

Chemical and Functional Properties of Food Lipids

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Preface

Lipids contribute very significantly to the sensory and nutritional properties of most kinds of foods, even if their content is not high. Nature has created a large number of lipids that perform various functions in plants and animal organisms. Furthermore, enzymatic and chemical processes applied in agriculture and in the food industry serve to generate novel, tailor-made lipids that fulfill the requirements of nutritionists, consumers, and processors. The changes that take place in raw materials post harvest, as well as chemical and biochemical interactions of lipids with other food components during storage and processing, affect all aspects of food quality. Most of these processes can be effectively controlled by those who know food chemistry.

This book differs from the standard text on lipid chemistry. It contains a concise, yet well-documented, presentation of the current state of knowledge on the occurrence, distribution, chemical and biochemical reactivity, functional properties, and biological role of lipids in food systems. The first two chapters describe the effect of lipids and their distribution in agricultural raw materials on food quality. [Chapter 3](#) contains a description of the current nomenclature, as well as the main chemical and physical properties of lipids, with [Chapter 4](#) extending the subject coverage to the rheological properties. [Chapters 5, 6, and 7](#) bring new information on phospholipids, sterols, and fat-soluble vitamins in foods. Of crucial significance for the sensory and nutritive aspects of food quality are the reactions and their effects described in [Chapter 8](#) on lipid oxidation. The current state of techniques used for lipid analysis is presented in [Chapter 9](#), while lipids in human nutrition are expertly treated in [Chapter 10](#). The lipids of plants, fish, milk, and eggs, as well as their roles in different foods are described in [Chapters 11 through 14](#), while modified lipids and frying fats are presented in [Chapters 15 and 16](#). Interactions of components are of crucial importance for food quality — these are the subject of [Chapter 17](#).

Most chapters are concise monographs prepared by renowned specialists in their respective areas. They are based on the personal research and teaching experience of the contributors, as well as on critical evaluations of the present state of knowledge as reflected in the current, extensive world literature. The references in the chapters include articles published in several languages in addition to English. This volume is addressed to food scientists in industry and academia, to food science graduate students, and also to nutritionists and all persons interested in the role and attributes of lipids as food components.

The editors have been fortunate to have had the opportunity to work with knowledgeable colleagues from universities and research institutions in Australia, the Czech Republic, Germany, Greece, Poland, and Spain. Their acceptance of our conception of the book and of the editorial guidelines is highly appreciated. Special thanks are due to those contributors who prepared their chapters well ahead of time. It was possible to publish the book without significant delay only because of the

understanding of Dr. Eleanor Riemer and Sara Kreisman of CRC Press, who agreed to accept several contributions even after the original deadline.

We are honored to dedicate this volume to the memory of Professor Henryk Niewiadomski, the organizer and head of the Department of Fat Technology at the Gdańsk University of Technology, and one-term president of the International Society of Fat Research. His books on fat raw materials and fat technology have been an invaluable source of information for generations of students and professionals. Thanks are also due to several of our co-workers, who willingly helped us in different ways, as well as to Krystyna and Edward, who tolerated their life partners engrossed in editing this book. We are most thankful to God for allowing us to bring the task to a positive end and for giving us the chance to learn to be more humble.

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Zdzisław E. Sikorski received his B.S., M.S., Ph.D., and D.Sc. degrees from the Gdańsk University of Technology (GUT), and doctor honoris causa from the Agricultural University in Szczecin, Poland. He served as organizer and head of the Department of Food Chemistry and Technology and Dean of the Faculty of Chemistry at GUT, and was visiting researcher/professor at the Ohio State University, Columbus, Ohio; CSIRO, Hobart, Australia; DSIR in Auckland, New Zealand; and National Taiwan Ocean University, Keelung. He is currently professor at GUT and, since 1996, the chairman of the Committee of Food Technology and Chemistry of the Polish Academy of Sciences. He has published about 200 journal articles, 11 books (in Polish, English, Russian, and Spanish), and eight book chapters in marine food science and food chemistry, and holds seven patents. His research deals mainly with food preservation, functional properties of food proteins, and interactions of food components.

Anna Kołakowska received her B.S. and M.S. degrees from the Faculty of Food Technology, University of Agriculture in Olsztyn, and Ph.D. and D.Sc. from the University of Agriculture in Szczecin, Poland. She is the first woman to have spent two winters in Antarctica, working at a research station.

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Contents

Chapter 1 The Role of Lipids in Food Quality

Anna Kołakowska and Zdzisław E. Sikorski

Chapter 2 Lipids in Food Structures

M. Ángeles Lluch, Isabel Hernando, and Isabel Pérez-Munuera

Chapter 3 The Nomenclature, Structure, and Properties of Food Lipids

David S. Nichols and Kevin Sanderson

Chapter 4 Rheological Properties of Lipids

Tadeusz Matuszek

Chapter 5 Phospholipids

Jan Pokorný

Chapter 6 Cholesterol and Phytosterols

Erwin Wąsowicz

Chapter 7 Fat-Soluble Vitamins

Małgorzata Nogala-Kalucka

Chapter 8 Lipid Oxidation in Food Systems

Anna Kołakowska

Chapter 9 Principles of Lipid Analysis

David S. Nichols

Chapter 10 Lipids in Human Nutrition

Roman M. Cichon

Chapter 11 Plant Lipids and Oils

Jan Pokorný and Lucie Parkányiová

Chapter 12 Fish Lipids

Anna Kołakowska, June Olley, and Graeme A. Dunstan

Chapter 13 Milk Lipids

Zofia A. Żegarska

Chapter 14 Egg Lipids

Waldemar Ternes

Chapter 15 Modified Lipids and Fat Mimetics

Włodzimierz Bednarski and Marek Adamczak

Chapter 16 Frying Fats

Dimitrios Boskou

Chapter 17 Lipid–Protein and Lipid–Saccharide Interactions

Jan Pokorný and Anna Kołakowska

1 The Role of Lipids in Food Quality

Anna Kołakowska and Zdzisław E. Sikorski

CONTENTS

- 1.1 Introduction
- 1.2 The Contents of Lipids in Food
- 1.3 The Composition of Lipids in Foods
 - 1.3.1 Lipid Classes
 - 1.3.2 Fatty Acids
- 1.4 The Effect of Lipids on the Nutritional Value and Safety of Foods
 - 1.4.1 The Beneficial Role of Fatty Components in Foods
 - 1.4.2 The Deleterious Effects of Lipids
- 1.5 The Effect of Lipids on the Sensory Attributes of Foods
 - 1.5.1 Introduction
 - 1.5.2 Color
 - 1.5.3 Texture
 - 1.5.4 Aroma and Flavor
- 1.6 Interactions of Lipids with Other Components
- References

1.1 INTRODUCTION

Lipids are important components that contribute very significantly to the nutritional and sensory value of almost all kinds of foods, except for most fruits, sweets, and beverages. Nature provides a large number of fats that differ in their chemical and functional properties. Furthermore, enzymatic and chemical processes applied in industry serve to create novel, tailor-made lipids that fulfill the requirements of the nutritionists, gourmets, and food processors.

The effect on food quality is predominantly related to the contents, distribution in the food matrix, chemical composition, and reactivity of the lipids, as well as to their physical changes due to processing, and the interactions with other components.

1.2 THE CONTENTS OF LIPIDS IN FOOD

All plants and animals eaten by humans contain lipids, which are the essential components involved in maintaining life activities. Only a few food products are devoid of lipids; for example, sugar, honey, and clarified juices. Various vegetables and most fruits also belong to the food commodities with very low amounts of fat, generally about 0.3%. A notable exception is the avocado, the edible part of which contains about 20% lipids. The lipid content in the muscle tissue of lean beef, fish, white poultry, and shellfish is about 2%, about 3.7% in cow's milk, about 2 to 4% in grains, about 30% in fatty pork, 32% in an egg yolk, and up to 35% in fillets of fatty fish. Oil-bearing nuts and seeds contain from 20% fat in soybeans to 65% fat in walnuts. The factors that affect the lipid content in food raw materials include the species, genotype, and variety of the plant or animal, as well as the part of the plant or organ of the carcass; for example, there is up to 70% oil in cod liver and only 1/100 of this amount in the fillet. The temperature and other conditions of vegetation or breeding are also important, as well as the maturity of the plant at harvest, feeding and age, sex, maturity of the slaughter animal, and stage of development of the gonads in fish.

In processed foods, the fat content depends on the raw material specificity and the required sensory properties of the products. It is also affected by the suggestions of nutritionists. The fat content in bread is 0.5 to 1.5%, in chocolate 22 to 36%, and in confectionery products from 3.4% for a wafer to 36% for a praline; margarine is 80% or 39 to 41%, depending on the type of a product; butter 81 to 85%; other dairy products from 0.5 to 30%; and meat products 13 to 50%.

1.3 THE COMPOSITION OF LIPIDS IN FOODS

1.3.1 LIPID CLASSES

Lipids occurring in plant and animal materials consist of structural lipids, which build the cell membranes, and depot fats. The cell and organelle membranes of animal organisms are made of phospholipids and non-esterified cholesterol, whereas in plants they consist of phospholipids and glycolipids. The latter are also found in the central nervous systems of some animals. If a muscle tissue like that in lean fish contains only 0.3% w/w of lipids, they consist almost entirely (90%) of phospholipids. Galactoglycerols and phospholipids serve as important factors in nutrient and antioxidant delivery systems (Herslof, 2000).

Triacylglycerols (TAG) in plants and animals and waxes in some organisms are a convenient means of storing food energy. Waxes are characteristic for organisms of polar regions and deep-ocean fish. Due to buoyancy wax, these species are able to quickly change their position vertically. Depot fats — plant and animal TAG — have been exploited by humans for centuries. During processing, the depot fats undergo more changes than structural lipids. They can be partially removed or added during technological processes and are used as a frying medium. Polar lipids, phospholipids, mono- and diacylglycerols are usually used in smaller amounts as functional additives. The intake of phospholipids with plant and animal

foods can be regarded as the only benefit of excessive consumption of invisible fats. However, most of the invisible fat consumed originates from added fats, particularly frying fats absorbed by the fried products. For example, in the raw state, fillets of cod, Alaska pollock, or hake contain less than 1% fat rich in n-3 polyenoic (polyunsaturated) fatty acids (PUFA); after frying, the lipid contents of these fillets is more than 20%, with only trace quantities of the beneficial PUFA (Kořakowska et al., 2001).

The content of cholesterol, which is principally bound to membrane lipids, cannot always be efficiently reduced by decreasing the proportion of lipids in a product. Therefore, the use of novel solutions, such as supercritical carbon dioxide extraction, has been suggested.

1.3.2 FATTY ACIDS

Fatty acid (FA) composition determines the physical properties, stability, and nutritional value of lipids. All lipids of natural origin consist of saturated FA, monoenoic FA, and PUFA in various proportions and differ in detailed FA composition. Variations in plant and animal lipid FA composition make it possible to determine the origin of the lipids. The profile of FA, especially in phospholipids, which due to their function are less prone to changes, is considered a species “identity card” (Grahl-Nielsen, 1999; Joenson and Grahl-Nielsen, 2001). The composition of depot fat reflects the composition of lipids in an animal’s diet and the vegetation conditions of the plant. This can be used to modify the FA pattern in the meat of farm animals, fish, poultry, and eggs. In ruminants, it requires special procedures to avoid the modifying influence of rumen microflora on fat composition. There are noticeable differences among hybrids in the susceptibility to modification according to species, variety, and genus. Changes in the FA composition of pig muscle fat induced by diet may increase the susceptibility to oxidation to a greater extent than would be expected by alterations in FA (Kořakowska et al., 1998). The composition of FA in plant lipids is generally altered at the molecular level and is the subject of intensive research that has already led to successful modification of rapeseed oil to the low erucic, high erucic, high oleic, high linoleic, and high lauric acid oils. Genetic modifications and spontaneous mutations of soybeans have produced low linolenic acid oil, high oleic acid oils, and high saturated FA oils (Orthoefer, 1996; Piazza and Foglia, 2001).

FA distribution in TAG as well as in phospholipids affects the physical properties, lipolytic and oxidative stability, and nutritional availability of lipids. In many TAG, the FA are arranged in a nonrandom distribution. In plants, monoenoic FA and PUFA are dominant at a *sn*-2 position (Orthoefer, 1996). In pig depot fat and in cow’s milk, the TAG *sn*-2 position is occupied by palmitic acid. The distribution is also different in cattle and sheep depot fats (Love, 1996). In blubber seals, long-chain n-3 PUFA are esterified rather in *sn*-1,3 positions, whereas, in muscle, TAG in the *sn*-2 position that is typical for the lipid muscles of nearly all fish (Ackman, 1994). Enzymatic syntheses of structured TAG containing *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) in the *sn*-2 position with medium-chain FA at the end positions are particularly interesting (Halldorsson

et al., 2001). However, the possibility of synthesis of structured TAG containing [60] fullerene is currently most interesting and thought-provoking (Lie Ken Jie et al., 2001)

1.4 THE EFFECT OF LIPIDS ON THE NUTRITIONAL VALUE AND SAFETY OF FOODS

1.4.1 THE BENEFICIAL ROLE OF FATTY COMPONENTS IN FOODS

Lipids play many essential roles in cell biology. They act as structural materials or as cell signals, provide a barrier for ion separation, and prevent cells from pathogen invasion and water loss. Among many other functions, they also act as temperature sensors and serve as a substrate for eicosanoids (Slabas et al., 2001). To fulfill these functions, n-6 and n-3 FA that cannot be synthesized by animal organisms need to be delivered in a diet. The majority of diet-supplied lipids serve as a source of energy and carriers of lipophilic, biologically active compounds, such as vitamins and antioxidants.

The increase in the number of obese people in the population and a relation between obesity and some diseases has led to a tendency to reduce fat intake. The actual trend with respect to fat content in the diet is to reduce the energy value derived from lipids to not more than 20% of total intake. It is constantly recommended to decrease the intake of saturated FA, although the hypercholesterolemic or neutral effect of each individual saturated FA is not clear (Khosla, 1995; Haumann, 1998). A recommended ratio of n-6/n-3 has not been established, although it has been suggested in the recent years that it should be reduced. Recently, it has become clear that this should refer to the ratio of long-chain n-6/n-3 PUFA. Considering the competitiveness in relation to delta-6 desaturase of n-3 and n-6, it is recommended to reduce the dietary intake of linoleic acid and increase that of fish lipids containing EPA and DHA. Excessive dietary supply of linoleic acid produces an unfavorable rise in this ratio, which might explain the increased incidence of chronic inflammatory conditions in modern societies (Lands, 1997).

1.4.2 THE DELETERIOUS EFFECTS OF LIPIDS

Health hazards posed by lipids may be the result of:

- The presence of natural harmful substances and unusual FA
- Lipid oxidation
- The formation of *trans* isomers
- Interactions with nutrients, especially proteins
- The accumulation of toxic lipophilic substances from the environment

Lipid oxidation *in vivo* is correlated with many diseases and is a base for the aging theory. The oxidation of food lipids depends on their FA composition but mostly on the pro- and antioxidant status in the food matrix, as well as on external factors such as light, oxygen pressure, and temperature. Lipid oxidation results in

sensory alterations of the product, even if the lipids constitute only a small percentage of that product, such as off-odor, off-flavor, discolorations, and deterioration of texture. There are only a few positive aspects of lipid oxidation, such as formation of conjugated linoleic acid in milk fat, promotion of weak interactions between fish lipids and proteins that can stabilize lipids during frozen storage (Kořakowska et al., 1995; Kořakowska and Szczygielski, 1994), bleaching of flour to produce white bread, and improvement in dough-forming properties (Hammer, 1993). The necrosis and apoptosis effect of EPA in lymphoma cells is explained by radical formation due to oxidation processes (Heimli et al., 2001).

There is only scarce evidence confirming the toxic influence of hydroperoxides from the diet. The toxicity of thermo-oxidized frying fats on humans is better verified. The toxicity of oxidized cholesterol, especially at temperatures higher than 100°C and in the presence of unsaturated lipids, has attracted particular attention, as has the role of oxy fitosterols. Antioxidants continue to be the subject of intensive researches and discussions (Pokorný et al., 2001). Their activity in food is different than *in vivo*. Although no clear understanding of the prooxidant activity of antioxidants exists, supplementation of antioxidants to food products and to animal organisms does take place.

Consumption of large amounts of lipids in fatty fish, meat, and dairy products may result in exceeding the permissible dose of dioxins and dioxin-like PCBs.

1.5 THE EFFECT OF LIPIDS ON THE SENSORY ATTRIBUTES OF FOODS

1.5.1 INTRODUCTION

In general, lipids have a desirable impact on the sensory properties of many foods by affecting the mouth-feel; for example, the richness of whole milk as opposed to the blank taste of skim milk, or the smoothness of high-quality ice cream. The particular sensory attributes of foods that are affected by lipids primarily include the color, rheological properties, and flavor. They depend on the content, chemical composition, physical changes due to processing, as well as the chemical and enzymatic transformations of the lipids during storage of the food and in manufacturing operations. The desirable contribution of fats as well as of lipochromes and lipid-soluble vitamins might be easily lost due to deteriorative reactions, predominantly oxidation and hydrolysis, during storage under abusive conditions.

1.5.2 COLOR

Lipids are involved in color formation in foods by carrying different colored substances and by participating as substrates in reactions leading to the generation of colored compounds.

