are sometimes subjected to ionizing radiation to destroy pathogens and extend their shelf-life. However, ionizing radiation can convert molecules to excited states, which produce free radicals such as the production of hydroxyl radical (•OH) from water. The hydroxyl radical is the most reactive radical known, so it is capable of abstracting hydrogen from lipids as well as molecules such as proteins and DNA. Therefore, it is not surprising that irradiation of foods, especially muscle foods that are high in lipids and prooxidants, can increase oxidative rancidity.

4.11.2.3 Prooxidants That Promote Decomposition of Hydroperoxides

Lipid hydroperoxides are found in essentially all lipid-containing foods. Hydrogen peroxide is also found in food when it is utilized as a processing aid and when enzymes such as superoxide dismutase produce it in foods such as meats, poultry, and seafood. High-quality lipid-containing foods contain 1–100 nmole lipid hydroperoxide per gram of lipid. These are an estimated 40–1000 times greater than the estimated lipid hydroperoxide concentrations found *in vivo* (e.g., plasma lipids), suggesting that oxidation occurs during the extraction and refining of fats and oils [17]. Lipid hydroperoxides can be decomposed by high temperatures during thermal processing or by a variety of prooxidants. Upon decomposition, they produce additional radicals, a factor that could be responsible for the exponential increase in oxidation that is seen in many foods. The decomposition of lipid hydroperoxides also leads to the formation of alkoxyl radicals that can enter into β-scission reactions, which are responsible for decomposing fatty acids into low-molecular-weight compounds that are volatile enough to be perceived as rancidity.

4.11.2.3.1 Transition Metals

Transition metals are found in all foods since they are common constituents of raw food materials, water, ingredients, processing equipment, and packaging materials. Transition metals are one of the major food prooxidants that decrease the oxidative stability of foods and biological tissues through their ability to decompose hydroperoxides into free radicals [29,36]. These reactive metals decompose hydrogen and lipid peroxides through the following redox cycling pathway:

$$Mn^{n+} + LOOH \text{ or } HOOH \rightarrow Mn^{n+1} + LO^{\bullet} \text{ or } HO^{\bullet} + OH^{-}$$

$$Mn^{n+1} + LOOH \rightarrow Mn^{n+} + LOO^{\bullet} + H^{+}$$

where

Mn^{n+} and Mn^{n+1} are transition metals in their reduced and oxidized states
LOOH and HOOH are lipid and hydrogen peroxide
$LO^{\bullet}$, $HO^{\bullet}$, and $LOO^{\bullet}$ are alkoxyl, hydroxyl, and peroxyl radicals, respectively

Hydroxyl radical is produced from hydrogen peroxide, while alkoxyl radicals are produced from lipid hydroperoxides. When iron and hydrogen peroxide are involved in this pathway, it is known as the *Fenton reaction*. The concentration, chemical state, and the type of metal will influence the rate of hydroperoxide decomposition. Copper and iron are the most common transition metals in foods capable of participating in these reactions, with iron generally being found at greater concentrations than copper. Copper is more reactive with cuprous ions (Cu^{1+}), decomposing hydrogen peroxide over 50-fold faster

than ferrous ions (Fe^{2+}). Redox state is also important, with Fe^{2+} decomposing hydrogen peroxide over 10^5 times faster than Fe^{3+}. In addition, Fe^{2+} is more water soluble than Fe^{3+}, meaning that it is more available to promote hydroperoxide decomposition in water-based foods. The peroxide type is also important, with Fe^{2+} decomposing lipid hydroperoxides ~10 times faster than hydrogen peroxide [29,36].

Since the reduced state of transition metals are more efficient at decomposing hydroperoxides, reducing compounds capable of promoting the redox cycling of transition metals can promote lipid oxidation. Examples of prooxidative reductants include superoxide anion ($O_2^{-\bullet}$) and ascorbic acid. Superoxide anion is produced by the addition of an electron to triplet oxygen. The added electron in superoxide anion can then be transferred to a transition metal to cause its reduction. Superoxide anion is produced by enzymes, by the release of oxygen from oxymyoglobin to produce metmyoglobin, or by cells such as phagocytes. The redox cycling of iron by superoxide anion to promote lipid oxidation is shown in the following pathways. This pathway is known as the Haber–Weiss reaction.

$$Fe^{3+} + O_2^{-\bullet} \rightarrow Fe^{+2} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{+3} + {}^{\bullet}OH + OH^-$$

$$\text{Net: } O_2^{-\bullet} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$$

Ascorbic acid can also participate in Haber–Weiss-like reactions; however, unlike superoxide anion, ascorbic acid can also act as an antioxidant. Thus, at high ascorbate concentration, its antioxidant activity may outweigh its ability to accelerate metal-promoted oxidation, resulting in a net antioxidant effect.

Transition metals associated with proteins can also promote hydroperoxide decomposition. The heme proteins are the best studied of this group, with the iron in myoglobin, hemoglobin, peroxidases, and catalase being able to promote both hydrogen and lipid hydroperoxide decomposition. In some cases, heme proteins have been suggested to cause homolytic scission of lipid hydroperoxides, meaning that the breakdown of the hydroperoxide will produce two free radicals (hydroxyl and alkoxyl). Thermal denaturation of these proteins can increase their prooxidant activity, presumably due to increased exposure of the heme iron that is able to more effectively interact with hydroperoxides. Thermal denaturation of myoglobin is believed to be one of the factors that cause the acceleration of lipid oxidation in cooked meats, a problem known as *warmed-over flavor*.

4.11.2.3.2 Light and Elevated Temperatures

UV and visible light can promote the decomposition of hydroperoxides to produce free radicals. Thus, packaging to decrease light exposure can reduce lipid oxidation rates. Increasing the temperature increases the lipid oxidation rate in essentially all foods, so temperature control is an important way to control rancidity. Elevated temperatures will also decompose lipid hydroperoxides. In fact, lipid hydroperoxide accumulation is often not seen in frying oils since the hydroperoxides breakdown very rapidly after formation.

4.11.3 Formation of Lipid Oxidation Decomposition Products

Once lipid hydroperoxides are decomposed into alkoxyl radicals, a number of different reaction schemes can occur. The products of these reaction schemes will depend on the fatty acid type as well as the location of the hydroperoxide on the fatty acid. In addition, decomposition products can be unsaturated and even have intact pentadiene structures, meaning that the oxidation products can be further oxidized. This results in literally hundreds of different fatty acid decomposition products. Since the type of fatty acid decomposition products will depend on the fatty acid composition of the food, lipid oxidation can have different effects on sensory properties. For example, oxidation of vegetable oils that have predominately ω-6 fatty acids will produce "grassy" and "beany" odors, while oxidation of the long chain ω-3 fatty acids in marine oils will produce "fishy" aromas.

One of the reasons why lipid hydroperoxide decomposition will cause decomposition of the fatty acid is that these pathways produce the alkoxyl radical (LO•). The alkoxyl radical is more energetic than both the alkyl and peroxyl radicals, meaning that it can attack other unsaturated fatty acids, other pentadiene groups within the same fatty acid, or the covalent bonds adjacent to the alkoxyl radical. This last reaction, known as the β-*scission reaction*, is important to food quality since it causes fatty acids to decompose into low-molecular-weight compounds, which are perceived as rancidity.

4.11.3.1 β-Scission Reaction

The highly energetic alkoxyl radical has the ability to abstract a hydrogen from the carbon–carbon bond on either side of the oxygen radical. Unesterified linoleic acid will be used to demonstrate more details of this reaction. One should remember that the decomposition product on the carboxylic acid end of the fatty acid would usually be esterified to the glycerol of a triacylglycerol or phospholipid. Thus, this decomposition product would not be volatile and thus would not contribute to rancidity unless it undergoes further decomposition reactions to form low-molecular-weight compounds. Figure 4.28 shows the oxidation of linoleic acid to produce a hydroperoxides at carbon 9. In step 1, the hydroperoxide decomposes into the alkoxyl radical. Step 2 shows the β-scission reaction that occurs because the high-energy alkoxyl radical can abstract an electron from adjacent carbon–carbon bonds to cleave the fatty acid chain. β-Scission breaks the fatty acid on either side of the alkoxyl radical. If cleavage of the fatty acid is on the

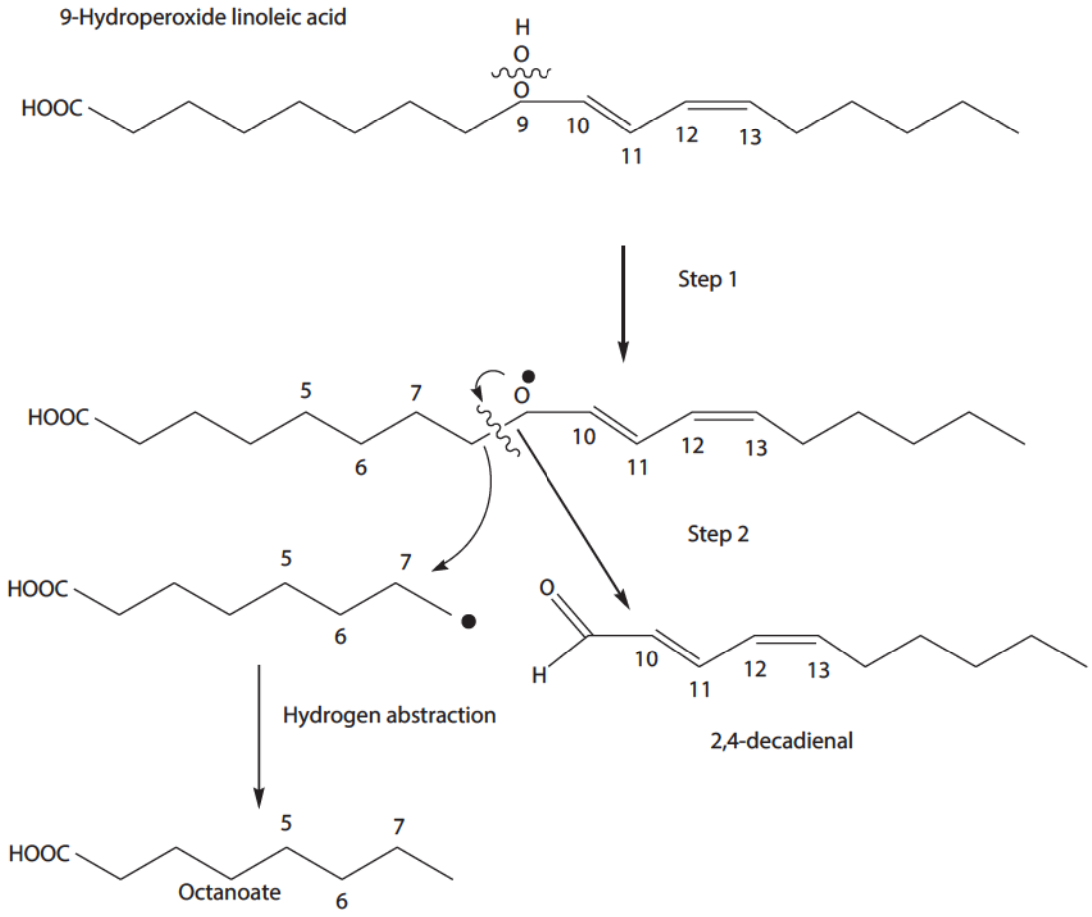


FIGURE 4.28 β-Scission decomposition products produced from 9-linoleic acid hydroperoxide when fatty acid cleavage occurs on the carboxylic acid side of the hydroperoxide. (From Frankel, E.N., *Lipid Oxidation*, 2nd edn., Oily Press, Scotland, 2005.)

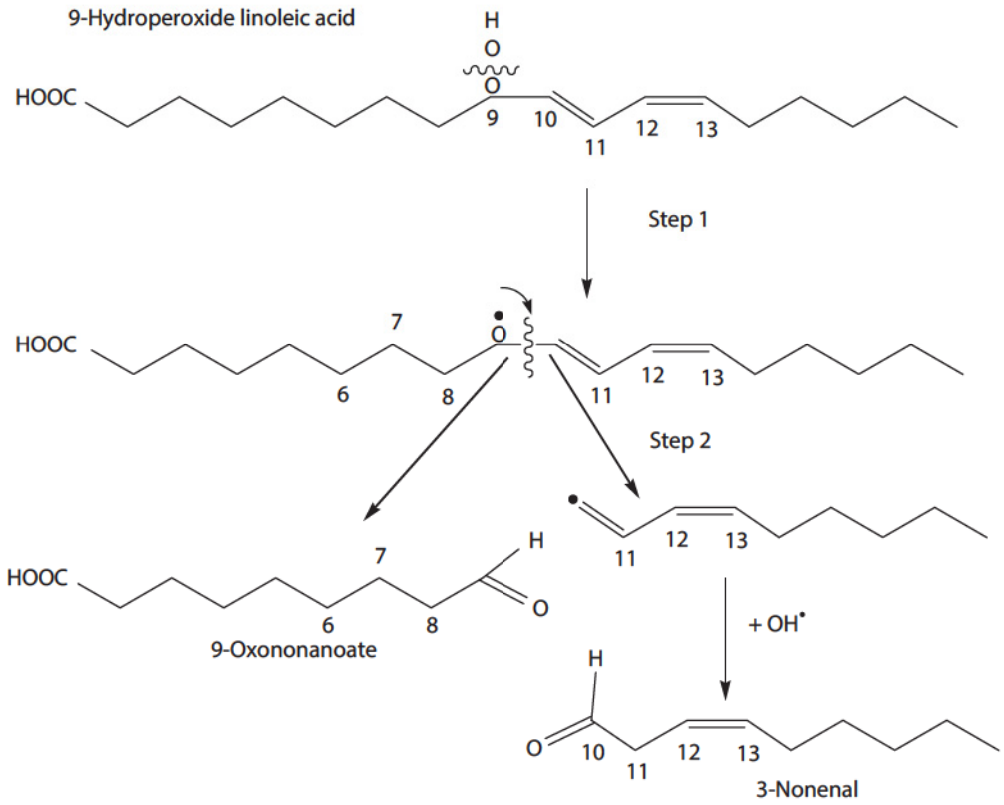


FIGURE 4.29 β-Scission decomposition products produced from 9-linoleic acid hydroperoxide when fatty acid cleavage occurs on the methyl side of the hydroperoxide. (From Frankel, E.N., *Lipid Oxidation,* 2nd edn., Oily Press, Scotland, 2005.)

carboxylic acid end of the fatty acid, the decomposition products will be octanoate and 2, 4-decadienal (Figure 4.28). Cleavage on the opposite side of the alkoxyl radical (Figure 4.29, methyl end of the fatty acid), 9-oxononanoate and a nine-carbon vinyl radical will be produced. Vinyl radicals can interact with hydroxyl radicals to form aldehydes, thus producing 3-nonenal. Similar pathways will occur if the hydroperoxide is on carbon 13. Cleavage on the carboxylic acid end will produce 12-oxo-9-dodecenoate and hexanal. Cleavage on the methyl end of the fatty acid will produce 13-oxo-9,11-tridecadienoate and pentane.

When singlet oxygen attacks linoleic acid, it will form hydroperoxides at all of the carbons associated with double bonds (Figure 4.27). This means that it will form hydroperoxides at carbons 9 and 13, as in free-radical-initiated oxidation, plus hydroperoxides at carbons 10 and 12. Typical products from the β-scission reaction from an alkoxyl radical at carbon 10 will produce 9-oxononanoate and 3-nonenal from cleavage on the carboxylic acid end and 10-oxo-8-decenoate and 2-octene from cleavage at the methyl end of the fatty acid. Typical products from the β-scission reaction from an alkoxyl radical at carbon 12 will produce 9-undecenoate and 2-heptenal from cleavage on the carboxylic acid end and 12-oxo-9-dodecenoate and hexanal from cleavage at the methyl end of the fatty acid.