The surface pigmentation of marine animals is largely due to different carotenoproteins, which may be yellow, orange, red, purple, blue, or green, depending on the structure of the complexes — the kind of carotenoid, predominantly astaxanthin, cantaxanthin, and β -carotene, as well as the properties of the proteinaceous compo-

ment. Dissociation of the protein moiety from the complex in bright light brings about fading of the colors. Carotenoid pigments are also responsible for the color of the flesh oil of redfish (*Sebastes marinus*). Vegetable oils also contain different carotenoids, generally in concentrations below 0.1%. In palm oil, the carotenoid pigments (about 0.3%) are responsible for the orange color.

1.5.3 TEXTURE

The rheological properties are affected by fat in meat and meat products, in fishery products, in dairy commodities, and in pastry, cakes, and mayonnaise. The desirable texture of culinary meat is due to proper marbling of the muscles with thin fat layers; that of comminuted sausages is conditioned by an adequate fat content in the formulation. Baltic sprats caught in the summer are unsuitable as raw material for the canned product known as smoked Baltic sprats in oil because, at a fat content of less than 6%, the texture of the fish is too hard. High-quality hot smoked mackerel can be assured only by using raw material containing about 30% fat. The cream for producing whipped cream without any whipping agents should contain about 30% fat. The desirable sensory sensation caused by melting of chocolate in one's mouth is due to the narrow range of melting temperature (28 to 36°C) of the lipids in cocoa butter.

The rheological characteristics of lipids in food systems are thoroughly discussed in [Chapter 4](#) of this volume.

1.5.4 AROMA AND FLAVOR

Lipid degradation products in low concentration contribute to the mild, rather pleasant, plant-like, melon-like, seaweedy aroma of very fresh fish. Due to reactions catalyzed by endogenous lipoxygenases, hydroxyperoxide lyases, Z,E-enal isomerases, and alcohol dehydrogenases, the PUFA of fish lipids are degraded to aldehydes, ketones, and alcohols with 6, 8, and 9 carbon atoms, respectively. The gradual loss in the intensity of the fresh fish aroma is caused, in part, by microbial conversion of the carbonyl compounds into alcohols, which have higher aroma threshold values. During the storage of frozen fish, an off-flavor develops due to the oxidation of lipids.

The desirable flavor in many cheeses is created, in part, by lipid oxidation products, such as ketones and aldehydes. In lipids consisting of short-chain FA, both oxidation and lipolysis influence off-flavor.

1.6 INTERACTIONS OF LIPIDS WITH OTHER COMPONENTS

Many of the lipids found in nature are intimately bound to proteins and saccharides. The interactions within such structures are usually weak, but covalent bonds can occur (e.g., lipid inclusion in amylose or some lipid fractions in fish muscle tissue). During the processing and storage of food, the lipids are released and new bonds

can be formed. Interactions are promoted purposely or occur spontaneously during processing and storage of foods.

Both the original lipids, the products of their chemical and enzymatic alterations, and added fats and lipid surfactants participate in interactions with proteins and saccharides in different foods. As a result of tissue disintegration due to mincing and the application of high shear forces in homogenization, the fats are emulsified in the food matrix in the presence of proteins and other surfactants, forming the desirable properties of mayonnaise and comminuted sausages. The interactions of lipids, proteins, and saccharides during mixing of a cake batter lead to the typical cake texture. In cereal products, various lipid–saccharide and lipid–protein interactions take place, although the effects caused by endogenous, native, neutral lipids, and by added fats and surfactants are different in several aspects. The interactions of lipids with starch affect the leaching of amylose out of the starch granules and swelling of the granules due to heating, decrease the rate of staling of bread and improve the texture of the loaf (Eliasson, 1998). Lipoprotein films formed in the bread dough by interactions of polar lipids with some wheat proteins are desirable in bread-making, while non-polar fats and fatty acids destabilize the protein foams (Marion et al., 1998).

The reactivity of fish lipids and proteins depends on variety of both the level of oxidation and reaction conditions. PUFA interact more easily and form stronger bonds with proteins and starches than other FA. DHA is preferentially bound. Fresh lipids are more reactive than oxidized lipids. The interactions of fish lipids with amylose are different than those with amylopectin. The effect of heating and freezing on lipid–protein, lipid–amylose, and lipid–amylopectin interactions is also different (Bienkiewicz and Kołakowska, 2001a,b). The interactions of lipids with other food components create new products attributes and also affect the properties of fats, particularly their extractability and availability in the human organism.

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2 Lipids in Food Structures

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CONTENTS

- 2.1 Introduction
- 2.2 Lipids in Oilseeds Structure
- 2.3 Lipids in Olives Structure
- 2.4 Lipids in Shell(Nut) Fruits Structure
- 2.5 Lipids in Milk Structure
- 2.6 Lipids in Dairy Products Structure
 - 2.6.1 Ice Cream
 - 2.6.2 Whipped Cream
 - 2.6.3 Cheese
 - 2.6.4 Butter
- 2.7 Lipids in Margarine and Shortenings Structure
- 2.8 Lipids in Egg Structure
- 2.9 Lipids in Meat and Meat Products Structure
- 2.10 Lipids in Chocolate Structure
- 2.11 Lipids in Mayonnaise and Sauces Structure
- 2.12 Oil Uptake in Fried Products
- References

2.1 INTRODUCTION

An appreciation of the microstructure of food and its components is now being recognized as a necessary prerequisite for understanding its properties. There are different methods that can be used to examine the components of food. Lipids can be studied by light microscopy (LM) and also by electron microscopy (EM). EM provides better resolution and higher magnification than LM, and can be divided into two different techniques: transmission electron microscopy (TEM), in which a two-dimensional image is formed, and scanning electron microscopy (SEM), in which a three-dimensional image is formed.

The preparation of samples for the observation of lipids in foods by both LM and EM involves the chemical fixation of the lipid. For this purpose, the most useful technique is osmium fixation. With this technique, excellent images can be obtained in which the lipids are suitably stabilized by the cross-linking properties



FIGURE 2.1 SEM micrograph of soybean cotyledon cells. Protein bodies (p), lipid bodies (l), cytoplasmic network (c).

of the osmium tetroxide with the double bonds of some fatty acids; the lipids are fixed and remain in place during fracturing for SEM or sectioning for TEM and LM.

Although neutral triacylglycerols (TAG), the most common form of storage lipid, are not considered a major structural element in food tissues, other lipids (e.g., phospholipids) are essential for the formation of cellular membranes. In addition, phospholipids play a decisive role in some food formulations by serving as emulsifiers.

This chapter discusses the structure of lipids in different types of food.

2.2 LIPIDS IN OILSEEDS STRUCTURE

There are a large number of oilseeds; some of them are very important because they yield edible oils. Among them are soybean (*Glycine max*), rapeseed (*Brassica campestris*), coconut (*Cocos nucifera*), and sunflower (*Helianthus annuus*). In addition to their lipid content, ranging from 20 to 55%, oilseeds often have substantial amounts of protein stored as protein bodies (30% or more v/v) and saccharides stored as cell wall components. Thus, the main subcellular structure consists of protein bodies 5 to 20 μm in diameter, surrounded by a cytoplasmic protein network in which are embedded the oil storage sites (Figure 2.1). These lipid bodies, sometimes referred to as spherosomes, are only 0.2 to 0.5 μm in diameter and are connected through a thin envelope.

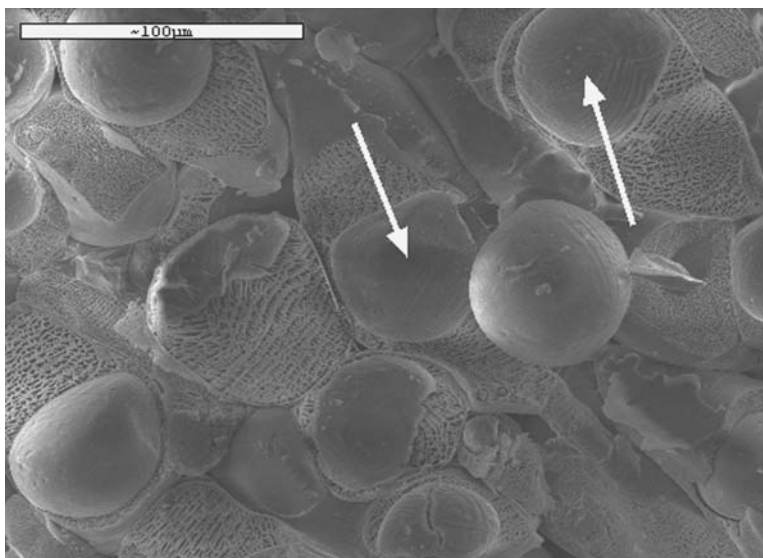


FIGURE 2.2 Cryo-SEM micrograph of olive. Storage lipids (arrows).

2.3 LIPIDS IN OLIVES STRUCTURE

The mesocarp, which constitutes more than 80% of the fruit, is parenchymal tissue composed of thin-walled pectocellulosic cells. These are storage cells containing inorganic and organic compounds that constitute the main tissue of the edible portion of the fruit. The lipids of olive (22%) are organized in nearly ellipsoidal isolated droplets enveloped by a lipoproteic membrane. These lipid droplets can coalesce to form larger droplets that can be stored in a central vacuole (Figure 2.2).

2.4 LIPIDS IN SHELL(NUT) FRUITS STRUCTURE

Shell(nut) fruits are variable in composition; their moisture content is less than 10%, N-compounds are about 20% and lipids are as high as 40%. The edible part of shell fruits primarily consists of two cotyledons. The cotyledon tissue of almond raw materials is made up of rather large, nearly isodiametric parenchymal cells with a thick cell wall. The cytoplasmic membrane delimits the subcellular organelles, with the protein bodies and lipid bodies (sphaerosomes) being the major components (Pascual-Albero et al., 1998). The spherical protein bodies (2.5 to 6 μm in diameter) seen in SEM (Figure 2.3) show a rather attractive “sculptured surface,” developed through the protein bodies being molded by the fat droplets in the almond cells pressing against them. During toasting of the almond, part of the cytoplasmic network is destroyed and larger fat droplets are formed by coalescence of the smaller ones observed in raw almond; these larger droplets emerge to the surface (Figure 2.4) and provide the typical oily aspect of toasted shell fruits.

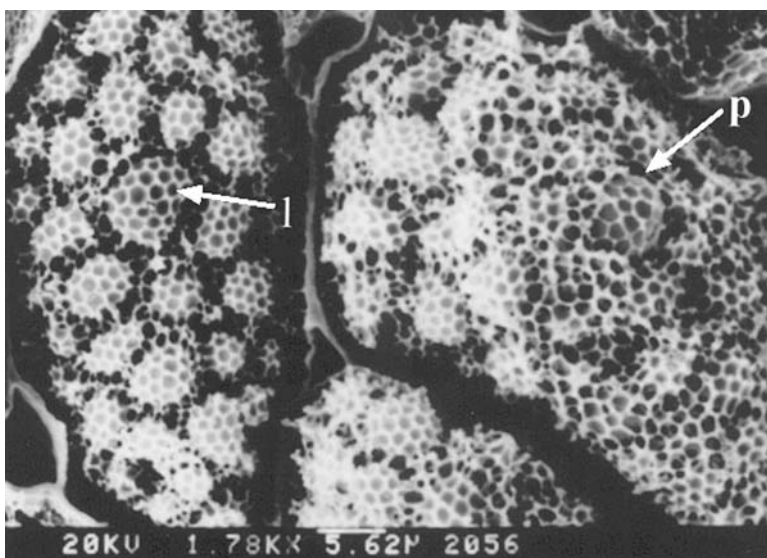


FIGURE 2.3 SEM micrograph of parenchymal tissue from raw almond cotyledon. Protein bodies (p) and lipid bodies (l) in cytoplasmic network.

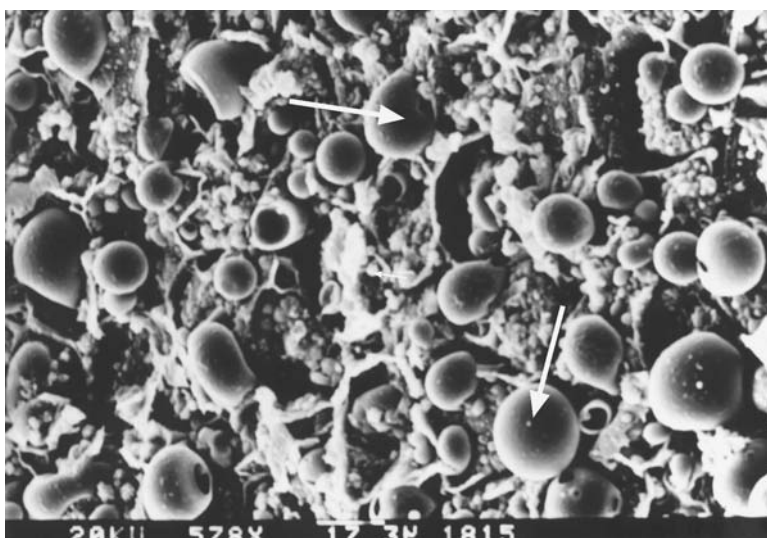


FIGURE 2.4 SEM micrograph of parenchymal tissue from toasted almond cotyledon. Fat droplets (arrows).

Almonds are the primary raw material in “Xixona Turrón,” a typical Spanish confectionery product of fatty aspect, made with concentrated syrup and ovalbumin. During the manufacturing process, the syrup is concentrated by heating and then mixed with almonds and ovalbumin. The structure of “Xixona Turrón” consists of twin continuous phases (Figures 2.5a and b) in that sugar, plant cell wall, and protein bodies form a “sugar phase” that is infiltrated by the lipid fraction (or “fat phase”). This structure would account for the relatively “crispy” texture of “Xixona Turrón,”

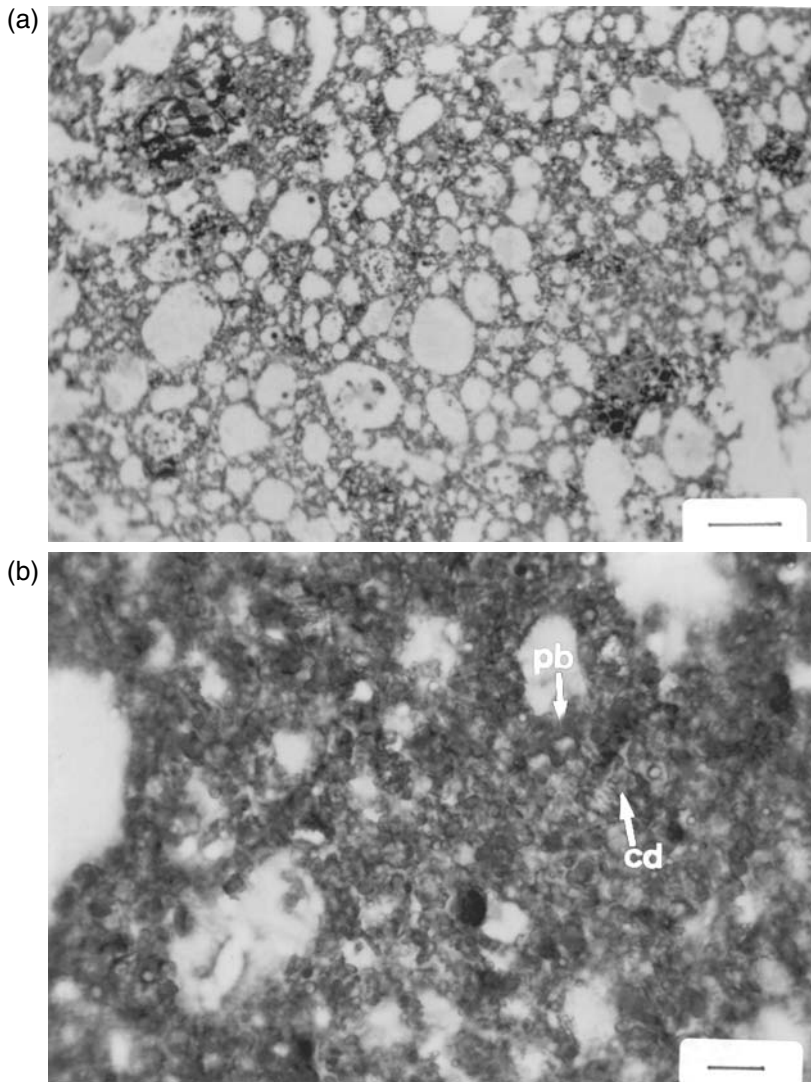


FIGURE 2.5 LM micrographs of “Xixona Turrón.” (a) fat (dark area), bar = 8 μm; (b) protein bodies (pb), cell debris (cd), bar = 15 μm.

although the “oily” feel of the product indicates that the lipid phase is not extensively encapsulated in the product (Pérez-Munuera et al., 2001).

2.5 LIPIDS IN MILK STRUCTURE

The lipid fraction in fresh milk is primarily TAG, about a third of the total dry matter in milk. Fat is present in the form of globules, 500 nm to 10 μm in diameter, encased in lipoprotein membranes, about 10 nm thick, which separate them from the aqueous milk serum (Figure 2.6). This membrane is assumed to consist of phospholipids and a double layer of proteins. The total area of the fat globule membrane is some 80 m^2 per liter, thus its important role in surface phenomena stabilizing the fat emulsion (Aguilera and Stanley, 1990).

Electron microscopy and biochemical methods have been combined to study the ultrastructure of the fat globule membrane. Several models have been proposed to explain its structure. One such model proposed that the main ultrastructural element was a bilayer membrane 10 to 15 nm thick that surrounded the globule and had inner and outer protein layers (Keenan et al., 1983). Buchheim (1986) demonstrated that the inner protein layer possessed a paracrystalline array that separated the membrane from the TAG core. This paracrystalline array was modified by heating or cooling the raw milk or by changing the ionic strength.