As one can see from the above discussion on the β-scission products and other free-radical reactions of linoleic acid, numerous products can be formed. For a detailed discussion on β-scission decomposition products, see Frankel [25]. Pathways similar to this will occur with other unsaturated fatty acids producing additional unique compounds. In addition, the decomposition products often contain double bonds and, in some cases, intact pentadiene systems. These double bond systems can undergo hydrogen abstraction or singlet oxygen attack, which will result in the formation of additional decomposition products. While the above discussion shows the theoretical decomposition

products of linoleic acid, in reality not all of these products have been detected. This is likely due to the ability of these compounds to undergo additional decomposition reactions.

4.11.3.2 Additional Reactions of Fatty Acid Decomposition Products

In addition to the fatty acid hydroperoxide products described previously, fatty acids radicals can undergo a series of other reactions to form compounds such as olefins, alcohols, carboxylic acids, ketones, epoxides, and cyclic products (for review see Ref. [25]). Alkyl radicals will react with hydrogen and hydroxyl radicals to produce olefins and alcohols. As mentioned earlier, alkoxyl radicals are high-energy radicals. Thus, they can abstract hydrogen from other molecules such as unsaturated fatty acids or antioxidants to produce fatty acid alcohols. Alkoxyl radicals can also lose an electron and be converted to a ketone or link to an adjacent carbon to form an epoxide. Peroxyl radicals can react with double bonds within the same fatty acid to produce cyclic products such as bicyclic endoperoxides.

Aldehydes produced from the oxidative decomposition of fatty acids are important because of their impact on off-flavor development. The aldehydes can also react with nucleophilic food components. In particular, they interact with sulfhydryls and amines in proteins, which may alter the functionality of the protein. One example is the ability of unsaturated aldehydes to react with histidine in myoglobin via a Michael addition–type reaction [22]. This reaction is thought to contribute to the conversion of myoglobin to metmyoglobin to produce meat discoloration.

Fatty acid decomposition products can also form dimers and polymers [25]. This can occur via radical–radical termination reactions. In the presence of oxygen (peroxyl and alkoxyl radicals), polymerization involves the formation of peroxide or ether linkages. In the absence of oxygen (alkyl radicals), polymerization occurs through carbon–carbon crosslinks. These carbon–carbon crosslinks often occur when oils are subjected to high temperatures where oxygen solubility is low. Methyl esters of fatty acids will crosslink much more readily than the fatty acids in triacylglycerols. Crosslinking of fatty acids in triacylglycerols is generally significant only in frying oils.

4.11.3.3 Cholesterol Oxidation

Cholesterol contains a double bond between carbons 5 and 6 (Figure 4.4). As with fatty acids, this double bond is susceptible to free-radical attack and can undergo decomposition reactions to produce alcohols, ketones, and epoxides [81]. The most notable of the cholesterol oxidation pathway begins with the formation of a hydroperoxide at carbon 7. This hydroperoxide can decompose into an alkoxyl radical, which in turn can undergo rearrangements to 5,6 epoxides, 7-hydroxylcholesterol, and 7-ketocholesterol. These cholesterol oxidation products are potentially cytotoxic and have been linked to the development of atherosclerosis. Cholesterol oxidation products have primarily been found in animal food products that have undergone thermal processing, such as cooked meats, tallow, lard, and butter, as well as dried dairy and egg products.

4.11.4 Antioxidants

Oxidative stress occurs in all organisms in an oxygenated environment. Thus biological systems have developed a wide variety of antioxidant systems to protect against oxidation. There is no uniform definition of an antioxidant because there are numerous chemical mechanisms that can be used to inhibit oxidation. The biological tissues from which foods are obtained generally contain several endogenous antioxidant systems. Unfortunately, food processing operations can remove antioxidants or cause additional oxidative stress that can overcome the endogenous antioxidants systems in the food. Therefore it is common to incorporate additional antioxidant protection into processed foods. Antioxidant mechanisms of compounds that are used to increase the oxidative stability of foods include the control of free radicals, prooxidants, and oxidation intermediates.

4.11.4.1 Control of Free Radicals

Many antioxidants slow lipid oxidation by inactivating or scavenging free radicals, thereby inhibiting initiation, propagation, and β-scission reactions. Free-radical scavengers (FRSs) or chain-breaking antioxidants can interact with peroxyl (LOO•) and alkoxyl (LO•) radicals by the following reactions.

$$\text{LOO}^{\bullet} \text{ or } \text{LO}^{\bullet} + \text{FRS} \rightarrow \text{LOOH or LOH} + \text{FRS}^{\bullet}$$

FRSs inhibit lipid oxidation by reacting faster with free radicals than unsaturated fatty acids. FRSs are thought to interact mainly with peroxyl radicals because propagation is the slowest step of lipid oxidation, meaning that peroxyl radicals are often found in the greatest concentration of all radicals in the systems; peroxyl radicals have lower energies than radicals such as alkoxyl radicals [10] and therefore preferentially react with the low-energy hydrogens of FRSs than polyunsaturated fatty acids; and FRSs are generally found at low concentrations and therefore do not compete effectively with initiating radicals (e.g., •OH) that can oxidize the first compound they come in contact with [52].

Antioxidant efficiency is dependent on the ability of the FRSs to donate hydrogen to a free radical. As the energy of a hydrogen bound to an FRS decreases, the transfer of the hydrogen to the free radical is more energetically favorable and therefore more rapid. The ability of an FRS to donate its hydrogen to a free radical can be predicted with the help of standard one-electron reduction potentials [10]. Any compound that has a reduction potential lower than the reduction potential of a free radical (or oxidized species) is capable of donating its hydrogen to that free radical unless the reaction is kinetically unfeasible. For example, FRSs including α-tocopherol ($E^{\circ\prime}$ = 500 mV), catechol ($E^{\circ\prime}$ = 530 mV), and ascorbate ($E^{\circ\prime}$ = 282 mV) all have reduction potentials below that of peroxyl radicals ($E^{\circ\prime}$ = 1000 mV) and are therefore capable of donating their hydrogen to the peroxyl radical to form a hydroperoxide.

The efficiency of the FRS is also dependent on the energy of the resulting free radical scavenger radical (FRS•). If the FRS· is a low-energy radical, then the likelihood of the FRS• catalyzing the oxidation of unsaturated fatty acids decreases. Effective FRSs form low-energy radicals due to resonance delocalization, as shown in Figure 4.30 [77]. Effective FRSs also produce radicals that do not react rapidly with oxygen to form hydroperoxides. When radical scavengers form hydroperoxides, they can undergo decomposition reactions that produce additional radicals which could cause oxidation of unsaturated fatty acids. FRS radicals may participate in termination reactions with other FRS• or lipid radicals to form nonradical species. This means that each FRS is capable of inactivating at least two free radicals, the first being inactivated when the FRS interacts with peroxyl or alkoxyl radicals, and the second when the FRS• enters a termination reactions with another FRS• or lipid radical (Figure 4.31).

Phenolic compounds possess many of the properties of an efficient FRS. These compounds donate a hydrogen from their hydroxyl groups, and the subsequent phenolic radical can have low energy as the radical is delocalized throughout the phenolic ring structure. The effectiveness of a phenolic FRS is often increased by substitution groups on the phenolic ring, which increase the ability of the FRS to donate hydrogen to lipid radicals and/or increase the stability of the FRS• [77]. In foods, the efficiency of phenolic FRSs is also dependent on their volatility, pH sensitivity, and polarity. In the following, we give examples of the most common FRSs in foods.

4.11.4.2 Tocopherols

Tocopherols are a group of compounds that have a hydroxylated ring system (chromanol ring) with a phytol chain (Figure 4.32). Differences in tocopherol homologs are due to differences in methylation on the chromanol ring, with α being trimethylated, β (positions 5 and 8) and γ (positions 7 and 8) being dimethylated, and δ being monomethylated (position 8). Tocotrienols differ from tocopherols in that they have three double bonds in their phytyl chain. Tocopherols have three asymmetric

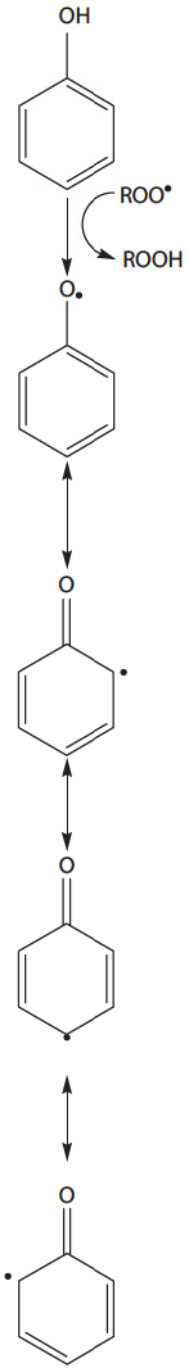


FIGURE 4.30 Resonance delocalization of phenol radical. (Adapted from Shahidi, F. and Wanasundara, J.P.K., *Crit. Rev. Food Sci. Nutr.,* 32, 67, 1992.)

carbons, and thus each homolog can have eight possible steroisomers. Natural tocopherols are found in the all *rac* or *RRR* configuration. Synthetic tocopherols have steroisomers with combinations of *R* and *S* configurations. The steroisomer configuration of α-tocopherol is important because only the *RRR* and 2*R*-steroisomers (*RSR*, *RRS*, and *RSS*) have significant vitamin E activity and can be used for the establishment of the recommended daily allowance (RDA) of vitamin E in the United States [23]. α-Tocopherol is commonly sold as an acetate ester when used as a nutritional supplement or a food ingredient. The acetate ester is hydrolyzed in the gastrointestinal tract by lipase to regenerate α-tocopherol. The acetate ester form of tocopherols blocks the hydroxyl group and decreases

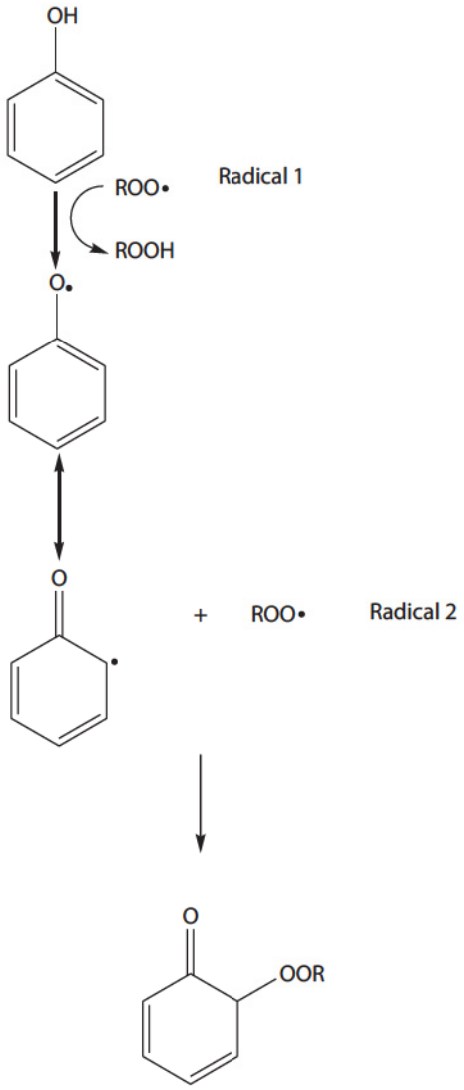


FIGURE 4.31 Termination reaction between and antioxidant radical and a lipid peroxyl radical (ROO•).

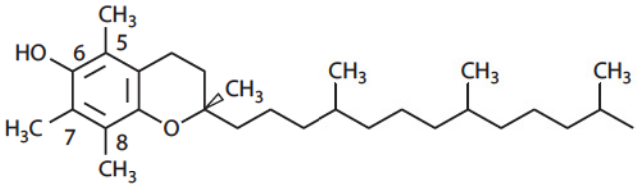


FIGURE 4.32 Structure of α-tocopherol.

the molecule's susceptibility to oxidative degradation. It should be noted that the blocking of the hydroxyl group by the acetate ester removes the antioxidant activity of tocopherol. The esterification of α-tocopherols also increases its stability, thereby maintaining vitamin E activity during storage.

Reactions between tocopherols and lipid peroxyl radicals lead to the formation of a lipid hydroperoxide and several resonance structures of tocopheroxyl radicals. These radicals can interact with other lipid radicals or with each other to form a variety of termination products. The types and amounts of these products are dependent on oxidation rates, radical species, physical location

(e.g., bulk vs. membrane lipids), and tocopherol concentration (see Ref. [52] for more details). Tocopherols are generally insoluble in water. However, they do vary in polarity, with α-tocopherol (trimethylated) being the most nonpolar and δ-tocopherol (monomethylated) being the most polar. These differences in polarity alter the surface activity of the tocopherols, a factor that may impact their antioxidant activity (see the section on physical location of antioxidants).

4.11.4.3 Synthetic Phenolics

Phenol is not a good antioxidant, but addition of substitution groups onto the phenolic ring can enhance its antioxidant activity. Thus the majority of synthetic antioxidants are substituted monophenolic compounds. The most common synthetic FRSs used in foods include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and propyl gallate (Figure 4.33). These synthetic FRSs vary in polarity in the order: BHT (most nonpolar) > BHA > TBHQ > propyl gallate. As with other FRSs, interactions between the FRS and lipid radicals result in the formation of a low-energy, resonance-stabilized phenolic radical, which neither rapidly catalyzes the oxidation of unsaturated fatty acids nor reacts with oxygen to form unstable antioxidant hydroperoxides that decompose into high-energy free radicals that can promote oxidation. Synthetic phenolics are effective in numerous food systems; however, their use in the food industry is declining due to the consumer demand for all natural products.

4.11.4.4 Plant Phenolics

Plants contain a diverse group of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydrocinnamic acid derivatives, and flavonoids. These phenolics are widely distributed in fruits, spices, tea, coffee, seeds, and grains. All phenolic classes have the structural requirements of FRSs, although their activity varies greatly. Factors influencing the FRS activity of plant phenolics include the position and degree of hydroxylation, polarity, solubility, reducing potential, stability of the phenolic to food processing operations, and stability of the phenolic radical.

Rosemary extracts are the most commercially important source of natural phenolics used as a foods additive to inhibit lipid oxidation by FRSs. Carnosic acid, carnosol, and rosmarinic acid are the major FRSs in rosemary extracts (Figure 4.34). Rosemary extracts can inhibit lipid oxidation in a wide variety of food products including meats, bulk oils, and lipid emulsions [4,26,60]. Utilization of phenolic antioxidants from crude herb extracts such as rosemary is often limited by the presence of flavor compounds such as monoterpenes. Phenolics found naturally in foods are important to the endogenous

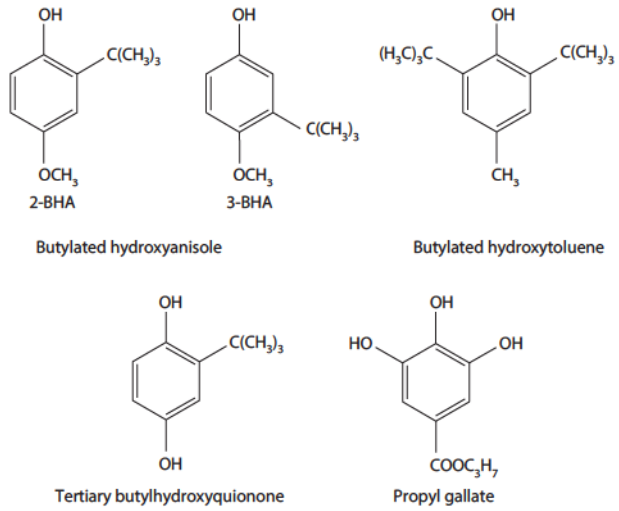


FIGURE 4.33 Structures of synthetic antioxidants used in foods.