The dried milk particles, varying widely in diameter between several micrometers and several tens of micrometers, have wrinkled exterior surfaces; many are hollow and porous. It is assumed that most pores are occupied by fat globules because the pores have approximately corresponding dimensions.

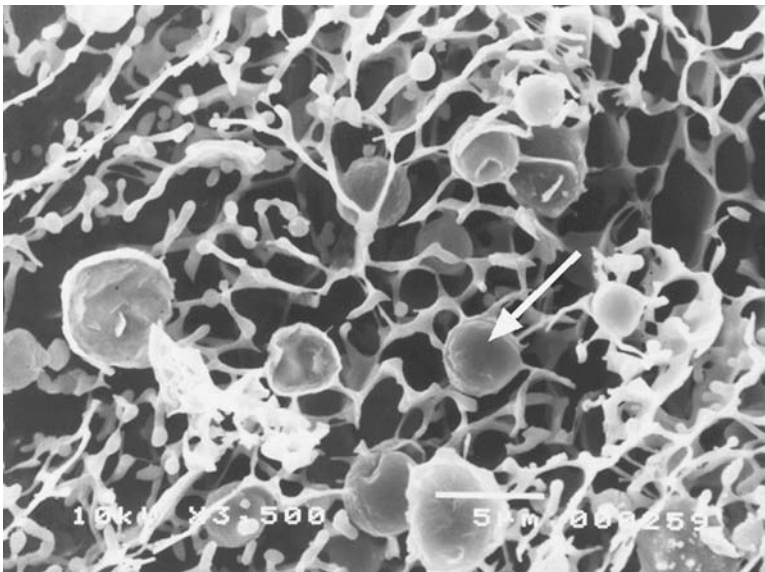


FIGURE 2.6 Cryo-SEM micrograph of fresh milk. Fat globule (arrow).

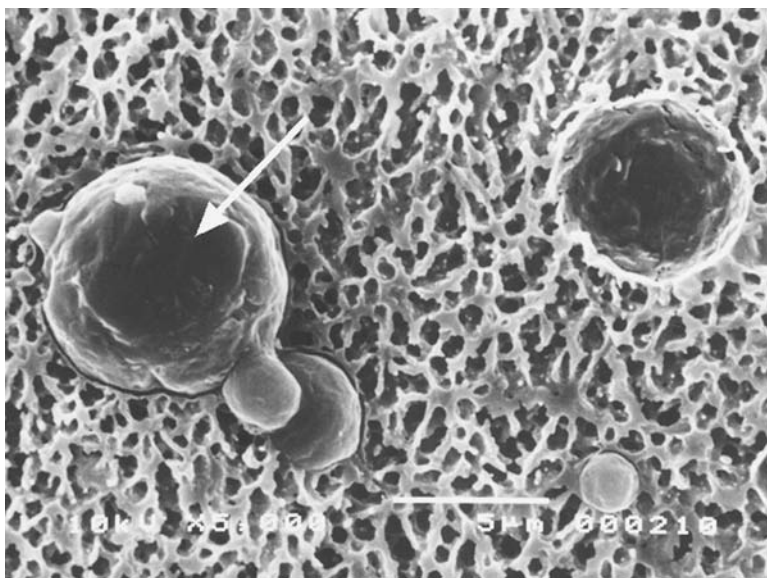


FIGURE 2.7 Cryo-SEM micrograph of ultrafiltered milk. Fat globule (arrow).

The ultrafiltered milk ([Figure 2.7](#)) shows a concentrated, continuous network, consisting primarily of casein micelles where some individual fat globules (1 to 18 μm in diameter) are included; the diameter of the fat globules is greater than that observed in raw milk (1 to 5 μm in diameter) because a mechanical breakdown of the double membrane, followed by coalescence of the original fat droplets, is produced under high ultrafiltration pressures (Hernando et al., 1999).

2.6 LIPIDS IN DAIRY PRODUCTS STRUCTURE

2.6.1 ICE CREAM

Ice cream ([Figure 2.8](#)) contains a large volume of air in the form of air cells, with fat globules at the interface and protruding into the air cells. To retain its form during freezing and low-temperature storage, ice cream is formulated as a complex system of genuine solutions (sugars and salt), colloidal solutions (proteins and stabilizers), and suspensions and emulsions (fat and emulsifiers) (Kalab, 1985). The fat content in ice cream is approximately 10 to 12%, and it may be regarded as a solid fat-foam containing air as a dispersed phase.

A number of changes occur in the ice cream emulsion during aeration and freezing that lead to the rupture of membranes on most fat globules and the release of liquid fat. The spreading of fat at the air bubble/globule interfaces cements the agglomerates of the remaining globules; something similar also occurs in whipped cream (Aguilera and Stanley, 1990). The mean diameter of air cells ranges from 20 to 100 μm , and the lamellae between air cells are 10 to 20 μm and sometimes even thicker.

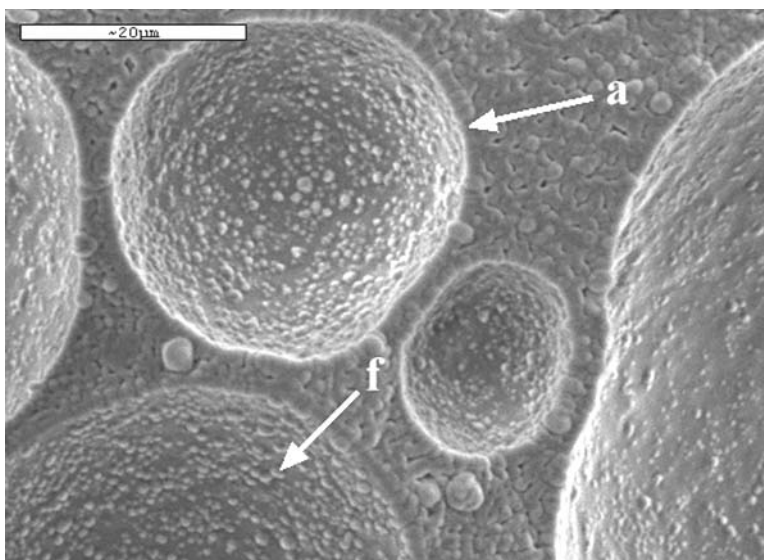


FIGURE 2.8 Cryo-SEM micrograph of ice cream. Air cell (a); fat globule at the interface (f).

Approximately 50% of the water content in ice cream is in the form of ice (mean size about 30 μm) at the temperature at which it is usually consumed. When ice crystals exceed 40 to 50 μm in length, the product acquires a coarse texture. Fat obstructs the growth of ice crystals and provides lubrication in the mouth, leading to the smooth texture of ice cream.

2.6.2 WHIPPED CREAM

Cream basically consists of all the milk components in which fat globules are dispersed at a high concentration; it is an oil-in-water (O/W) emulsion containing 30 to 40% fat. Whipping incorporates a large volume of air into the cream and produces a fat-rich foam. The fat globules are absorbed and densely packed at the air/serum interface of the air cells and partially protruding into the air cell, similar to ice cream structure. In the lamellae between the air cells, membranes of some fat globules are ruptured, so some fat globules are observed in coalescence.

2.6.3 CHEESE

Fresh cheese observed by Cryo-SEM ([Figure 2.9](#)) shows the distribution of fat that is organized in globules (1 to 5 μm in diameter) occluded in the protein network. There is a protein shell (0.2 μm thick) deposited around the membrane of the fat globules; the protein shells of the membranes of the different fat globules appear to interact with each other and with the casein micelles, thus fortifying the structure of the cheese ([Figure 2.10](#)). In addition, these shells prevent the fat from migrating from the interior of the globules, which could explain why fresh cheeses do not have an oily appearance (Hernando et al., 2000).

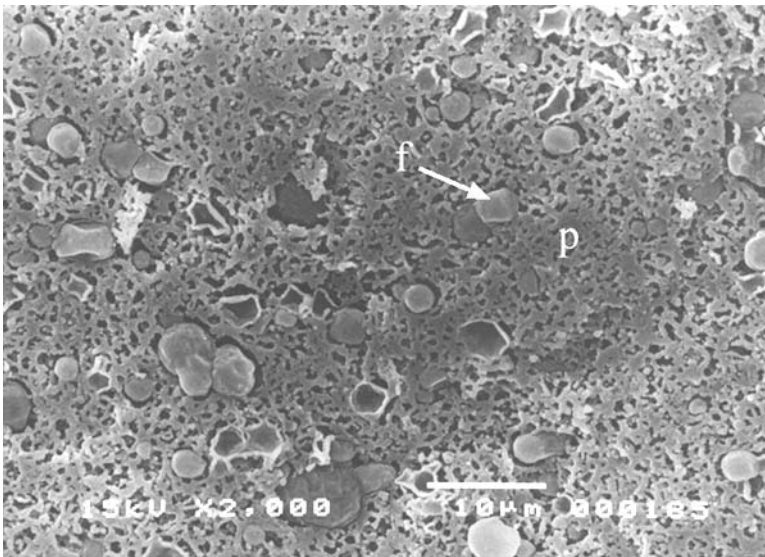


FIGURE 2.9 Cryo-SEM micrograph of fresh cheese. Fat globule (f); protein network (p).

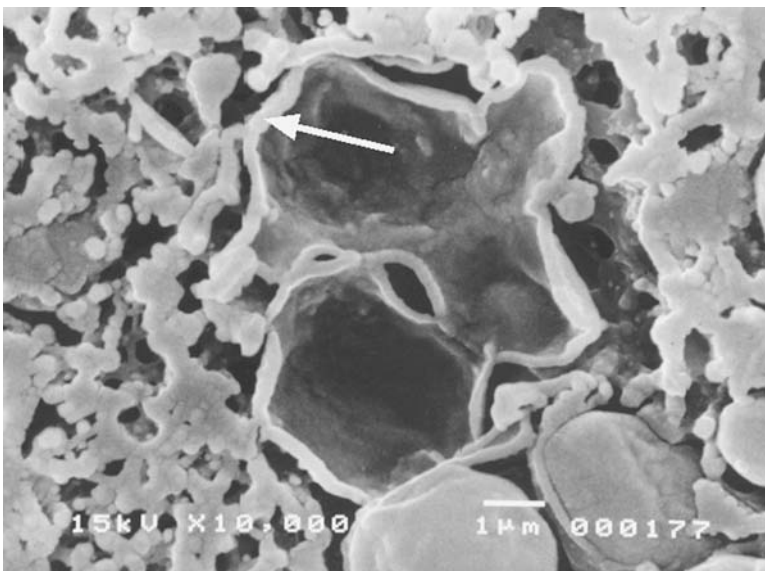


FIGURE 2.10 Cryo-SEM micrograph of fresh cheese. Protein shell of the fat globule membrane (arrow).

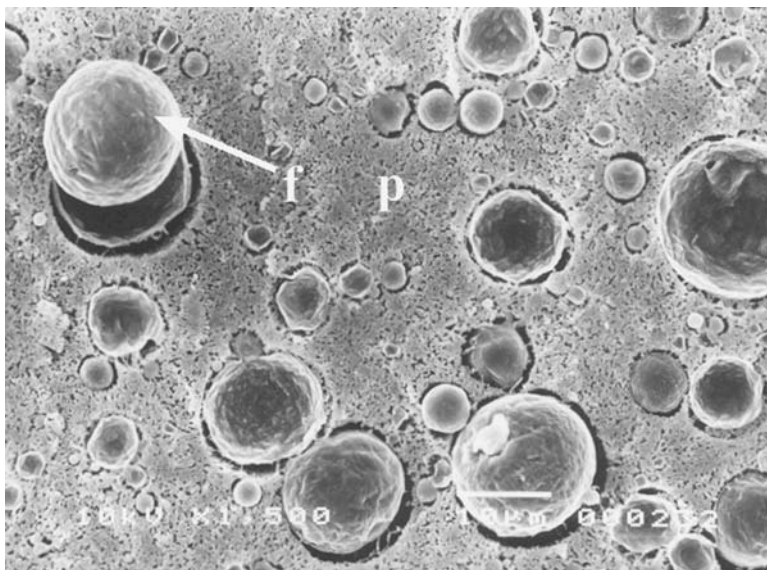


FIGURE 2.11 Cryo-SEM micrograph of fresh cheese elaborated with ultrafiltered milk. Fat globule (f); protein network (p).

Fresh cheese elaborated with ultrafiltered milk (Figure 2.11) is composed of a continuous network of protein in which some individual fat globules (1 to 18 μm in diameter) are occluded. During cheese manufacture, fat globules retain the size originated during the ultrafiltration process (Hernando et al., 1999).

In fresh cheese elaborated with pectin (Figure 2.12), the hydrocolloid is observed to form a network that interacts not only with the protein matrix, but also with the protein shell surrounding fat globules (Hernando et al., 1998).

In ripened cheeses (Figure 2.13), the fat migrates to the surface because of the breakdown of the protein shell that was covering the fat globule. This event results in the oily appearance of ripened cheeses.

The fat content in cream cheese is greater than 30%. The protein network is absent because the curd is disrupted by stirring and homogenization. The microstructure is composed of compact fat–casein aggregates with large spaces filled with whey; this is what makes this cheese spreadable (Kalab, 1985). Buchheim and Thomasow (1984) demonstrated that emulsification and heating increased the disintegration into fat globules and non-micellar casein, resulting in a very fine, homogeneous structure.

2.6.4 BUTTER

Butter is a water-in-oil (W/O) emulsion, which means that water (approximately 20% of the total mass of butter) is in the form of a very fine dispersion in semisolid milk fat; water droplets are smaller than 10 μm in diameter. Fat lamellae separate the water droplets from each other and prevent them from coalescing. This fine dispersion contributes to the “keeping” quality of butter. The microstructure of butter

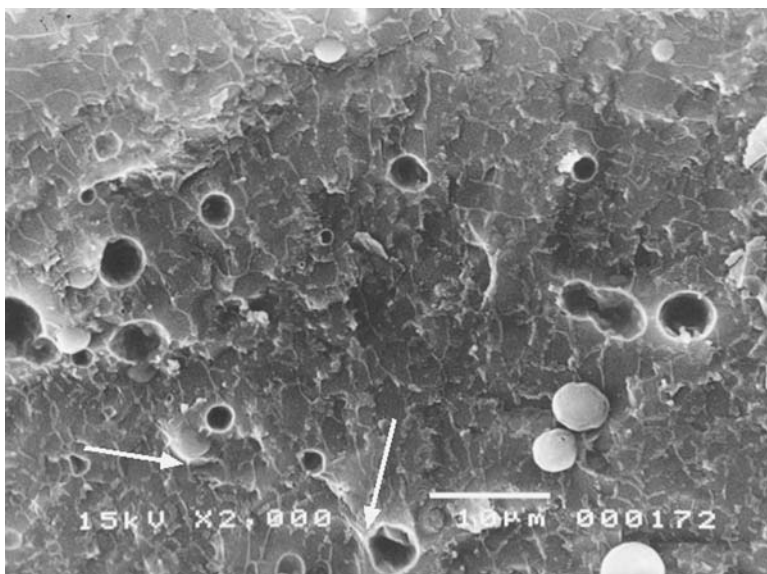


FIGURE 2.12 Cryo-SEM micrograph of fresh cheese elaborated with pectin. Pectin network interacting with the protein shell surrounding fat globules (arrows).

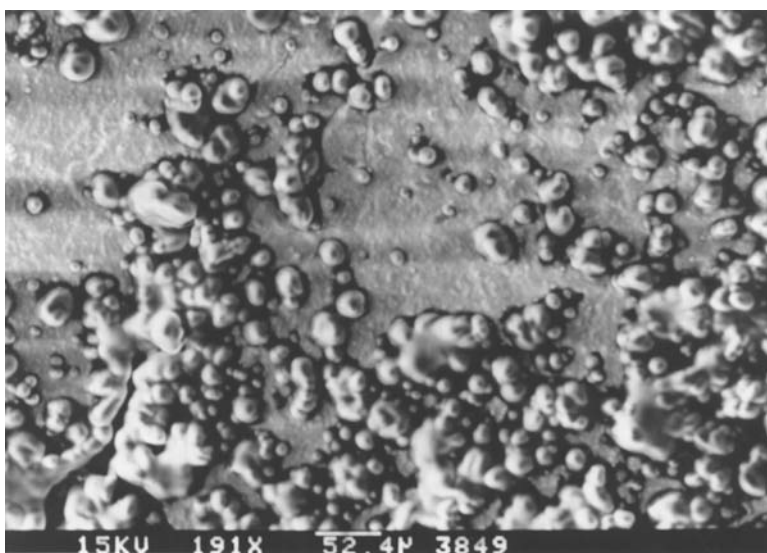


FIGURE 2.13 Cryo-SEM micrograph of ripened cheese. Fat globules on the surface.