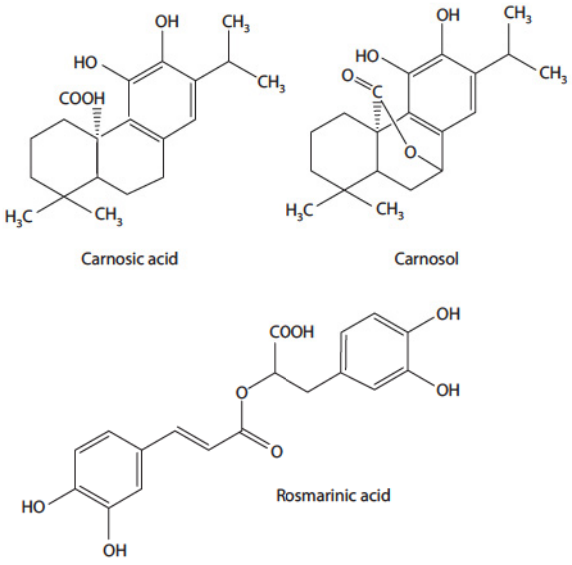


FIGURE 4.34 Structures of phenolic antioxidants found in rosemary extracts.

oxidative stability of foods. Natural phenolic levels in foods can vary as a function of plant maturity, variety, tissue type, growing conditions and postharvest age, and storage conditions [9,41,84].

4.11.4.5 Ascorbic Acid and Thiols

Free radicals are generated in the water phase of foods by processes such as the Fenton reaction, which produces hydroxyl radicals from hydrogen peroxide. In addition, free radicals can be surface-active, meaning that they would migrate from the lipid phase toward the water phase in lipid dispersions. Since free radicals are found in the aqueous phase, biological systems containing water-soluble compounds are capable of free-radical scavenging. Ascorbic acid and thiols scavenge free radicals, resulting in the formation of low-energy radicals [18]. Thiols such as cysteine and glutathione may contribute to the oxidative stability of plant and muscle foods, but they are rarely added to foods as antioxidants. One exception to this is the thiols found in proteins that can inhibit lipid oxidation in food products [20,87]. Ascorbate and its isomer erythorbic acid can both scavenge free radicals. Both have similar activity, but erythorbic acid is more cost effective. Ascorbic acid is also available as a conjugate with palmitic acid. The conjugate is lipid-soluble and surface-active, meaning that it can be effective in bulk oils and emulsions. In the gastrointestinal tract, ascorbyl palmitate is hydrolyzed to ascorbic and palmitic acids, and thus there are no restrictions on its usage levels.

4.11.4.6 Control of Prooxidants

The rate at which lipids oxidize in foods is very much dependent on prooxidant concentrations and activity (e.g., transition metals, singlet oxygen, and enzymes). Control of prooxidants is therefore a very effective strategy to increase the oxidative stability of foods. Both endogenous and exogenous antioxidants will impact the activity of transition metals and singlet oxygen.

4.11.4.7 Control of Prooxidant Metals

Iron and copper are examples of important prooxidant transition metals that accelerate lipid oxidation by promoting hydroperoxide decomposition. The prooxidative activity of metals is altered by chelators or sequestering agents. Chelators inhibit the activity of prooxidant metals by one or more of the following properties: prevention of metal redox cycling; occupation of all metal coordination sites; formation of insoluble metal complexes; and/or steric hinderance of interactions between metals and lipids or oxidation intermediates (e.g., hydroperoxides) [18]. Some metal chelators can increase oxidative reactions by increasing the metal solubility and/or altering the redox potential.

The tendency of a chelator to accelerate or inhibit prooxidant activity depends on the metal and chelator concentrations. For instance, ethylenediaminetetraacetic acid (EDTA) is ineffective or prooxidative when the EDTA/iron ratios are ≤1 and antioxidative when the ratio is >1 [53].

The main metal chelators found in foods contain multiple carboxylic acid (e.g., EDTA and citric acid) or phosphate groups (e.g., polyphosphates and phytate). Most chelators act in the aqueous phase of foods, but some will also partition into the lipid phase (e.g., citric acid), thus allowing them to inactivate lipid-soluble metals. Chelators must be ionized to be active, and therefore their activity decreases at pH values below the pK_a of the ionizable groups. The most common chelators used as food additives are citric acid, EDTA, and polyphosphates. The effectiveness of phosphates increases with increasing number of phosphate groups. Thus tripolyphosphate and hexametaphosphate are more effective than phosphoric acid [83]. Prooxidant metals can also be controlled by binding to proteins such as transferrin, phosvitin, lactoferrin, ferritin, and casein (for review see Ref. [18]).

4.11.4.8 Control of Singlet Oxygen

As mentioned previously, singlet oxygen is an excited state of oxygen that can promote the formation of lipid hydroperoxides. Carotenoids are a diverse group (>600 different compounds) of yellow to red colored polyenes. The activity of singlet oxygen can be controlled by carotenoids by both chemical and physical quenching mechanisms [51,67]. Carotenoids chemically quench singlet oxygen when singlet oxygen attacks the double bonds of the carotenoid. This reaction will lead to the formation of carotenoid breakdown products containing aldehydes, ketones, and endoperoxide. These reactions cause carotenoid decomposition, leading to loss of antioxidant activity and color. The more effective mechanism of singlet oxygen inactivation by carotenoids is physical quenching. Carotenoids physically quench singlet oxygen by a transfer of energy from singlet oxygen to the carotenoid to produce an excited state of the carotenoid and ground-state triplet oxygen. Energy is dissipated from the excited carotenoid by vibrational and rotational interactions with the surrounding solvent to return the carotenoid to the ground state. The presence of nine or more conjugated double bonds in a carotenoid is necessary for physical quenching. Carotenoids that have six carbon oxygenated ring structures at the end their polyenes are often more effective at physically quenching singlet oxygen. Carotenoids can also physically absorb the energy of photoactivated sensitizers, preventing them from promoting the formation of singlet oxygen.

4.11.4.9 Control of Lipoxygenases

Lipoxygenases are active lipid oxidation catalysts found in plants and some animal tissues. Lipoxygenase activity can be controlled by heat inactivation and plant-breeding programs that decrease the concentration of these enzymes in edible tissues.

4.11.4.10 Control of Oxidation Intermediates

Compounds are found in foods that indirectly influence lipid oxidation rates by interacting with prooxidant metals or oxygen to form reactive species. Examples of such compounds include superoxide anion and hydroperoxides.

4.11.4.11 Superoxide Anion

Superoxide participates in oxidative reactions by reducing transition metals to a more active state or by promoting the release of iron bound to protein. In addition, at low pH values, superoxide will form its conjugated acid, the perhydroxyl radical, which can directly catalyze lipid oxidation [46]. Due to the prooxidant nature of superoxide anion in oxidative reactions, biological systems contain superoxide dismutase (SOD). SOD catalyzes the conversion of superoxide anion to hydrogen peroxide by the following reaction:

$$2O_2^{-\bullet} + 2H^+ \rightarrow O_2 + H_2O_2$$

4.11.4.12 Peroxides

Peroxides are important intermediates of oxidative reactions since they decompose via transition metals, irradiation, and elevated temperatures to form free radicals. Hydrogen peroxide exists in foods as a result of direct addition (e.g., aseptic processing operations) and formation in biological tissues by mechanisms including the dismutation of superoxide by SOD and the activity of peroxisomes and leukocytes. The inactivation of hydrogen peroxide is catalyzed by catalase, a heme-containing enzyme, by the following reaction [46]:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione peroxidase is a selenium-containing enzyme that can decompose both lipid hydroperoxides and hydrogen peroxide using reduced glutathione (GSH) as a cofactor [46]:

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

or

$$LOOH + 2GSH \rightarrow LOH + H_2O + GSSG$$

where
GSSG is oxidized glutathione
LOH is a fatty acid alcohol

4.11.4.13 Antioxidant Interactions

Food systems usually contain endogenous, multicomponent antioxidant systems. In addition, exogenous antioxidants can be added to processed foods. The presence of multiple antioxidants will enhance the oxidative stability of the product due to interactions between antioxidants. Synergism is often used to describe antioxidant interactions. For an antioxidant interaction to be synergistic, the effect of the antioxidant combination must be greater than the sum of the effects of the two individual antioxidants. The effectiveness of many antioxidant combinations often is equal to or less than their additive effect. Thus caution should be used when claiming synergistic activity.

Enhanced antioxidant activity can be observed in the presence of two or more different FRSs. In the presence of two FRSs, it is possible that one FRS (the primary FRS) will react more rapidly with lipid free radicals than the other due to lower bond disassociation energies or due to the fact that its physical location is closer to the site where free radicals are being generated. In the presence of multiple FRSs, the primary FRS that is rapidly oxidized can be regenerated by a secondary FRS, with the free radical being transferred from the primary to the secondary FRS. This process is seen with α-tocopherol and ascorbic acid. In this system, α-tocopherol is the primary FRS due to its presence in the lipid phase. Ascorbic acid then regenerates the tocopheroxyl radical or possibly the tocopherylquinone back to α-tocopherol, resulting in the formation of the dehydroascorbate [10]. The net result is that the primary FRS (α-tocopherol) is maintained in an active state where it can continue to scavenge free radicals in the lipid phase of the food.

Chelators and FRS combinations can result in synergistic inhibition of lipid oxidation [25]. These enhanced interactions occur by a "sparing" effect provided by the chelator. Since the chelator will decrease the amounts of free radicals formed in the food by inhibiting metal-catalyzed oxidation, the eventual inactivation of the FRS through reactions such as termination or autoxidation will be slower. Thus, by decreasing free-radical generation and thus decreasing FRS inactivation, concentrations of the FRSs will be higher.

Since multicomponent antioxidant systems can inhibit oxidation by many different mechanisms (e.g., FRS, metal chelation, and singlet oxygen quenching), the use of multiple antioxidants

can greatly enhance the oxidative stability of foods. Thus when designing antioxidant systems, the antioxidants used should have different mechanisms of action and/or physical properties. Determining which antioxidants would be the most effective depends on factors such as type of oxidation catalysts, physical state of the food, and factors that influence the activity of the antioxidants themselves (e.g., pH, temperature, and ability to interact with other compounds/antioxidants in the foods).

4.11.4.14 Physical Location of Antioxidants

Antioxidants can show a wide range of effectiveness depending on the physical nature of the lipid [25,71]. For example, hydrophilic antioxidants are often less effective in O/W emulsions than lipophilic antioxidants, whereas lipophilic antioxidants are less effective in bulk oils than hydrophilic antioxidants [25,71]. This observation has been coined the "antioxidant paradox." Differences in the effectiveness of the antioxidants in bulk oils and emulsions are due to their physical location in the two systems. Polar antioxidants are more effective in bulk oils presumably because they can accumulate in reverse micelles within the oil [12], the locations where lipid oxidation reactions would be greatest due to the coexistence of surface active hydroperoxides and prooxidants such as metals [90]. In contrast, predominantly nonpolar antioxidants are more effective in O/W emulsions because they are retained in the oil droplets and/or accumulate at the oil–water interface, the location where, again, lipid oxidation reactions are prevalent. Conversely, in O/W emulsions, polar antioxidants would tend to partition into the aqueous phase where they would not be able to protect the lipid.

4.11.5 Other Factors Influencing Lipid Oxidation Rates

4.11.5.1 Oxygen Concentration

Reduction of oxygen concentrations is a common method used to inhibit lipid oxidation. However, the addition of oxygen to the alkyl radical is a diffusion-limited reaction, so it has been suggested that to effectively inhibit lipid oxidation, most of the oxygen must be removed from the system. Since oxygen solubility is higher in oil than water, removal of oxygen to stop lipid oxidation can be difficult unless vacuum conditions are used. Unfortunately, very little research has been conducted on lipid oxidation at intermediate oxygen concentrations.

4.11.5.2 Temperature

Increasing temperature generally increases lipid oxidation rates. However, increasing temperatures also decrease oxygen solubility, so in some cases high temperatures can slow oxidation. This can happen in heated bulk oil. However, if food is fried in heated oil, aeration of the oil occurs, leading to acceleration of oxidation. Elevated temperatures can also cause antioxidants to degrade, volatilize, and, in the cases of antioxidant enzymes, become inactivated through denaturation.

4.11.5.3 Surface Area

Increasing the surface area of lipids can increase lipid oxidation rates since this can lead to increased exposure to oxygen and prooxidants. This has recently been observed in bulk oils that contain nanostructures formed by naturally occurring surfactants (e.g., phospholipids) and water [12].

4.11.5.4 Water Activity

As water is removed from a food system, lipid oxidation rates generally decrease. This is likely due to a decrease in the mobility of reactants such as transition metals and oxygen. In some foods, continued removal of water will result in an acceleration of lipid oxidation. This acceleration of lipid oxidation at very low water activity is thought to be due to the loss of a protective water solvation layer surrounding lipid hydroperoxides [12,13].

4.11.6 Measurement of Lipid Oxidation

As one can see from the foregoing discussion on lipid oxidation pathways, numerous oxidation products can be formed from a single fatty acid. In addition, these decomposition products often contain double bonds and in some cases intact pentadiene systems. These double bond systems can undergo hydrogen abstraction or singlet oxygen attack, which will result in the formation of additional decomposition products. Since food lipids can contain many different unsaturated fatty acids and can be exposed to several different prooxidants, hundreds of decomposition products can be formed. In addition, many oxidation products are unstable (hydroperoxides) and can react with other food components (aldehydes). Thus the complexity of these pathways and factors make analysis of lipid oxidation very challenging. In the following, we give a summary of the most common analytical techniques used to monitor the oxidation products in food lipids.

4.11.6.1 Sensory Analysis

The gold standard of lipid oxidation measurements is sensory analysis, since this is the only technique that directly monitors the off-aromas and off-flavors generated by oxidative reactions. In addition, sensory analysis can be highly sensitive because humans can detect certain aroma compounds at levels below or close to detection levels that can be achieved by chemical and instrumental techniques. Sensory analysis of oxidized lipids must be done with a panel that is trained in the identification of oxidation products. This training is usually product-specific, since the oxidation products from different fatty acids can produce different sensory profiles. Due to the necessity for extensive training, sensory analysis is often time consuming and cost prohibitive and obviously is not suitable for the rapid and extensive analysis required for quality control operations. Thus, many chemical and instrumental techniques have been developed. In the best case scenario, these chemical and instrumental techniques are most useful when correlated with sensory analysis. Numerous tests exist for the measurement of oxidative deterioration of foods. The most common methods and their advantages and disadvantages are discussed in the following.

4.11.6.2 Primary Lipid Oxidation Products

Primary lipid oxidation products are compounds that are produced by the initiation and propagation steps of lipid oxidation. Since these are the first oxidation products produced, they can appear early in the oxidative deterioration of lipids. However, during the latter stages of oxidation, the concentrations of these compounds decrease, as their formation rates become slower than their decomposition rates. A disadvantage of using primary products to measure oxidation is that primary products are not volatile and thus do not directly contribute to off-flavors and aromas. In addition, under certain conditions such as high temperatures (frying oils) or high amounts of reactive transition metals, the concentration primary products may not increase since their decomposition rates are high. This would produce misleading results since a very rancid oil could have very low concentrations of primary lipid oxidation products.