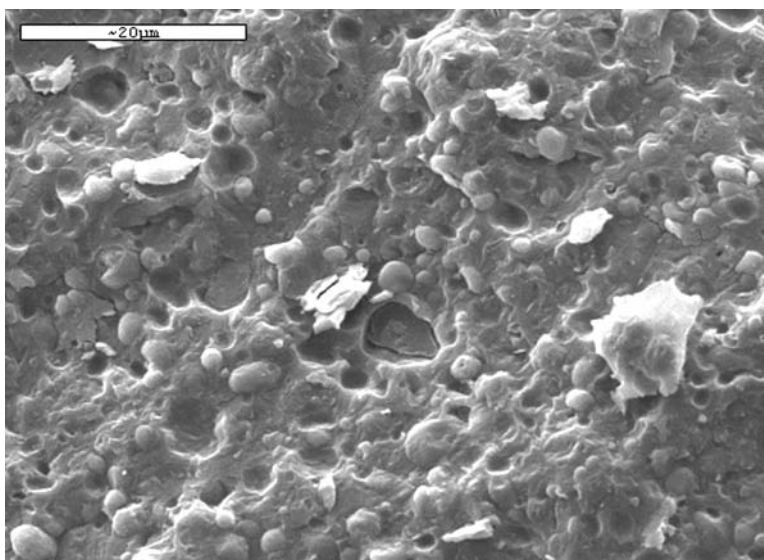


FIGURE 2.14 Cryo-SEM micrograph of butter. Fat globules embedded in a matrix of free fat. Void spaces left by etched water droplets.

(Figure 2.14) shows fat globules embedded in a matrix of free fat; these fat globules remain largely intact after butter manufacturing. These “survivors” have been observed by TEM to have a thick outer shell (approximately 0.1 to 0.5 μm thick) of fat crystals and a core of liquid fat and small crystalline aggregates (Aguilera and Stanley, 1990).

2.7 LIPIDS IN MARGARINE AND SHORTENINGS STRUCTURE

Products such as margarine and butter contain, apart from oil and fat, about 20% water. This water is present as finely dispersed droplets (a few micrometers in diameter) covered by a shell of fat crystals. Typical margarine is formed by a continuous fat matrix (Figure 2.15) that appears to be an interconnected network structure composed of single crystals and sheet-like crystal aggregates. The shells of the water droplets, showing the crystalline nature of the interface, are inserted in this fat network.

There are important structural differences, as observed by Heertje (1993), between margarine and butter in de-oiled samples. While butter shows a discontinuous structure of fat globules, margarine is composed of a continuous network structure of fat crystals or fat crystal aggregates.

Shortenings have the simplest product structure of all fat spreads because they are only composed of liquid oil and solid fat crystals. Appropriate preparation techniques, which extract the oil from the product, reveal that the fat

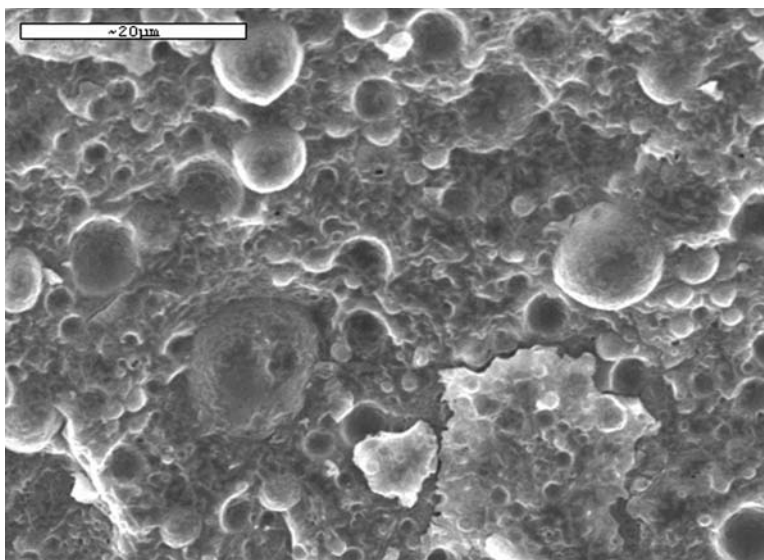


FIGURE 2.15 Cryo-SEM micrograph of margarine. Fat globules embedded in a matrix of free fat. Void spaces left by etched water droplets.

crystals form a three-dimensional crystalline network (Heertje, 1993). This fat network consists of sintered crystals, and the type of aggregation that they undergo depends on processing.

2.8 LIPIDS IN EGG STRUCTURE

The yolk contains the majority of lipids present in an egg, in the form of lipoprotein complexes. These complexes exist as lipoproteins dissolved in the aqueous phase and as particles in suspension.

Although the intact yolk appears to the naked eye to be a homogeneous liquid contained within the vitelline membrane, it in fact consists of an internal structure made up of alternating layers of yellow and white yolk. These layers of yellow and white yolk are 2.0 mm and 0.25 to 0.40 mm thick, respectively, and each layer has been shown to contain numerous spheres of yolk and granules suspended in a continuous plasma.

Yolk is an oil-in-water (O/W) emulsion with about 50% dry matter content, and consists of proteins (one third) and lipids (two thirds, being 6% cholesterol). Yolk contains particles of different sizes that can be classified into two groups: yolk droplets and granules. Yolk droplets are of highly variable size, with a diameter range of 20 to 150 μm ; they resemble fat droplets, consist mostly of lipids (lipoproteins with a low density), and some have protein membranes (Figure 2.16). Granules have a diameter of 0.3 to 0.6 μm ; they are smaller than yolk droplets and more uniform in size, but less uniform in shape. They have a substructure that consists of proteins, but also contains lipids and minerals.

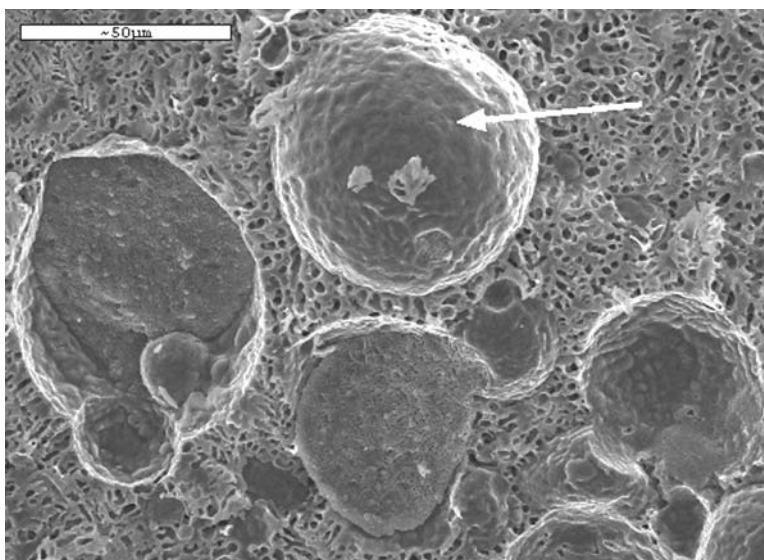


FIGURE 2.16 Cryo-SEM micrograph of egg yolk. Yolk droplet (arrow).

2.9 LIPIDS IN MEAT AND MEAT PRODUCTS STRUCTURE

Lipids in meat are not considered a main structural element, although they are important constituents of the cellular membranes. In addition, lipids in meat are relevant because they are necessary to produce the typical pleasant flavor and mouth sensation. However, excessive amounts of lipids decrease the lean usable portion without improving the quality.

Adipose cells are located outside the muscular bundles (Figure 2.17); they are situated in the perimysial and subcutaneous spaces, but not in the endomysial area. Lipids in the endomysial area are usually membrane lipids or very small lipid droplets of fat distributed among the muscle fibers.

The adipose tissue of pork consists of cells with connective tissue surrounding them. The cell contents are almost entirely lipid in nature. Hard fats (flare or mesenteric fat) have highly crystalline fat within the cells, thin connective tissue cell walls with poorly organized collagen fibers, and tend to have quite large cells. Soft fats (jowl or head fats) have much less crystalline fat, smaller cells, and thicker and more-organized cell walls. Intermediate fats (leg or shoulder fat) generally have features part way between the hard and soft fats; however, they often show a specific crystalline fat layer at the edge of each cell (Lewis, 1981). In cured ham, the proteolysis and lipolysis phenomena that occur during curing produce the release of intracellular lipids. These lipids tend to coalesce, forming fat droplets that confer the typical high-quality mouth sensation of cured hams (Figure 2.18).

Fatty tissue and its interaction with lean meat in the production of comminuted meat products play an interesting functional role. Comminuted meat products are

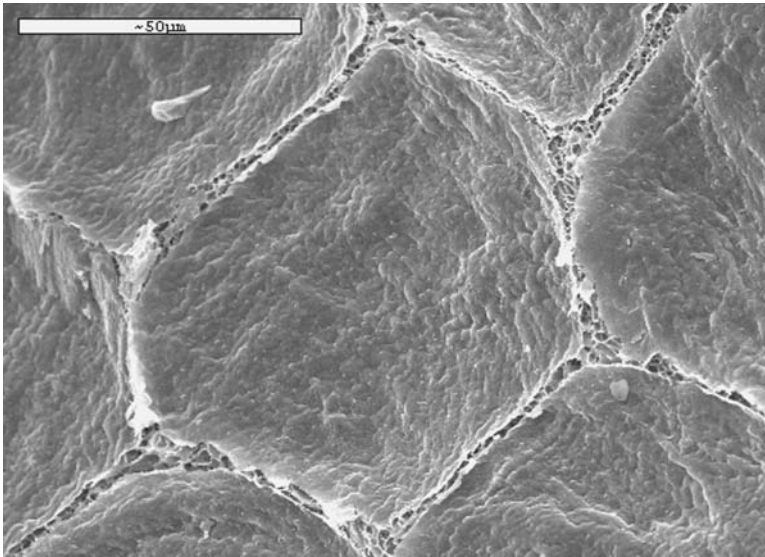


FIGURE 2.17 Cryo-SEM micrograph of pork meat. Adipose cells.

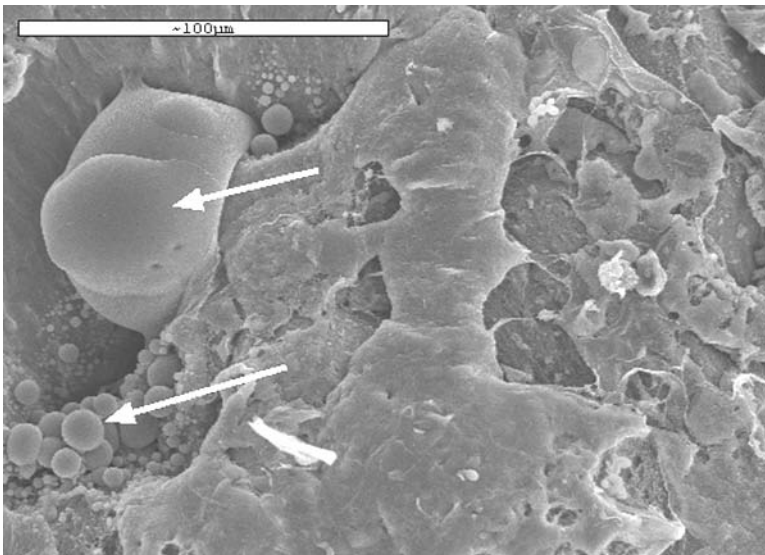


FIGURE 2.18 SEM micrograph of cured ham. Fat globules (arrows).

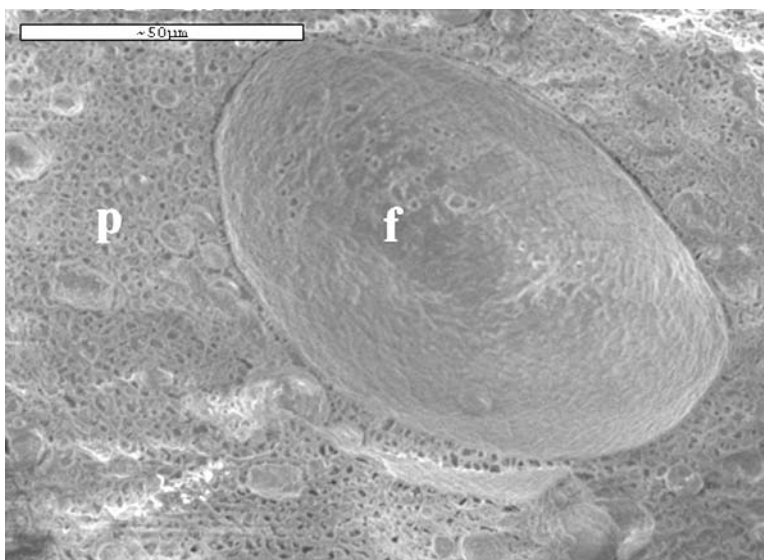


FIGURE 2.19 Cryo-SEM micrograph of a frankfurter. Fat (f); protein matrix (p).

composite foods in which an O/W emulsion is entrapped in a gel formed by insoluble proteins and muscle fibers. Fat, which usually ranges between 20 and 45% of the total weight, is the dispersed phase in the emulsion and fat droplets are surrounded by a film of proteins extracted by salt. Myofibrillar proteins also contribute to formation of the gel matrix. The stability of so-called “meat emulsions” is different from that of normal emulsions in that coalescence may occur, but the gel matrix confines movement of the fat globules (Figure 2.19). Structure stabilization is favored by fat droplets of appropriate hardness and uniform distribution, and the presence of a continuous protein matrix.

Some changes have been observed in the emulsion structure with increasing processing temperature. A critical maximum chopping temperature for emulsion stabilization is found to occur at 16°C (Jones and Mandingo, 1982), where the protein coating surrounding the fat droplets is sufficiently thin and elastic to accommodate volume changes, and the protein matrix formed is dense enough to retain its integrity during heat treatment.

2.10 LIPIDS IN CHOCOLATE STRUCTURE

Chocolate (Figure 2.20) consists of a continuous phase of cocoa butter, substantially crystalline at room temperature and comprising 30 to 35% of the whole, in which sugar and cocoa solids are suspended. Milk chocolate also contains milk fat and solids. Lipids in chocolate have great importance in bloom formation. Bloom is a defect in the surface appearance of chocolate that appears as a white, powdery deposit. It is known to be associated with changes in the polymorphic forms of cocoa butter.

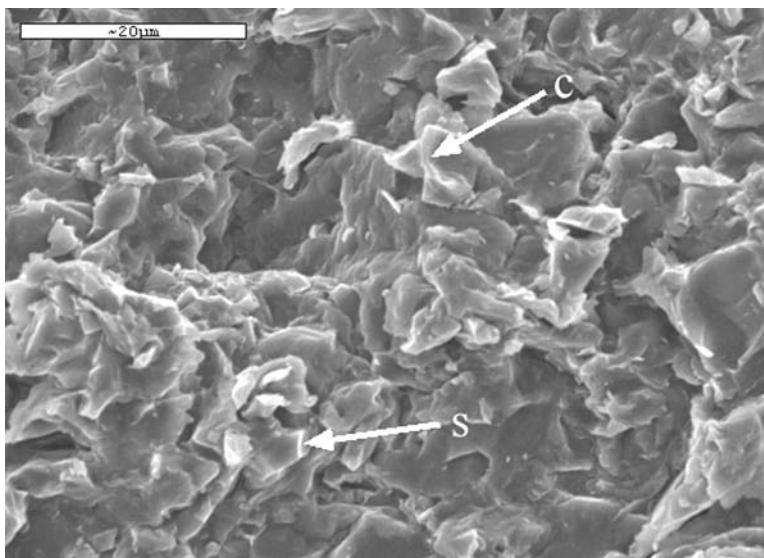


FIGURE 2.20 Cryo-SEM micrograph of plain chocolate. Continuous phase of cocoa butter with crystals (s) and cocoa residues (c).

Fat crystals observed in milk chocolate are more uniform in size than those in plain chocolate. The milk chocolate fat crystals are between 0.5 and 1 μm long and between 0.2 and 0.5 μm wide, while those in plain chocolate are up to 5 μm long. All the fat crystals are between 3.0 nm and 5.0 nm thick, and all are form V polymorphs, which is the “normal” form for chocolate.

2.11 LIPIDS IN MAYONNAISE AND SAUCES STRUCTURE

Mayonnaise and sauces vary widely in their composition, texture, and flavor, but they are all O/W emulsions. The distribution of the two phases has an impact on the stability and texture of the product. A full-fat mayonnaise contains the same volume fraction of fat as margarine, but is the reverse type of emulsion. The distribution of the dispersed fat phase, the size of droplets, as well as the amount of fat crystals and the interaction between droplets influence the properties of the product. The size of the oil droplets is primarily affected by the emulsifying equipment and the viscosity of the water phase.

Mayonnaise (Figure 2.21) is an O/W emulsion consisting of 50 to 85% edible oil, 5 to 10% egg yolk, vinegar, salt, and seasonings. The emulsion is stabilized by egg yolk phospholipids. Tung and Jones (1981) studied the mayonnaise microstructure by conventional SEM and observed a high concentration of dispersed phase (oil) in the form of small lipid droplets. They discussed the possibility of an interfacial film, observed by TEM, which might be formed of low-density lipoproteins and microparticles of egg yolk granules. Langton et al. (1999) identified in TEM

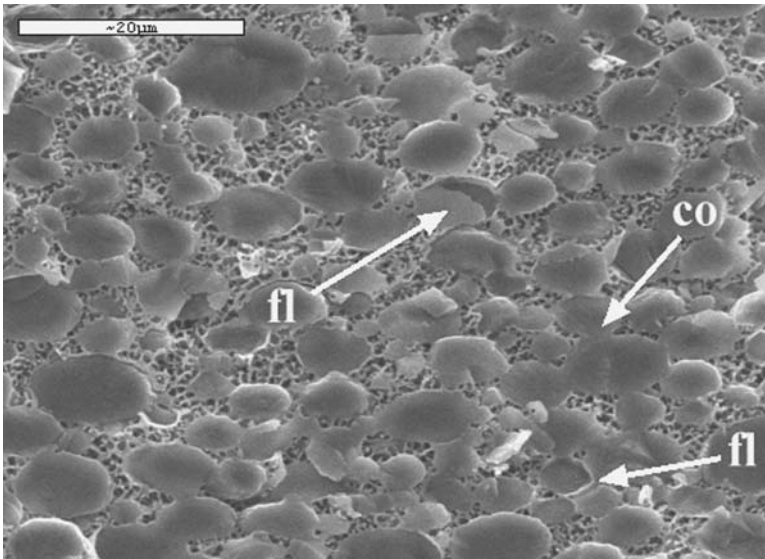


FIGURE 2.21 Cryo-SEM micrograph of mayonnaise. Film surrounding lipid droplets (fl); connection between droplets (co).