4.11.6.3 Conjugated Double Bonds

Conjugated double bonds are rapidly formed in polyunsaturated fatty acids upon the abstraction of hydrogen in the initiation step. Conjugated dienes have an absorption maximum at 234 nm with a molar extinction coefficient of $2.5 \times 10^4\ M^{-1}\,cm^{-1}$ [7]. This extinction coefficient gives an intermediate level of sensitivity compared to other techniques. Conjugated dienes can be useful for simple oil systems, but it is often ineffective in complex foods where many compounds exist that also absorb at similar wavelengths and thus cause interference. Sometimes conjugated diene values are used interchangeably with lipid hydroperoxides since many lipid hydroperoxides will contain a conjugated diene system. However, this equivalence should be discouraged because fatty acid breakdown products can also contain conjugated double bonds and also because monounsaturated fatty acids (e.g., oleic) will form hydroperoxides that do not have a conjugated diene system. Conjugated trienes

can also be measured in foods at 270 nm. This technique is useful only with lipids that have more than or equal to three double bonds and thus is limited to highly unsaturated oils such as those from linseed and fish.

4.11.6.4 Lipid Hydroperoxides

A very common method to measure the oxidative quality of lipids is to measure fatty acid hydroperoxides. Most methods that measure lipid hydroperoxides rely on the ability of the hydroperoxides to oxidize an indicator compound. Peroxide values are expressed as milliequivalents (meq) of oxygen per kg of oil, with 1 meq equal to 2 mmol of hydroperoxide. The most common titration method uses the hydroperoxide-promoted conversion of iodide to iodine. Iodine is then titrated with sodium thiosufite to produce iodide, which is measured with a starch indicator [70]. This method is relatively insensitive (0.5 meq/kg oil) and can require up to 5 g of lipid; thus it is practical only for isolated fats and oils. Lipid hydroperoxide–promoted oxidation of ferrous to ferric ions can also be used, with ferric ions being detected with ferric ion–specific chromophores such as thiocyanate or xylenol orange [79]. These methods are much more sensitive than the titration methods, with the thiocyanate method having an extinction coefficient of $4.0 \times 10^4\ M^{-1}\ cm^{-1}$ allowing analysis to be performed with milligram quantities of lipids [79].

4.11.6.5 Secondary Lipid Oxidation Products

Secondary lipid oxidation products are compounds that arise from the decomposition of fatty acid hydroperoxides via reactions such as β-scission. As described previously, these reactions can generate hundreds of different compounds, both volatile and nonvolatile, from the oxidation of food lipids. Since it is virtually impossible to measure all of these compounds simultaneously, these methods generally focus on the analysis of a single compound or a class of compounds. A drawback of these methods is that the formation of secondary products relies on the decomposition of lipid hydroperoxides. Thus in certain cases (e.g., presence of antioxidants), the concentrations of secondary products can be low while the primary product concentrations are high. In addition, compounds in foods containing amine and sulfhydryl groups (e.g., proteins) can interact with secondary products that contain functional groups such as aldehydes, thus making them difficult to measure. An advantage of these measurements is that they measure many of the products from fatty acid decomposition which are responsible for the off-flavors and aromas in rancid oils and thus have higher correlation with sensory analysis.

4.11.6.6 Analysis of Volatile Secondary Products

Volatile lipid oxidation products are typically measured by gas chromatography using direct injection, static or dynamic headspace, or solid-phase microextraction (SPME) [48]. Using these systems, lipid oxidation can be measured using specific products (e.g., hexanal for lipids high in ω-6 fatty acids and propanal for lipids high in ω-3 fatty acids), product classes (e.g., hydrocarbons or aldehydes), or by total volatiles. Each method can give different profiles of volatiles due to the differences in their ability to drive the volatiles out of the sample. The advantage of measuring volatile lipid oxidation products is a stronger correlation with sensory analysis compared to primary oxidation products. The disadvantage is the expense of instrumentation and the difficulty in analyzing large amounts of samples especially in lipids that oxidize rapidly (these techniques are often time consuming). In addition, these methods often use heating steps to drive the volatiles into the headspace. In some foods such as meats, these heating steps may increase lipid oxidation rates by cooking the food. In general, lipids should be sampled at the lowest temperature possible. An additional problem is the loss of volatiles by processes such as steam distillation in frying oils.

4.11.6.7 Carbonyls

Carbonyls arising from lipid oxidation can be determined by reacting lipids with 2,4-dinitrophenylhydrazine to form a complex that absorbs light at 430–460 nm. This method is

limited by the presence of other carbonyls in foods that can cause interference [70]. HPLC techniques have been developed to separate carbonyls arising from lipid oxidation from interfering compounds. However, these techniques are expensive and time consuming, and are therefore not routinely used in food lipids.

Carbonyls can also be measured by conjugation with anisidine to form products that absorb at 350 nm. This method is useful because it can measure nonvolatile, high-molecular-weight carbonyls. Thus, anisidine is used to measure oxidation in products such as fish oils since these oils commonly undergo steam distillation during refining. Anisidine is therefore useful in fish oils because it can give an indication of the quality of the oil prior to steam distillation, since nonvolatile, high-molecular-weight compounds are retained in the oil.

4.11.6.8 Thiobarbituric Acid (TBA)

The TBA assay is based on the reaction between TBA and carbonyls to form red fluorescent adducts under acidic conditions [94]. The assay can be conducted on whole samples, sample extracts, or distillates, and adduct formation can be conducted under a number of varying temperature (25°C–100°C) and time (15 min–20 h) protocols. The compound often attributed to be the primary lipid oxidation product detected by TBA is malonaldehyde (MDA) whose TBA adduct absorbs strongly at 532 nm. MDA is a dialdehyde produced by a two-step oxidative degradation of fatty acids with three or more double bonds. This means that MDA yield during the oxidation of lipids is dependent on the fatty acid composition, with highly unsaturated fatty acids producing high amounts of MDA. TBA can also react with aldehydic lipid oxidation products other than malonaldehyde, especially unsaturated aldehydes.

The TBA assay suffers from nonspecificity due to its ability to react with nonlipid carbonyls such as ascorbic acid, sugars, and nonenzymic browning products. These compounds can form TBA adducts, which absorb over the range 450–540 nm. Often, it is more appropriate to refer to TBA reactive substances (TBARSs) to acknowledge that compounds in addition to MDA can generate pink chromophores. To decrease problems with interfering compounds, the TBA–MDA complex can be measured directly by fluorescence or HPLC techniques.

The TBA assay can be a useful method for analysis of lipid oxidation in foods since it is simple and inexpensive. However, the nonspecificity of the method requires an understanding of the test's limitations, so improper comparisons and conclusions are not made. To minimize potential misinterpretation of TBA analysis, it is suggested that analysis of fresh, nonoxidized samples be conducted to obtain baseline data on TBA reactive substances in product that do not arise from lipid oxidation. However, the TBA method should be avoided in foods where concentrations of interfering compounds are high. In addition, attempts to use TBA to compare oxidation between products with different fatty acid compositions are inappropriate since the MDA yield varies with the fatty acid composition.

4.11.7 Summary

Hydrolysis of triacylglycerol can impact food quality by releasing fatty acids, which negatively impact flavor, physical properties, and oxidative stability of fats and oils.

- Oxidation rancidity occurs via autocatalytic free-radical reactions.
- Oxidative rancidity occurs when fatty acids are decomposed into low-molecular-weight aldehydes and ketones.
- Prooxidant such as transition metals, singlet oxygen, and enzymes are often the major cause of lipid oxidation in foods.
- Antioxidants slow oxidation by scavenging free radicals and/or decreasing the activity of prooxidants.
- Lipid oxidation is also influenced by factors such as oxygen concentrations, fatty acid unsaturation, temperature, and water activity.

- Sensory analysis is the gold standard for measuring oxidative rancidity.
- Lipid oxidation can be monitored by measuring the primary oxidation products, but these tend to not be strongly correlated with rancidity.
- Secondary lipid oxidation products originate from fatty acid decomposition and can be more strongly correlated with rancidity.