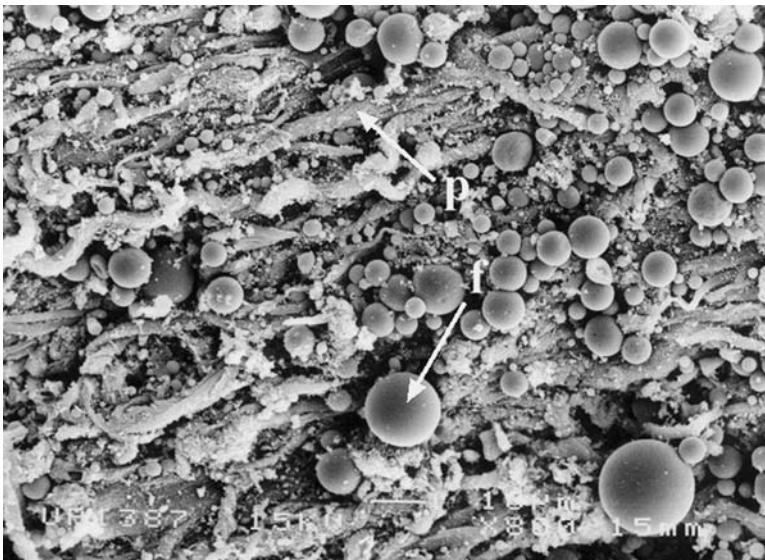


FIGURE 2.22 SEM micrograph of fried battered squid. Frying oil globule (f); protein fiber of squid (p).

images connections between droplets, as well as droplets within droplets. A crystalline appearance is observed in the interior of droplets and at the surface, forming bridges between droplets.

2.12 OIL UPTAKE IN FRIED PRODUCTS

The study of oil uptake during the frying of foods is rather complex and significantly depends on the substrate to be fried. For example, immersion in hot oil causes several transformations within a tortilla chip (McDonough et al., 1993). The oil coats and adheres to the surface of the chip, and free moisture in the chip turns to steam and exits, leaving behind a uniform sponge-like tunnel network. As the steam exits the chip, the oil is drawn inside the tunnels. Starch chains, protein, and lipids interact to form a continuous phase that becomes firm upon dehydration.

Studies on the transfer of fat point out that breading and battering are good methods for inhibiting the absorption of oil, forming a barrier that reduces the access of fat into the food. Llorca et al. (2001) describe that, during the deep fat frying of battered squids, the absorption of fat reaches the surface of the food substrate, and that as the oil penetrates, it also draws with it other components of the batter, such as starch granules (Figure 2.22).

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3 The Nomenclature, Structure, and Properties of Food Lipids

David S. Nichols and Kevin Sanderson

CONTENTS

- 3.1 Introduction
- 3.2 Fatty Acids
 - 3.2.1 General Features
 - 3.2.2 Saturated Fatty Acids
 - 3.2.3 Monoene Fatty Acids
 - 3.2.4 Polyene Fatty Acids
 - 3.2.5 Branched-Chain Fatty Acids
 - 3.2.6 Oxygenated Fatty Acids
- 3.3 Acylglycerols
 - 3.3.1 Monoacylglycerols
 - 3.3.2 Diacylglycerols
 - 3.3.3 Triacylglycerols
- 3.4 Other Fatty Acid Esters
 - 3.4.1 Waxes
- 3.5 Isoprenoid Lipids
 - 3.5.1 Introduction
 - 3.5.2 Steroids
 - 3.5.2.1 Structural Nomenclature of Steroids
 - 3.5.2.2 Named Nomenclature of Steroids
 - 3.5.3 Carotenoids
 - 3.5.3.1 Introduction
 - 3.5.3.2 Nomenclature
 - 3.5.3.3 Stereochemistry of Carotenoids
- 3.6 Properties of Lipids
 - 3.6.1 Chemical Indices
 - 3.6.1.1 Introduction
 - 3.6.1.2 Iodine Value
 - 3.6.1.3 Acid Value or Neutralization Value
 - 3.6.1.4 Saponification Value

3.6.2	Physical Properties
3.6.2.1	Introduction
3.6.2.2	Density
3.6.2.3	Surface Tension
3.6.2.4	Viscosity
3.6.2.5	Polarity
3.6.2.6	Melting Point
3.6.2.7	Refractive Index
3.6.3	Absorption Spectra
3.6.3.1	Ultraviolet and Visible Spectra
3.6.3.2	Infrared Spectra
3.7	Conclusion
	References

3.1 INTRODUCTION

Lipids, or those classes of organic compounds that may be, have been, or are referred to as fats, are an essential component of many foods. Their use as ingredients in foods, cosmetics, and medicinals dates to prehistoric times. It is thus not surprising that knowledge of the chemical nature of some lipids predates that of other food components, e.g., saccharides and proteins.

The term “lipids” is defined as those organic compounds that are insoluble in water, soluble in organic solvents (e.g., chloroform, ether), contain hydrocarbon groups as primary parts of the molecule, and are present in or derived from living organisms. Compound classes covered in this definition include fatty acids (FA), acylglycerols, FA esters (e.g., waxes), and isoprenoid hydrocarbons. Other compounds also included are often considered as belonging to different classes, such as carotenoids, sterols, and the vitamins A, D, E, and K.

Lipids tend to be classified as “simple” or “complex,” referring to the size or structural detail of the molecule. Simple lipids include FA, hydrocarbons, and alcohols, all of which are relatively “neutral” in terms of charge. Complex lipids, such as phospholipids and glycolipids, are relatively more charged and are also referred to as “polar.”

3.2 FATTY ACIDS

3.2.1 GENERAL FEATURES

There are many features of FA that can be described in general terms. First, FA are characterized by consisting of a long chain of carbon atoms (generally 12 to 22 carbon atoms) ending in a carboxyl functionality (Figure 3.1). Substitutions or variations in chain structure may occur, yielding a diversity of FA types (Figure 3.1 and Table 3.1). These include branched-chain FA, unsaturated FA, and those possessing oxygenated groups. While over 500 individual FA have been described from plant and microbial sources, only a few are quantitatively significant. For

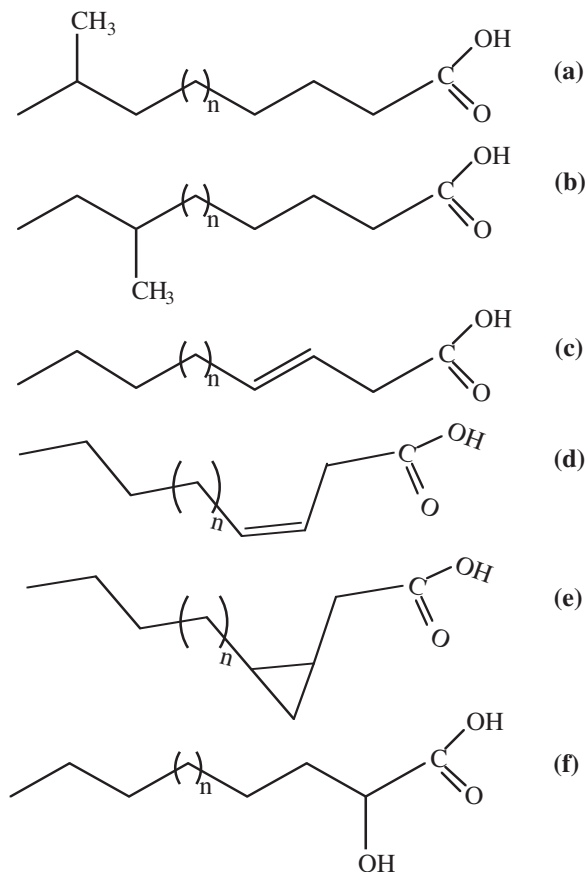


FIGURE 3.1 Generic structures of various fatty acid types: (a) *iso*-branched, (b) *anteiso*-branched, (c) *trans*-monounsaturated, (d) *cis*-monounsaturated, (e) cyclopropane, and (f) hydroxylated.

example, approximately 95% of the FA from plant leaves or oils consist of only seven components: lauric, myristic, palmitic, stearic, oleic, linoleic, and α -linolenic acids (Tables 3.1 and 3.3).

The nomenclature of FA reflects the long history of their study and description. Most FA were originally described under “trivial” names prior to the adoption of the international molecular nomenclature rules in 1892 (Table 3.1). Even after adopting the International Union of Pure and Applied Chemistry (IUPAC) system for nomenclature (IUPAC-IUB, 1977), the habit of assigning trivial names to FA continues. The basis of the systematic nomenclature system is an extension of that accepted for hydrocarbon (alkane/alkene) naming. Hence, the descriptive name is based on the number of carbon atoms contained in the molecule, with the suffix “-e” replaced with “-oic acid.” However, in most instances, FA are referred to by their formula notations. As is the case with systematic and trivial names, there exists an IUPAC accepted formula notation nomenclature and several earlier versions (Table 3.2). Each system

TABLE 3.1

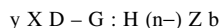
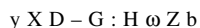
The Formula, Nomenclature, and Names of Representative Fatty Acids that May Be Present in Foods

Formula	Notation ^a		Name		Discovered
	Omega	Delta	Systematic	Trivial	
	Saturated				
C ₁₂ H ₂₄ O ₂	12:0	12:0	Dodecanoic acid	Lauric acid	1842
C ₁₄ H ₂₈ O ₂	14:0	14:0	Tetradecanoic acid	Myristic acid	1841
C ₁₅ H ₃₀ O ₂	15:0	15:0	Pentadecanoic acid	Pentadecylic acid	1926
C ₁₆ H ₃₂ O ₂	16:0	16:0	Hexadecanoic acid	Palmitic acid	1816
C ₁₈ H ₃₆ O ₂	18:0	18:0	Octadecanoic acid	Stearic acid	1816
Branched-chain					
C ₁₅ H ₂₉ O ₂	i15:0	i15:0	13-Methyltetradecanoic acid	Isopentadecanoic acid	1960
C ₁₅ H ₂₉ O ₂	a15:0	a15:0	12-Methyltetradecanoic acid	Sarcinic acid	1960
C ₁₉ H ₃₈ O ₂	10-Me18:0	10-Me18:0	10-Methyloctadecanoic acid	Tuberculostearic acid	1929
Monounsaturated					
C ₁₆ H ₃₀ O ₂	16:1ω7c	16:1Δ9c	cis-9-Hexadecenoic acid	Palmitoleic acid	1854
C ₁₈ H ₃₄ O ₂	18:1ω7c	18:1Δ11c	cis-11-Octadecenoic acid	Oleic acid	1815
C ₁₈ H ₃₄ O ₂	18:1ω9c	18:1Δ9c	cis-9-Octadecenoic acid	Vaccinic acid	1844

Polyunsaturated					
$C_{18}H_{32}O_2$	18:2 ω 6	18:2 Δ 9,12c	<i>cis</i> -9,12-Octadecenoic acid	α -Linoleic acid	1844
$C_{18}H_{32}O_2$	18:2 ω 3	18:2 Δ 12,15c	<i>cis</i> -12,15-Octadecenoic acid	γ -Linoleic acid	1844
$C_{18}H_{30}O_2$	18:3 ω 3	18:3 Δ 9,12,15c	<i>cis</i> -9,12,15-Octadecenoic acid	α -Linolenic acid	1887
$C_{18}H_{30}O_2$	18:3 ω 6	18:3 Δ 6,9,12c	<i>cis</i> -6,9,12-Octadecenoic acid	γ -Linolenic acid	1887
$C_{20}H_{32}O_2$	20:4 ω 6	20:4 Δ 5,8,11,14c	<i>cis</i> -5,8,11,14-Eicosatetraenoic acid	Arachidonic acid	1909
$C_{20}H_{30}O_2$	20:5 ω 3	20:5 Δ 5,8,11,14,17c	<i>cis</i> -5,8,11,14,17-Eicosatetraenoic acid	Timnodonic acid	1948
Oxygenated					
$C_{16}H_{32}O_3$	16-OH16:0	16-OH16:0	16-Hydroxyhexadecanoic acid	Juniperic acid	1909
$C_{18}H_{36}O_6$	9,10-OH18:0	9,10-OH18:0	9,10-Hydroxyhexadecanoic acid	Dihydroxystearic acid	1925
$C_{18}H_{28}O_3$	4-O-18:3 ω 6	4-O-18:3 Δ 9,11,13c	4-Keto-9,11,13-octadecatrienoic acid	Licaqnic acid	1931

^aThe two notation forms displayed are no longer recommended for usage. They are used here to highlight earlier descriptions of fatty acids that may be encountered elsewhere. The currently recommended IUPAC notation system for fatty acids (the (n-) notation) is described in [Table 3.2](#).

Data compiled from Deuel, H.J., Jr. 1951. *The Lipids, Their Chemistry and Biochemistry, Volume 1: Chemistry*, Interscience, New York.

TABLE 3.2**Schematic Formulae for the Two Main Types of Fatty Acid Systematic (n) and Non-systematic (ω) Structural Notations**

where:

y = i (*iso*-) or a (*anteiso*-) branching (if present)

X = carbon number of additional functional group (if present) from the carboxyl carbon

D = abbreviation of additional functional group (if present)

G = total number of carbon atoms

H = total number of double bonds

Z = carbon number of first double bond from the methyl position (if present)

b = c (*cis*-) or t (*trans*-) double-bond geometry (if present)

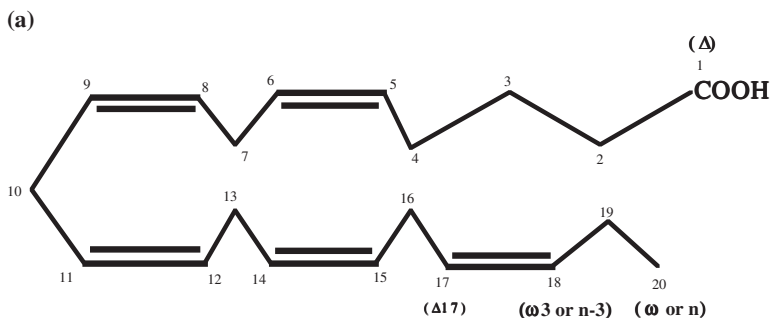
Note: Refer to [Figure 3.2](#) as an example.

is interchangeable, and it is important to recognize each naming system because the earlier literature may use either system.

The three nomenclature notation systems commonly used for FA are described in [Figure 3.2](#) and [Table 3.2](#). Each has certain advantages in the description of structural features. However, the current IUPAC system of (n-) nomenclature for unsaturated FA should be used in preference to the ω or Δ notations. The systems of formula notation are based on a common core. Each denotes the number of carbon atoms and the number of double bonds in the molecule, with these numerics separated by a colon. A term then follows that describes the location of any double bonds in the carbon chain. It is the method of this description that differs between the various notation systems. One nomenclature system describes double bond location with respect to the methyl (ω or n) end of the carbon chain. Where more than one double bond is present, only the first from the (n) position is noted, as subsequent double bonds are assumed to be present in a methylene interrupted series (see [Section 3.2.4](#)). The second nomenclature system describes the double bond location with respect to the carboxyl (Δ) carbon. Here, the location of each double bond is listed in order. Each system is the same with regard to using a series of general prefix and suffix terms to describe additional structural details. The prefixes “i” and “a” refer to *iso*- and *anteiso*-branching, respectively. The suffixes “c” and “t” denote *cis* and *trans* double-bond geometry, respectively (see [Figure 3.1](#)). Specialized terms specific to unusual FA structures or functionalities are discussed in the following relevant sections.