4.12 FOOD LIPIDS AND HEALTH

4.12.1 Bioactivity of Fatty Acids

Dietary lipids have often been negatively associated with health. Since obesity is highly correlated with numerous diseases such as heart disease and diabetes, the negative role of lipids in health is often attributed to their high caloric density of 9 kcal g^{-1}. Specific dietary lipids have also been associated with the risk of heart disease due to their ability to modulate low-density lipoprotein (LDL)-cholesterol levels in the blood. Since LDL-cholesterol levels are often associated with the development of heart disease, several dietary strategies have been proposed to decrease LDL-cholesterol, including consumption of dietary saturated fatty acids at <10% of calories, limiting dietary cholesterol to <300 mg day^{-1}, and keeping dietary *trans* as low as possible [19]. Recently, the role of dietary saturated fatty acids in heart disease has been questioned, since saturated fatty acids raise "good" HDL-cholesterol. In addition, the biological effect of saturated fatty acids varies with fatty acid type [14].

4.12.2 *Trans* Fatty Acids

Trans fatty acids have gained attention because of their unique role in heart disease through their ability to both increase LDL-cholesterol and decrease HDL-cholesterol [47]. This behavior is partially due to the geometric configuration of *trans* fatty acids, which is more similar to that of saturated fatty acids than of unsaturated fatty acids. Because of the potential harm of *trans* fatty acids, foods are required to disclose *trans* fatty acid concentrations on their nutritional labels in many countries. In the United States, foods with less than 0.5 g of fat/serving do not have to label *trans* fatty acids as long as no claims are made about fat, fatty acids, or cholesterol content. This is because refining causes the formation of *trans* fatty acids, so most commercial oils are not *trans*-free. Because of the labeling requirement, *trans* fatty acid concentrations in processed foods have decreased dramatically [75].

While a large amount of research has been devoted to the negative aspects of dietary lipids on health, more recently evidence is growing that some dietary lipids can reduce the risk of several diseases. These bioactive lipids include ω-3 fatty acids, phytosterols, carotenoids, and conjugated linoleic acid.

4.12.3 ω-3 Fatty Acids

As agricultural practices have advanced, the profile of dietary lipids in Western societies has changed dramatically. Our ancestors are thought to have consumed diets with approximately equal amounts of ω-6 and ω-3 fatty acids. The development of modern agriculture increased the availability of refined fats, especially vegetable oils, changing our dietary ω-6 to ω-3 ratio to >7:1. This is an extremely rapid change on an evolutionary timescale, and is problematic since humans interconvert ω-6 and ω-3 fatty acids at slow rates. A lower amount of ω-3 fatty acids in the diet is important because these bioactive lipids play a vital role in membrane fluidity, cellular signaling, gene expression, and eicosanoid metabolism. Therefore, consumption of dietary ω-3 fatty acids (ω-3s) is essential for the promotion and maintenance of good health, especially for pregnant and lactating women and individuals with coronary heart disease, immune-response disorders, and compromised

mental health. There is strong evidence that the level of ω-3s currently consumed by the general population is inadequate [19]. Numerous food companies are attempting to increase the levels of these bioactive lipids in their products by direct incorporation of ω-3 fatty acids into foods or by feeding ω-3 fatty acids to livestock. These approaches are commonly impeded by the oxidative deterioration of ω-3 fatty acids during the processing and storage of fortified food products.

4.12.4 Conjugated Linoleic Acid

The two double bonds of linoleic acid are normally in a methylene-interrupted system, where two single bonds separate the double bonds. However, the double bond system is sometimes altered, resulting in isomerization of the double bonds to a conjugated configuration. This isomerization can occur during processes such as hydrogenation, and is common during the biohydrogenation process promoted by bacteria in ruminants. These isomers, known as *conjugated linoleic acid* (CLA), have gained widespread interest due to their ability to inhibit cancer [35], lower blood cholesterol [49], inhibit the onset of diabetes, and influence weight gain [69]. The different isomers have different biological effects, with 9-*cis*, 11-*trans* linoleic acid showing anticarcinogenic activity and 10-*trans*, 12-*cis* linoleic acid having the ability to influence body fat accumulation. The 9-*cis*, 11-*trans* isomer of CLA is the predominant isomer found in dairy and beef products [80]. Molecular mechanisms of CLA bioactivity have been attributed to their ability to modulate of eicosanoid formation and gene expression. A meta-analysis of dietary CLA consumption by humans indicated that CLA had little impact on body composition [65].

4.12.5 Phytosterols

The major phytosterols are sitosterol, campesterol, and stigmasterol. Dietary phytosterols have little to no absorption into the blood. Their bioactivity lies in the fact that they can inhibit the bioavailability of both dietary and biliary (produced by intestinal cells) cholesterol [72,73]. Intake of 1.5–2 g day^{-1} of phytosterols can reduce LDL-cholesterol by 8%–15%. Since phytosterols primarily inhibit cholesterol absorption, they are most effective when consumed with a cholesterol-containing meal. Phytosterols have very high melting points, and therefore exist as lipid crystals at the temperatures common to many foods. To minimize crystallization, phytosterols are commonly esterified to unsaturated fatty acids to increase their lipid solubility.

4.12.6 Carotenoids

Carotenoids are a diverse group (>600 different compounds) of yellow to red colored polyenes that are lipid-soluble. Vitamin A is an essential nutrient obtained from carotenoids such as β-carotene. The bioactivity of other carotenoids has been a research area of great interest. This interest was initially focused on the antioxidant activity of carotenoids. However, when clinical trials were conducted to evaluate dietary β-carotene in subjects at risk to free-radicals stress (smokers), β-carotene was found to increase lung cancer rates [6]. Other carotenoids have been found to have health benefits. Lutein and zeaxanthin can enhance visual activity [31]. The health benefits of tomatoes have been attributed to the carotenoid lycopene [62]. Interestingly, cooked tomatoes have greater lycopene bioavailability, presumably due to the thermally induced conversion of *trans*-lycopene to *cis*-lycopene.

4.12.7 Low-Calorie Lipids

One of the other health concerns of dietary triacylglycerols is their high caloric density. Many attempts have been made to produce low-fat foods that have the same sensory attributes as their full-fat counterparts by using fat mimetics. Fat mimetics are nonlipid compounds such as proteins

or carbohydrates that can produce fat-like properties at lower caloric values. A similar approach has been attempted to produce lipid components with no calories or with lower caloric contents (fat substitutes). The first commercial non-caloric lipid was sucrose fatty acid esters (Proctor & Gamble's Olestra). This compound is non-caloric because the presence of more than or equal to six fatty acids esterified to sucrose sterically prevents lipase from hydrolyzing the ester bond to release free fatty acids that can be absorbed into the blood. The nondigestibility of sucrose fatty acid esters means that they pass through the gastrointestinal tract and are excreted in the feces. This property can cause gastrointestinal problems such as diarrhea.

Structured lipids with lower caloric density have also been used in the food industry (e.g., Nabisco's Salatrim). These products are based on the principle that fatty acids at *sn*-1 and *sn*-3 of triacylglycerol are released as free fatty acids upon hydrolysis by pancreatic lipase. If *sn*-1 and *sn*-3 have long-chain saturated fatty acids (≥16 carbons), their release can lead to interactions with divalent cations to form insoluble soaps that are not readily bioavailable. Structured low-calorie fats also use short-chain fatty acids (≤6 carbons) at the *sn*-2 position. After hydrolysis by pancreatic lipase, *sn*-2 monoacylglycerol is absorbed into the intestinal endothelial cells. The short-chain fatty acids at *sn*-2 eventually are metabolized in the liver where they yield fewer calories than long-chain fatty acids. The combination of both long-chain saturated fatty acids at *sn*-1 and *sn*-3 and short-chain fatty acids at *sn*-2 produces a triacylglycerol with 5–7 cal g^{-1}.

4.12.8 Summary

Lipids can be both deleterious and beneficial to health.

- *Trans* fatty acid have been negatively associated with health because they increase LDL- and decrease HDL-cholesterol.
- Omega-3 fatty acids, conjugated linoleic acid, phytosterols, and carotenoids are examples of lipids that positively influence health.
- The caloric content of lipids can be decreased by altering their digestion and metabolism.

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