3.2.2 SATURATED FATTY ACIDS

Saturated FA are those fatty acids containing only single bonds between carbon atoms, and hence the molecule is “saturated” or contains the maximum possible



Eicosapentaenoic Acid

[20:5ω3; EPA] or [20:Δ5,8,11,14,17; EPA] or [20:5(n-3); EPA]

(b)

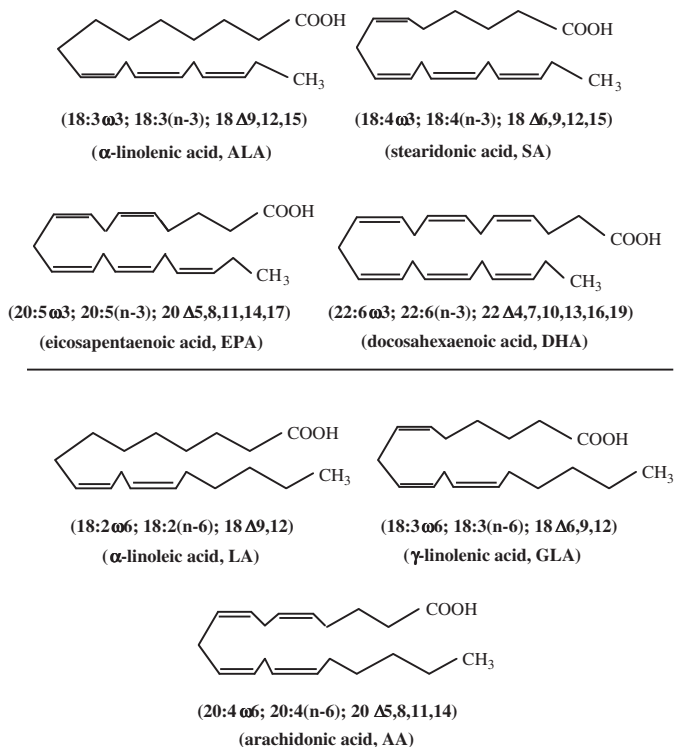


FIGURE 3.2 (a) The relationship between the three nomenclature notations for fatty acids. Eicosapentaenoic acid (EPA) is shown as an example. (b) Representative structures of the common polyene fatty acids of the n-3 and n-6 families.

number of hydrogen atoms per carbon. In practice, the term also tends to be limited to those FA that do not possess any other structural feature or functionality. The formula for such molecules therefore follows the series $C_nH_{2n}O_2$ (Table 3.1) and

consists of a linear chain of carbon atoms (Figure 3.1). In the case of most food lipids, saturated FA possess an even carbon number.

3.2.3 MONOENE FATTY ACIDS

Monoene or monounsaturated FA are defined as components containing one double bond between adjacent carbon atoms. They therefore follow the generalized formula of $C_nH_{(2n-2)}O_2$. The presence of even this simple functionality introduces two important variables into monoene structure and nomenclature. First, the double bond may be present in a number of possible locations along the carbon chain, giving rise to different monounsaturated isomers (e.g., 16:1(n-9) and 16:1(n-11); see Table 3.1). In practice, $\Delta 9$ and $\Delta 11$ isomers are the most common although other variations do occur. Second, the double bond may be in either the *cis* or *trans* geometry (Figure 3.1). Hence, the addition of the formula notation suffix “c” or “t” is required to define this important difference (Table 3.2). In nature, the vast majority of all double bonds in FA are of the *cis* geometry. However, *trans* components do occur, particularly where food lipids have been exposed to heating (e.g., vegetable oils used for cooking).

3.2.4 POLYENE FATTY ACIDS

Polyene FA (PEFA), or polyunsaturated FA (PUFA), are classified as those fatty acids containing more than one double bond in the carbon chain. As such, the class does not possess a generic formula. While *trans* bonds are possible, PUFA from all natural sources contain *cis* double bonds. In addition, the majority of natural PUFA contain double bonds in a methylene interrupted pattern (Figure 3.2) yielding a generic isomeric distribution of $\Delta x, (x+3), (x+6), (x+9), (x+12)$. Hence, the maximum number of double bonds possible in a PUFA is theoretically limited by the chain length and the position of the first double bond from the carboxyl carbon. In practice, natural PUFA are limited to the last double bond involving the (n-3) position and the first involving either the $\Delta 4$, $\Delta 5$, or $\Delta 6$ position for C_{18} , C_{20} , and C_{22} , respectively (Figure 3.2). Therefore, the maximum number of double bonds in naturally occurring PUFA is four for C_{18} , five for C_{20} , and six for C_{22} .

3.2.5 BRANCHED-CHAIN FATTY ACIDS

Branched-chain FA do not commonly occur in food products, but may be widely distributed in the environment and are therefore of relevance. Branched-chain FA can be considered to fall within two categories. The first category occurs where a methyl group replaces one or more of the hydrogen atoms of the carbon chain. The majority are produced by microorganisms and contain a single methyl branch on the second-last (*iso*-branched) or third-last (*anteiso*-branched) carbon of the chain, which is usually odd numbered (i.e., C_{13} , C_{15} , or C_{17} ; Figure 3.1). Microorganisms of the genus *Mycobacterium* also produce a range of mid-chain methyl-branched FA, such as 10-Me18:0 (tuberculostearic acid; see Table 3.1) and a range of polymethyl-branched FA (e.g., 3,13,19-trimethyltricosanoic acid) collectively known as mycolic acids (Harwood and Russell, 1984).

The second category of branched-chain FA consists of the isoprenoid acids, derived from isoprene units (see [Section 3.5](#)). They are widely distributed in both terrestrial and marine organisms. Examples include 2,6,10,14-tetramethylpentadecanoic acid (pristanic acid) and 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid).

3.2.6 OXYGENATED FATTY ACIDS

FA containing an oxygen functionality exist in a number of forms, most commonly as a hydroxyl or keto group. Both types mirror the general structural theme of branched-chain FA, in which an oxygenated functionality replaces one or more hydrogen atoms on one carbon atom of the chain. Oxygenated fatty acids occur as natural products from many organisms but can also be produced from the partial oxidation of unsaturated FA during cooking or heating processes.

Hydroxy FA occur in both saturated and unsaturated forms, with various positional isomers. There are three major types. First, the 2-hydroxy (2-OH) or α -hydroxy FA ([Figure 3.1](#)). These saturated components occur as a series of even numbered acids from C_{10} to C_{26} and are components of animal tissues (within cerebroside) and certain plants. Second, 3-hydroxy (3-OH) or β -hydroxy FA exist as a series of even-numbered acids from C_{10} to C_{18} . They are ubiquitous components of many bacteria and yeasts, where they exist as ester-linked residues of extracellular lipids. Third, the hydroxyl group may be present at the penultimate carbon from the carboxyl group (i.e., ω 2, or otherwise referred to as ω -1) of the chain series C_{11} to C_{19} . In most instances, these compounds represent intermediates in the ω -oxidation of FA by microorganisms.

Analogous to the isomer distribution of hydroxy FA, the major types containing a ketone functional group occur as 2-keto (α -keto), 3-keto (β -keto), and ω -1 keto acids. The chain-length distribution for each type is C_6 to C_{20} or greater for 3-keto acids and C_7 to C_{20} or greater for both 2-keto and ω -1 keto acids. In addition, 4-keto, 5-keto, and 6-keto acids are found in heated milk and pork (Deuel 1951).

3.3 ACYLGLYCEROLS

Acylglycerols consist of one, two, or three FA (acyl) residues esterified to the hydroxy residue(s) of a glycerol molecule ([Figure 3.3](#)). They are commonly referred to as complex lipids due to the presence of one or more individual acyl residues in the molecule that can be removed by chemical reaction. Acylglycerols represent the most common type of complex lipid in which FA are present. In the past, they were referred to as neutral lipids, fats, or glycerides (mono-, di-, and triglycerides). They represent the most common lipid class present in foods. From natural sources, mono- and diacylglycerols are usually present as precursors to the formation of triacylglycerols (TAG) or phospholipids. In foods, their presence may also indicate the degradation of TAG components by chemical or enzymatic deacylation.

The nomenclature of acylglycerols is based on the concept of substitution of the glycerol “backbone” by acyl residues in a manner similar to substitutions along a FA carbon chain. Acylglycerols can introduce the further complication of asymmetric

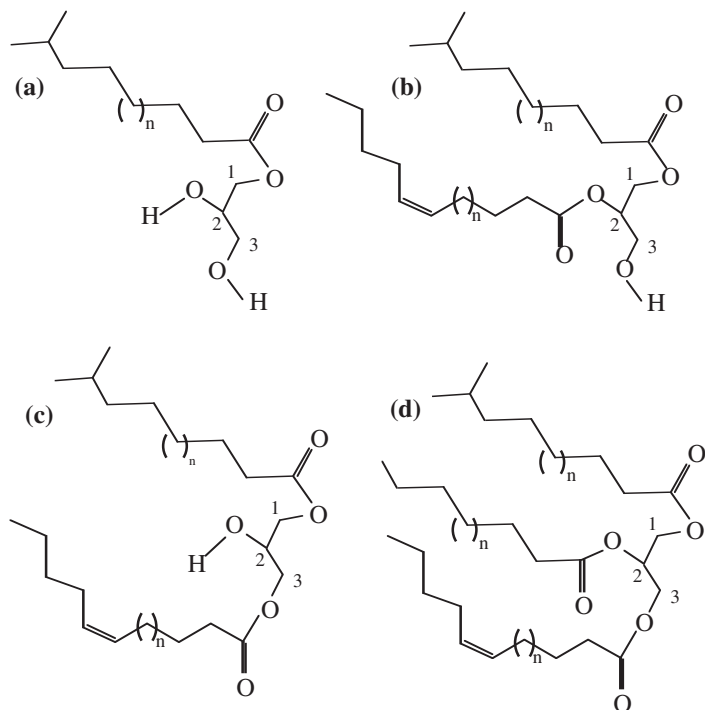


FIGURE 3.3 Schematic structures of the major acylglycerol classes: (a) 1-monoacyl-*sn*-glycerol; (b) 1,2-diacyl-*sn*-glycerol; (c) 1,3-diacyl-*sn*-glycerol; and (d) 1,2,3-triacyl-*sn*-glycerol.

substitution, forming a chiral center at the central carbon of the glycerol backbone when one of the hydroxyl groups is substituted (monoacylglycerols) or when two or all three hydroxyl groups are substituted by different acyl residues (diacyl- and TAG). To address this issue, the stereospecific numbering (*sn*) system is used to differentiate the carbinol groups. This defines a fixed numbering system for the glycerol backbone that is independent of the substituents. An example is given in [Figure 3.4](#). Using a derived Fischer projection, the orientation of the secondary hydroxyl group to the left of the second carbon defines the upper carbon atom of the glycerol backbone as *sn*-1 and the lower carbon as number *sn*-3. Reversal of the hydroxyl position at the second carbon reverses the numbering sequence. The prefix “*sn*–” denotes the use of stereospecific numbering. In previous literature, the carbon positions were referred to as α , β , and α' ([Figure 3.4](#)).

3.3.1 MONOACYLGLYCEROLS

Monoacylglycerols consist of a single hydroxyl group substitution of the glycerol molecule with an acyl residue via an ester linkage ([Figure 3.3](#)). Three positional isomers are therefore possible for the substitution: *sn*-1, the central carbon position *sn*-2, or the terminal carbon position *sn*-3. Unlike the *sn*-1 and *sn*-3 isomers, the *sn*-2 isomer retains its molecular symmetry and is therefore nonchiral.

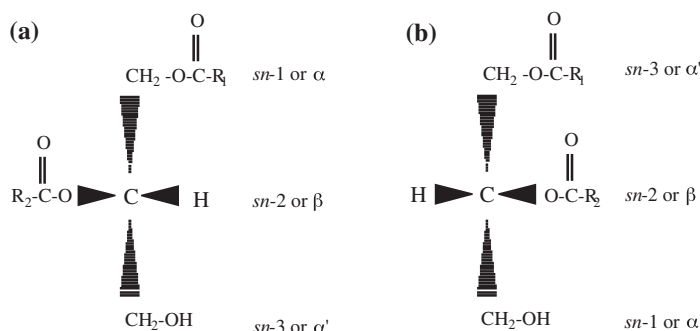


FIGURE 3.4 Stereochemical projection of a generic diacylglycerol illustrating the stereochemical numbering (*sn*) system and the chiral center at the *sn*-2 carbon. Each molecule, although identical in formula, is a stereoisomer (specifically an enantiomer, a nonsuperimposable mirror image). R_1 and R_2 represent substituent alkyl chains.

3.3.2 DIACYLGLYCEROLS

Diacylglycerols result from the substitution of two hydroxyl groups of the glycerol molecule with an acyl residue via an ester linkage (Figure 3.3). For diacylglycerols containing a single FA, three isomers are possible (*sn*-1, *sn*-2; *sn*-1, *sn*-3; and *sn*-2, *sn*-3). Where two different FA are involved, six positional isomers are possible.

3.3.3 TRIACYLGLYCEROLS

Triacylglycerols (TAG) contain full substitution of all three hydroxyl groups of the glycerol molecule with ester-linked acyl residues (Figure 3.3). They have commonly been referred to as oils or fats, depending on their melting points, and represent the depot lipids of both plants (in seeds) and animals (in adipose tissue). As such, they are common components of food systems. A large variety of positional isomers are possible, depending on the diversity of component FA and their positional distribution within the molecule.

TAG derived from the seeds of tropical trees are unusual in possessing a large ratio of saturated to unsaturated FA. Hence, they form a solid at normal temperatures but possess a very narrow melting range due to the limited positional isomerism exhibited. A good example of this is cocoa butter (Table 3.3). In general, oils derived from vegetables contain high proportions of unsaturated FA. The more unsaturated components are concentrated in the *sn*-2 position with saturated FA more common at the *sn*-1 and *sn*-3 locations. However, TAG of plant origin can also be highly saturated, as demonstrated by the coconut, which is dominated by short-chain saturated FA with specific isomeric distributions (Table 3.3).

TAG from animals are more dominated by C_{16} and C_{18} saturated FA, including significant proportions of fully saturated TAG, giving rise to solid substances at room temperature (e.g., tallow, lard). In general, the saturated content of the *sn*-2 position is greater than that from plants and 16:0 is concentrated at *sn*-1. Pork fat exists as a special case where 16:0 is concentrated at *sn*-2, 18:0 at *sn*-1 and a high proportion of monounsaturated FA at *sn*-1 and *sn*-3 (Table 3.3).

TABLE 3.3
Fatty Acid Positional Distributions in Triacylglycerols from Various Sources

Lipid Source	sn-Position	Fatty Acid (%)								
		8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3
Coconut	1	4	4	39	29	16	3	4	—	—
	2	2	5	78	8	1	1	3	2	—
	3	32	13	38	8	1	1	3	2	—
Coca butter	1	—	—	—	—	34	50	12	1	—
	2	—	—	—	—	2	2	87	9	—
	3	—	—	—	—	37	53	9		—
Corn	1	—	—	—	—	18	3	28	50	—
	2	—	—	—	—	2	—	27	70	—
	3	—	—	—	—	14	31	52	1	—
Soybean	1	—	—	—	—	14	6	23	48	9
	2	—	—	—	—	1	—	22	70	7
	3	—	—	—	—	13	6	28	45	8
Olive	1	—	—	—	—	13	3	72	10	1
	2	—	—	—	—	1	—	83	14	1
	3	—	—	—	—	17	4	74	5	1
Peanut	1	—	—	—	—	14	5	59	19	—
	2	—	—	—	—	2	—	59	39	—
	3	—	—	—	—	11	5	57	10	—
Beef fat	1	—	—	—	4	41	17	20	4	1
	2	—	—	—	9	17	9	41	5	1
	3	—	—	—	1	22	24	37	5	1
Pig (outer back)	1	—	—	—	1	10	30	51	6	—
	2	—	—	—	4	72	2	13	3	—
	3	—	—	—	—	—	7	73	18	—

Data compiled from Deuel, H.J., Jr. 1951. *The Lipids, Their Chemistry and Biochemistry, Volume 1: Chemistry*, Interscience, New York.

3.4 OTHER FATTY ACID ESTERS

3.4.1 WAXES

Waxes can be defined as FA esters of alcohols possessing a higher molecular weight. Waxes are chemically stable and insoluble in water and many organic solvents. Due to these properties, waxes are widely distributed in both plants and animals as protective coverings for tissues. Simple waxes are classified as monoesters of normal FA and normal long-chain alcohols. Complex waxes also exist, in which either the FA or alcohol components possess complex structures in their own rights (e.g., vitamin esters or sterol esters, see [Section 3.5.1](#)).

The nomenclature of simple waxes is based on the stem name of the FA. The FA suffix “-ic acid” is replaced with “-ate.” This stem term is then followed by

the name of the alcohol component with the suffix modified from the “-ol” of the free alcohol to “-yl.” Many examples exist in bacteria and marine organisms where waxes serve as short-term storage lipids or as aids in buoyancy. Compounds with C_{21} to C_{44} have been reported from marine organisms; however, the usual range from copepods or fish is C_{30} to C_{42} . Component alcohols are usually saturated or monounsaturated, with 16:0 and 18:1 predominating. The FA involved are more diverse and may include PUFA, although 16:1 and 20:1 are frequently major components.

3.5 ISOPRENOID LIPIDS

3.5.1 INTRODUCTION

Isoprenoid lipids represent a diverse collection of compound types, some of which are present in nearly every organism. The group includes the major classes of compounds known as terpenoids, steroids, and carotenoids. The latter two classes are dealt with in more detail in the following sections. Although there is great diversity among the isoprenoid lipids, they do share a common structural origin. The basis of this is the biosynthesis of the “backbone” structure from multiple, repeating branched-chain unsaturated C_5 units called isoprene or isopentenyl units (Figure 3.5). Differing numbers of isoprene units can combine in a variety of ways, yielding a diversity of chain structures. Hence, isoprenoid compounds usually contain a carbon number divisible by five and are broadly classified by a terpenoid nomenclature based on this fact (Table 3.4). Indeed, many isoprenoid lipids can be easily identified by the ability to neatly divide the compound structure into its original isoprene synthetic units (the so-called isoprene rule). However, subsequent cyclization and/or addition of further functional groups at sites of unsaturation result in the large diversity and complex nomenclature of isoprenoid lipids.

3.5.2 STEROIDS

3.5.2.1 Structural Nomenclature of Steroids

The steroids comprise a large group of compounds sharing a particular structural motif of C_{30} (triterpenoid) derived isoprenoid lipids. They include a number of ubiquitous compound classes such as sterols, adrenal steroids, sex hormones, bile acids, saprogenins, and others. As a group, they share the common feature of a tetracyclic ring system (three six-membered rings and one five-membered ring) derived from lanosterol, itself a cyclization product of the triterpenoid squalene (Figure 3.5). However, significant modification of the ring system gives rise to the diversity of steroid classes. Due to the high degree of modification of the original ring system, the majority of steroids do not conform to the isoprene rule. The use of common or trivial names for steroids is widespread and beyond the scope of this chapter (refer to Briggs and Brotherton, 1970). The following sections concentrate solely on an explanation of the IUPAC systematic nomenclature, which the reader will be able to relate to other descriptions.

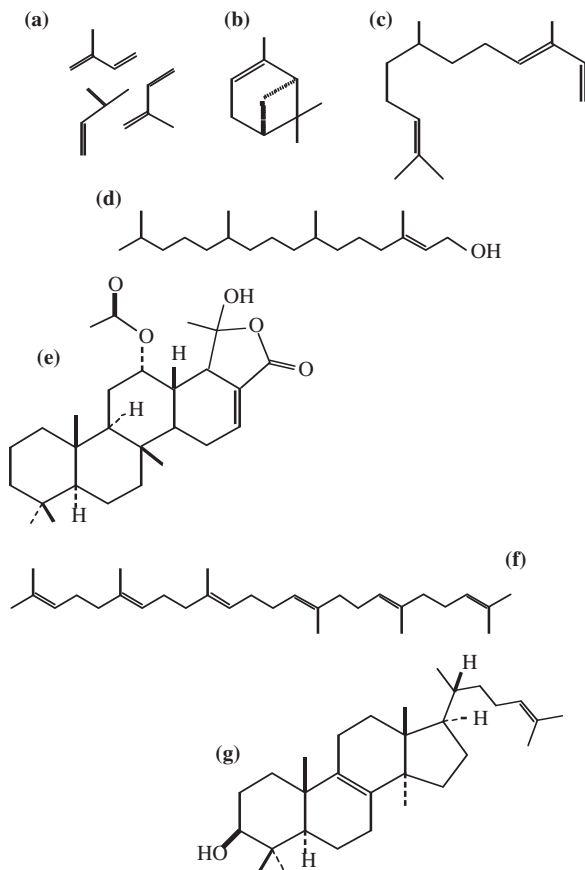


FIGURE 3.5 Examples of isoprenoid lipids illustrating the structural diversity of the group. (a) Three isoprene units (2-methyl-1,3-butadiene) in various conformations; (b) α -pinene (2,6,6-trimethylbicyclo[3.1.1]hept-2-ene); (c) β -farnescene (3,7,11-trimethyl-1,3,10-dodecatriene); (d) phytol (3,7,11,15-tetramethyl-2-hexadecenol); (e) scalarin (12 α -acetoxy-25 α -hydroxyscalar-16-en-25,24-olide); (f) squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene); and (g) lanosterol (4,4,14 α -trimethyl-5 α -cholest-8,24-diene-3 β -ol).

The systematic nomenclature of steroids is complex due to the diversity of substituents and the stereochemistry of the ring system. The carbon numbering of the generic steroid ring system is shown in Figure 3.6. The carbon atoms are numbered in cyclic fashion, beginning with the combined rings A and B anticlockwise, ring C clockwise, and then ring D anticlockwise. The carbon substituents of ring junctions are then numbered, working backward from ring D to ring A. The isoprenoid side-chain at C-17 is numbered last in a specific fashion to accommodate the presence or absence of common substituents. Further carbon substitutions may also occur in some cases at C-24 (then numbered C-28, C-29) and C-4 (then numbered C-30, C-31) (e.g., scalarin, Figure 3.5).

TABLE 3.4
Isoprenoid Lipids Classified in Terms of Terpenoid Class, with Common Examples

Terpenoid Class	Carbon	Examples
Hemiterpenes	5	Isoprene
Monoterpenes	10	α -Pinene
Sesquiterpenes	15	β -Farnesene
Diterpenes	20	Phytol
Sesterterpenes	25	Scalarin
Triterpenes	30	Squalene, lanosterol
Polyterpenes	>30	Carotenes

Note: Refer to [Figure 3.5](#) for structures.

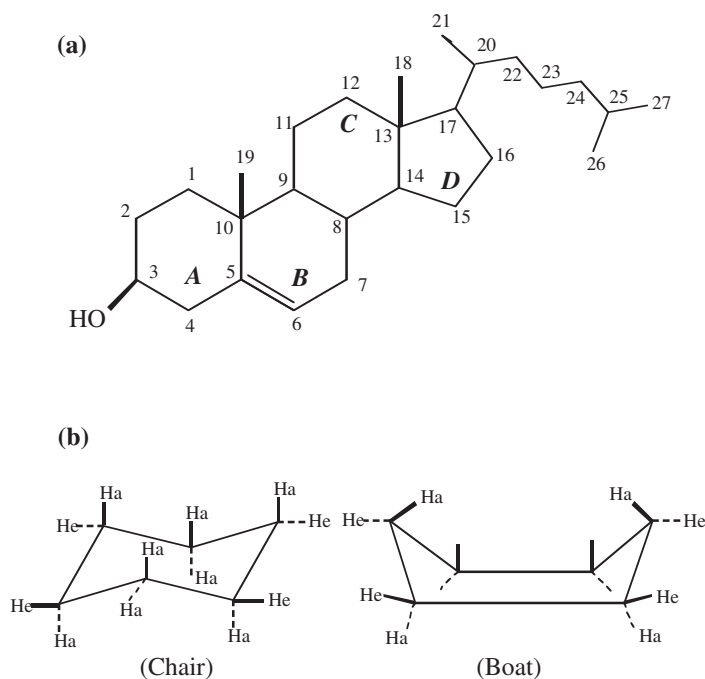


FIGURE 3.6 Steroids. (a) The carbon numbering system for steroids using cholesterol (cholest-5-en-3 β -ol) as an example. The individual rings of the carbon skeleton are labeled A to D. (b) Representations of the “chair” and “boat” conformations for a six-membered carbon ring illustrating equatorial (He) and axial (Ha) hydrogen atoms.

A description of the molecular stereochemistry is also included in both the structural formula and nomenclature. Each ring junction can exist in either a *cis* or *trans* conformation, yielding six centers of asymmetry or chirality (C-5, 8, 9, 10, 13, 14). Hence, 64 stereoisomers are possible for the ring system alone. With the C-17 side-chain forming a seventh site of asymmetry, the theoretical number of stereoisomers increases to 128. However, in practice, the isomeric possibilities are restricted by the overall conformational limitations of the ring system.

In isolation, each six-membered ring system can exist in either the well-known “chair” or “boat” conformation (Figure 3.6). While the chair conformation is favored, in either case the two hydrogen atoms attached to each carbon take up positions either in the general plane of the ring, termed equatorial (e), or perpendicular to the plane of the ring, termed axial (a). Equatorial or axial positions can be oriented above (β -configuration) or below (α -configuration) the general plane of the ring. By convention, the β -configuration is represented by a heavy line and the α -configuration by a dotted line in the structural formula. The junction between rings *A* and *B* can be in one of two orientations: with substituent hydrogens in a *trans* position (i.e., C-5 α , C-10 β configuration) or in a *cis* position (i.e., C-5 β , C-10 β configuration). Hence, the orientation of the C-5 hydrogen defines the type of *A/B* ring junction, a *trans* junction is defined by a 5 α orientation (previously termed “allo”) and a *cis* junction is defined by the 5 β position (previously termed “normal”).

In most naturally occurring steroids, sterols, and bile salts, the *C/D* ring junction is in the *trans* conformation. This is also the case for the *A/B* ring junction in most biologically active steroids. These junctions make the tetracyclic ring system planar in orientation. To aid in deducing the stereochemistry from the structural formulae, it is common to show the orientation of the C-5 hydrogen atom (e.g., Figures 3.5 and 3.6). The orientation of the remaining ring junctions can then be assumed in most cases. That is, unless otherwise indicated, substituent atoms at ring junctions are assumed to be C-8 β , C-9 α , C-10 β , C-13 β , C-14 α , and the C-17 side-chain in the β conformation.

The stereochemistry of side-chain substituents is described by the sequence rule procedure, where any chiral centers formed by substituents at C-20 or above are assigned the (*R*) or (*S*) configuration. This designation, when employed, precedes the entire nomenclature name (see Section 3.5.2.2). For brevity, the side-chain stereochemistry will not be described in further detail.

3.5.2.2 Named Nomenclature of Steroids

3.5.2.2.1 Introduction

Steroids also possess their own set of nomenclature stem names based on the carbon number of the central ring system (Figure 3.7). These names are equivalent to the stem names for n-alkane hydrocarbons. Many of the standard IUPAC prefixes and suffixes are then applied directly to describe the related structural details, in a similar manner as for other compounds (Table 3.5). However, there are some variations. For the following discussion, the parent ring system cholestane will be used to highlight examples.

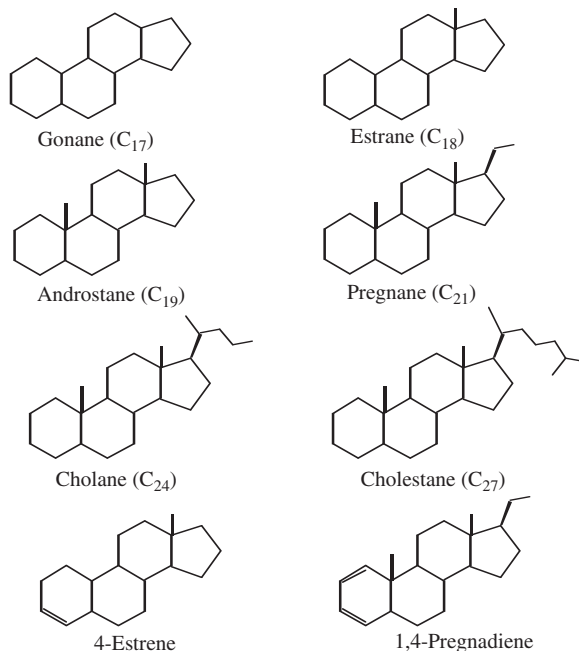


FIGURE 3.7 Example generic carbon ring systems for saturated and unsaturated steroids of C₁₇ to C₂₇ with the systematic nomenclature stem names.

TABLE 3.5
Functional Groups in Order of Priority for the
Selection of the Name Suffix in Systematic Steroid
Nomenclature

Functional Group	Prefix	Suffix
Carboxylic acid	—	-oic acid / -carboxylic acid ^a
Lactone	—	-lactone / -carbolactone ^a
Ester or salt of acid	alkyl group ^a	-oate
Aldehyde	oxo-	-al
Ketone	oxo-	-one
Hydroxyl	hydroxy-	-ol

^aRefer to [Section 3.5.2.2.2](#)

Information compiled from Kirk, D.N. and Marples, B.A. 1995. *Steroid Analysis*, Makin, H.L.J., Gower, D.B., and Kird, D.N., Eds, Blackie Academic and Professional, London, Chapter 1.

3.5.2.2.2 Carboxylic Acids and Derivatives (Esters and Lactones)

Substitution of a carboxylic acid functionality to a methyl carbon employs the use of the “-oic acid” suffix (e.g., 5 β -cholest-26-oic acid, Figure 3.8). When substitution occurs on a methylene carbon, the suffix employed is “-carboxylic acid” (e.g., 5 β -cholestane-24-carboxylic acid, Figure 3.8). Esters of either type of carboxylic acid are referred to in the same manner as other acids, by changing the “-oic acid” or “-ic acid” suffix to “-oate” or “-ate” and utilizing the ester as the prefix. Hence, the methyl esters of the previous examples become methyl 5 β -cholest-26-oate and methyl 5 β -cholestane-24-carboxylate, respectively. For lactone derivatives, the suffix “-ic acid” is replaced by “-lactone” and “carboxylic acid” changed to “-carbolactone.” The suffix is preceded by the acid group location and then the hydroxyl group location (e.g., 5 β -cholesto-26,17 α -lactone, Figure 3.8).

3.5.2.2.3 Aldehydes, Ketones, and Ethers

When a methyl group changes to an aldehyde, the suffix used is “-al.” When an acid group is changed to an aldehyde, the suffix becomes “-aldehyde” but the name is derived from that of the acid (e.g., 5 β -cholest-26-aldehyde). The prefix “oxo-” denotes the change of a methyl or methylene group to an aldehyde or ketone group, respectively. If additional carbon atoms are introduced as aldehyde groups, a separate nomenclature system is used. For ketone groups, the standard suffix “-one” and prefix “oxo-” are used as appropriate (e.g., 4-

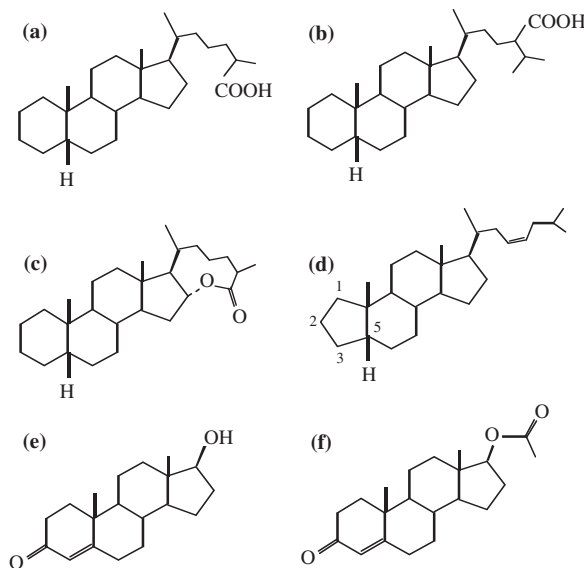


FIGURE 3.8 Example steroid structures with systematic nomenclature names: (a) 5 β -cholest-26-oic acid; (b) 5 β -cholestane-24-carboxylic acid; (c) 5 β -cholesto-26,17 α -lactone; (d) 23(Z)-4-nor-5 β -cholest-23-ene; (e) 17 β -hydroxyandrost-4-en-3-one; and (f) 3-oxo-androst-4-en-17 β -yl acetate.

oxo-5 β -cholest-26-oic acid, 5 β -cholest-4-one). Ethers are named as alkoxy group prefixes (i.e., methoxy-, ethoxy-, etc.).

3.5.2.2.4 Alcohols and Derivatives (Esters and Ethers)

Alcohol groups are designated as for other lipid components, with the suffix “-ol” and prefix “hydroxy-” where appropriate (e.g., cholest-5-en-3 β -ol, [Figure 3.6](#)). Esters are named by replacing the “-ol” suffix of the parent alcohol with “-yl” to generate the radical name. The acyloxy group is then denoted in anionic form following the main name (e.g., cholest-5-en-3 β -yl acetate). As ester groups take naming precedence over oxo (aldehyde and ketone) groups, the systematic names of many steroid esters do not necessarily derive from the parent alcohol. An example is the addition of an acetyl group to the C-17 hydroxy group of 17 β -hydroxyandrost-4-en-3-one (testosterone), which forms the compound named 3-oxo-androst-4-en-17 β -yl acetate ([Figure 3.8](#)).

3.5.2.2.5 Unsaturation and Skeletal Modifications

The insertion of double bonds in the ring system does not require further nomenclature description because the geometry is fixed by the ring itself. For double bonds on the side-chain, the older terms *cis* and *trans* used for FA nomenclature are recommended to be replaced with the more precise sequence rule terms (*Z*), usually equivalent to *cis*, and (*E*), usually equivalent to *trans*, respectively. Where the ring system has been modified by the loss of a carbon atom (either a methyl group or ring carbon), the prefix “nor-” is used preceded by the location of the missing carbon ([Figure 3.8](#)). Where additional carbons are added to the ring structure, the prefix “homo-” is used as noted previously. Scission of a ring system is denoted by the prefix “seco-” with the location of the two carbon atoms where the break occurs.

3.5.3 CAROTENOIDS

3.5.3.1 Introduction

Carotenoids consist of a group of pigments with an extremely wide distribution in natural products. The term is used to describe several groups of compounds derived from eight isoprene units in a manner such that the arrangement of the units is reversed at the center of the molecule. They are also characterized by the presence of a conjugated double bond system. All members of the carotenoids can be formally derived from the acyclic precursor lycopene ([Figure 3.9](#)) by reactions involving one or more options of hydrogenation, dehydrogenation, cyclization, insertion of oxygen, double bond migration, methyl migration, chain elongation, or chain shortening. Different groups of carotenoids are defined by these various modifications. The reader is referred to Goodwin (1980) for further details.

3.5.3.2 Nomenclature

Carotenes are a specific group of carotenoids that retain their hydrocarbon character (i.e., without the addition of other functional groups) and can be described by the type of terminal chain structures (end groups) connected to the central

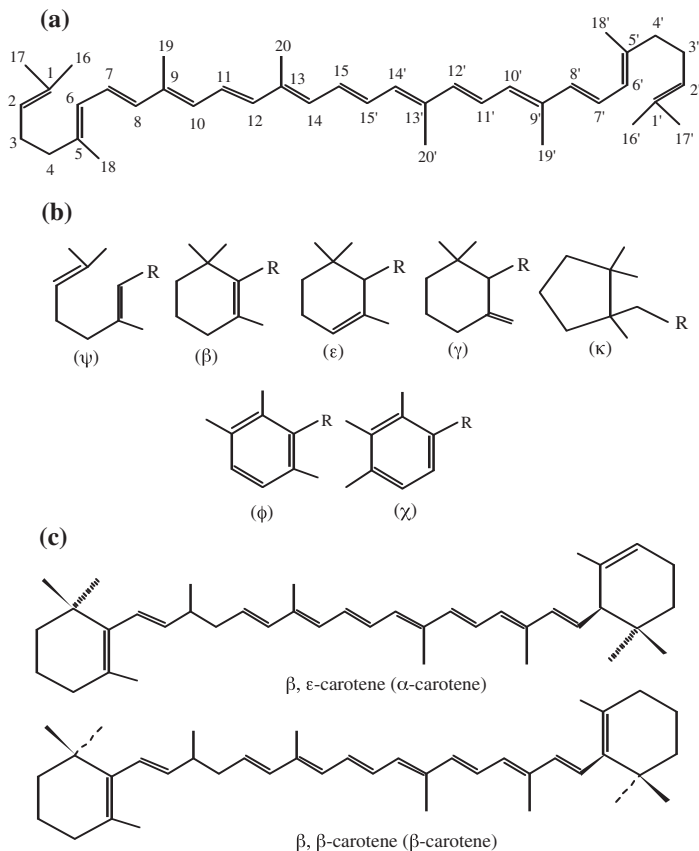


FIGURE 3.9 Examples of carotene structural diversity: (a) lycopene (ψ,ψ -carotene) displaying the carbon number system for carotenes; (b) the systematic nomenclature end group designations for carotenes; and (c) α -carotene (β,ϵ -carotene) and β -carotene (β,β -carotene).

$C_{22}H_{26}$ linear chain. End group designations for carotenes are given in Figure 3.9. As for steroids, trivial names for carotenes are commonly employed. Again, we concentrate on the IUPAC systematic nomenclature. The stem name “carotene” is employed for this and the following groups, preceded by the designation of the end groups. Hence, lycopene is systematically described as ψ,ψ -carotene, the well-known α -carotene is β,ϵ -carotene, and β -carotene is β,β -carotene (Figure 3.9). When a carotene is referred to in most texts, the systematic name will be denoted in addition to the trivial name. However, following this, the compound is likely to be referred to only by its trivial name.

Carotenoids that contain an oxygenated functionality are collectively referred to as xanthophylls. They retain the systematic naming system of carotenes, with the additional functionalities named according to the usual nomenclature rules previously discussed. For example, β,β -carotene-3,3'-diol (zeaxanthin) (Figure 3.10a). Methoxy, carboxy, aldehyde, epoxy, and ketone derivatives are also common.

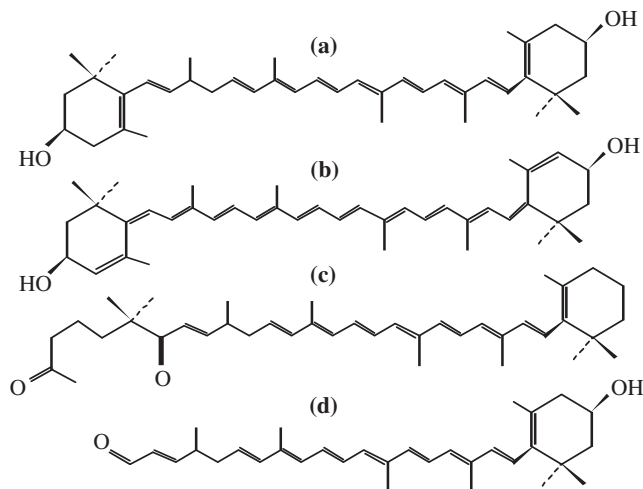


FIGURE 3.10 Examples of oxygenated carotenoids (xanthophylls): (a) zeaxanthin (β,β -carotene-3,3'-diol); (b) eschscholtzianthin (4',5'-didehydro-4,5'-retro- β,β -carotene-3,3'-diol); (c) 5,6-seco- β -carotene-5,6-dione; and (d) 3'-hydroxy-8-apo- β -carotene-8-al.

In *retro*-carotenoids, all the single or double bonds of the conjugated polyene system have shifted by one position. The carbon atoms defining the new conjugated system are indicated in the nomenclature as a prefix prior to the term “-retro-.” For example, 4',5'-didehydro-4,5'-retro- β,β -carotene-3,3'-diol (eschscholtzianthin) (Figure 3.10b).

Seco carotenoids have undergone oxidative fission of a ring system without the loss of any carbon atoms. In these cases, the prefix “seco-” is utilized, preceded by the numbers of the carbon atoms involved in the fission. The remainder of the stem name is taken from the description of the remaining molecule. For example, 5,6-seco- β -carotene-5,6-dione (Figure 3.10c). Where oxidative fission of a ring system results in the loss of any carbon atoms, the resulting compound is termed an apo-carotenoid. The prefix “apo-” is preceded by the number of the carbon atom from which the remainder of the molecule has been removed, with the stem name taken from the description of the remaining molecule. For example, 3'-hydroxy-8-apo- β -carotene-8-al (Figure 3.10d). Nor-carotenoids are compounds in which carbon atoms have been removed by procedures other than the cleavage of carbon-carbon bonds. They are named according to the normal standard system of nomenclature retaining the “carotene” stem name.

3.5.3.3 Stereochemistry of Carotenoids

The configuration of chiral centers is designated as described previously, using the (*R*) and (*S*) designations, together with locations, preceding the full systematic name. Stereochemistry also affects the carbon numbering of the end groups. When a potential chirality at C-1 is structured as in the β end group (Figure 3.9), with the polyene chain to the right of C-1, then the methyl group below the plane of the ring

is numbered C-16 and the one above is numbered C-17. When the polyene chain is to the left of C-1, the numbering of the methyl groups is reversed. If the end group is acyclic (e.g., ψ end group, Figure 3.9), the methyl group that is *trans* to the polyene chain is numbered C-16 and the *cis* methyl group is numbered C-17.

The stem name “carotene” brings with it an implication of *trans* double bond geometry for all bonds. Note that this is opposite to that of polyene FA discussed earlier (see Section 3.2.4). If a *cis* double bond is present, this is distinguished in the structural name as either “*cis*–” or “*Z*–” following the appropriate carbon location. Compounds known to contain a *cis* double bond but where the location is unknown, or isomers of carotenes with an unlocated *cis* double bond, are given the prefix “neo–” and an alphabetical suffix.

3.6 PROPERTIES OF LIPIDS

3.6.1 CHEMICAL INDICES

3.6.1.1 Introduction

Chemical indices are a measure of several specific physical/chemical properties of lipids. Indices are widely used in the fat and oil industry because they are useful for describing the characteristics of lipid mixtures used in food manufacturing and processing. The composition of natural oils and fats is affected by cultivar and growing conditions, as well as handling and processing. Chemical indices are a practical way of describing and quantifying this diversity. They are, however, seldom used for the characterization of individual lipids because, in isolation, they do not provide proof of the identity or purity of an individual compound.

A large number of indices have been proposed and used by different industry sectors. Standard methods for the assays have been published by The American Oil Chemists’ Society (AOCS, <http://www.aocs.org>) and the International Organisation for Standardization (ISO, <http://www.iso.org>), among others. The following section focuses on indices particularly relevant to food processing and manufacturing.

3.6.1.2 Iodine Value

The iodine value (IV) is an important parameter in trade specifications and may provide an indication of the adulteration of an oil or fat. It is also useful in determining the ability of a lipid to be hardened by hydrogenation and for monitoring hydrogenation process control. The iodine value is the number of grams of iodine absorbed by 100 g of lipid. It is based on the ability of an unsaturated carbon-to-carbon bond to add halogen atoms and provides a measure of the degree of unsaturation of a lipid (Table 3.6). Saturated compounds have a zero iodine value. The Wijs method, which involves reacting a sample with iodine monochloride in a solution of glacial acetic acid and detecting liberated iodine with standardized thiosulfate, is the standard chemical method (see, for example, ISO 3961:1996; AOCS Cd 1d-92).

Fatty acids containing acetylenic bonds, double bonds close to the carboxyl group, conjugated unsaturation, or other reducible (e.g., keto) groups rarely add the

TABLE 3.6
Effect of Unsaturation on the
Iodine Value (IV) of Individual
Fatty Acids

Fatty Acid	IV
16:1(n-9)c	99
18:1(n-9)c	89
18:2(n-6)	181
18:3(n-3)	273
20:4(n-6)	320

Data compiled from Deuel, H.J., Jr. 1951.
The Lipids, Their Chemistry and Biochemistry, Volume 1: Chemistry, Interscience, New York.

theoretical amount of iodine. Samples containing significant proportions of these compounds will give lower than expected iodine values. The presence of conjugated double-bond systems can be detected by ultraviolet spectroscopy. Hydroxyl groups in some lipids may interact with the Wijs reagent, giving a higher than expected IV.

Because the IV is a measure of the relative unsaturation of a compound or sample, other analytical techniques (e.g., gas chromatography of FA composition, AOCS Cd 1c-85) can be used to estimate the value. Interest in recent years has focused on spectroscopic techniques for the rapid determination of IV. Fourier transform (FT)-near-infrared, near-infrared, FT-Raman, and ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopic techniques have all been investigated (Ng and Gee, 2001). The most promising results have been obtained with FT-near-infrared spectroscopy, which only takes a few minutes to determine the iodine value (Cox et al., 2000).

3.6.1.3 Acid Value or Neutralization Value

The neutralization or acid value (AV) of a lipid sample gives an indication of the unbound or free FA content. This is achieved by direct titration of the sample in an appropriate solvent with alkali (e.g., ISO 660:1996). The AV is defined as the number of milligrams of potassium hydroxide required to neutralize a 1-g sample. Although refined oils are largely devoid of free FA, considerable amounts may be present in crude oils. Their presence may be an index of oil purity. The degree of edibility of a fat is generally considered to be inversely proportional to the total amount of free FA.

3.6.1.4 Saponification Value

The saponification value (SV) gives a measure of the average length of the FA acyl residues present in complex lipid components. It is defined as the number of milligrams of potassium hydroxide necessary to saponify a 1-g lipid sample. The SV is

inversely proportional to the molecular weight of the lipid. Samples consisting largely of lipids containing C₁₈ FA along with some 16:0, a little unsaponifiable material, and possessing low AV have saponification values around 290. Higher values indicate the presence of increased amounts of longer-chain FA (e.g., canola oil, ca. 320), hydroxy acids (e.g., castor oil, ca. 310), or unsaponifiable material. Lower values are characteristic of samples rich in shorter-chain fatty acids (e.g., palm oil, 260 to 280; or butter fat, 240 to 260). [Table 3.7](#) lists some typical saponification values.

3.6.2 PHYSICAL PROPERTIES

3.6.2.1 Introduction

The physical properties of lipids derive directly from their chemical structures and functional groups. Physical properties greatly influence the functions of lipids in foods and the methods required for manipulation and processing. They can also be used to assess the purity or quality of lipid material in reference to known standards or preferred characteristics.

3.6.2.2 Density

For FA that are solid at room temperature, the density is best determined well above the melting point, usually 40 to 50°C. The relative density of a complex lipid increases with increasing molecular weight of the component FA and with larger proportions of unsaturated or hydroxy acids ([Table 3.7](#)). It also tends to increase with oxidation (rancidity) of a lipid.

3.6.2.3 Surface Tension

Surface tension decreases with increasing temperature. This relationship is linear over a large temperature range. The measurement of surface tension is markedly affected by the presence of sample impurities.

3.6.2.4 Viscosity

Viscosity is an important parameter for the design of industrial processes; for example, it determines the rate at which an oil drains from a fried food. It is also an important factor affecting the stability of foods. In general, saturated fatty acids have higher viscosities than unsaturated fatty acids because their molecular structure enables close proximity of the carbon chains, which allows intermolecular interactions such as Van der Waals forces to establish. Conversely, the *cis* configuration of double bonds within FA prevents close alignment of molecules and hence results in weaker intermolecular interactions. For example, olive oil (10% unsaturated FA) has a higher viscosity than sunflower oil (70% unsaturated FA). Some typical viscosity values are given in [Table 3.7](#). Viscosity can also be used to evaluate the quality of fats used in frying because these show an increased viscosity as they approach heat-induced breakdown (reviewed by Gertz, 2000). The increased viscosity is coincident with polymerization, oxidation, gumming, and foaming tendencies.

TABLE 3.7
Physical and Chemical Properties of Lipids from Different Sources

Lipid Source	IV ^a	Sap ^b	SG ^c (20/20°C)	Viscosity (20°C)	Refractive Index (40°C)	Melting Point (°C)
Olive oil	75–94	184–196	0.910–0.916	63.28	1.468–1.471 ^d	–3–0
Coconut oil	5–13	248–265	0.908–0.921 ^e	—	1.448–1.450	23–26
Palm kernel oil	14–21	230–254	0.899–0.914 ^e	—	1.448–1.452	24–26
Chicken fat	76–80	—	0.914–0.924 ^f	—	1.452–1.460	30–34
Tallow (mutton)	35–46	—	0.938–0.955 ^g	—	1.452–1.458	44–51
Tallow (beef)	33–47	190–200	0.903–0.907 ^h	—	1.450–1.458	45–48
Salmon oil	130–160	183–186	0.924–0.926 ⁱ	—	1.472–1.477	—
Sunflower seed oil	118–145	188–194	0.918–0.923	49.14	1.467–1.469	—
Rapeseed oil	94–120	168–181	0.910–0.920	—	1.465–1.469	—
Canola oil	110–126	182–193	0.914–0.920	—	1.465–1.467	—
Palm oil	49–55	190–209	0.891–0.899 ^j	—	1.454–1.456	33–40
Castor oil	81–91	176–187	0.945–0.965 ^h	—	1.466–1.473	—
Pumpkin seed oil	116–133	174–197	0.903–0.926 ⁱ	54.82	1.466–1.474	—
Maize oil	107–135	187–195	0.917–0.925	51.44	1.465–1.468	—

^aIodine value; ^bsaponification value; ^cspecific gravity; ^d20°C; ^e40/20°C; ^f10/10°C; ^g55/55°C; ^h25/25°C; ⁱ15/15°C; ^j50/20°C.

Data compiled from Anon. 1997. *Physical and Chemical Characteristics of Oils, Fats and Waxes*, AOCS Press; Abramovic, H. and Klofuta, C. 1998. *Acta Chim. Sloven.*, 45, 69; Firestone, D. 1999. *Physical and Chemical Properties of Oils, Fats, and Waxes*, AOCS Press.

TABLE 3.8
Examples of Lipid Classes
Listed in Order from Most
Polar to Least Polar

Sphingomyelin
Phosphatidylinositol
Phosphatidylserine
Sulfolipids
Monoacylglycerols
Diacylglycerols
Sterols
Triacylglycerols
Aldehydes
Hydrocarbons

3.6.2.5 Polarity

The polarity of a lipid affects its volatility, solubility, and nonspecific binding to other polar compounds. Lipids are often functionally classified as neutral or polar on the basis of their mobility on thin-layer chromatography using a neutral or polar solvent system. Neutral lipids such as wax esters, steryl esters, ether lipids, and TAG are chemically neutral while other neutral lipids, such as free FA, fatty alcohols, and monoacylglycerols, are actually slightly polar due to the presence of hydroxyl or carboxyl groups. [Table 3.8](#) lists the relative polarities of some common lipid classes.

3.6.2.6 Melting Point

Natural lipid mixtures do not have definite melting points because they are a mixture of various compounds. When heated, they soften due to the melting of individual components before becoming fully liquid. Some fats have a double melting point where they melt and then solidify before melting again. Therefore, empirical methods have been adopted to characterize the melting of mixtures such as TAG. Commonly employed methods include the drop point, the temperature at which the first drop of liquid falls from an open capillary containing the solid mixture, and the slip point, the temperature at which a solid mixture in an open capillary placed in a water bath begins to move upward. These methods are generally reproducible for homogenous mixtures but can give quite variable results with substances formed from a number of lipid sources ([Table 3.7](#)).

Purified lipids have sharp and reproducible melting points. The melting points of saturated FA do not increase uniformly with increasing chain length but form two alternate series with even chain length FA having a higher melting point. These series converge as the chain length increases ([Figure 3.11](#)). The introduction of a double bond leads to a decrease in the melting point. FA with a double bond in the *cis* configuration have a lower melting point than the corresponding isomer with a *trans* configuration. The position of the double bond also affects the melting point.

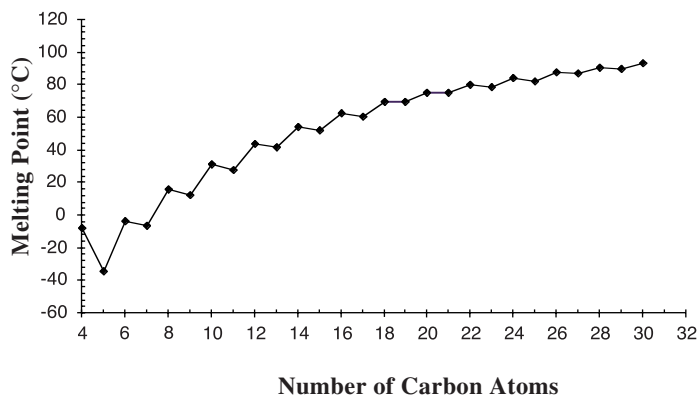


FIGURE 3.11 Melting points of saturated straight-chain fatty acids demonstrating the difference between even- and odd-chain length series.

Melting points decrease as the double bond moves toward the center of the carbon chain. This effect is much greater for double bonds in the *cis* configuration than in the *trans* configuration (Gunstone, 1958). These acyl chain effects have been interpreted in terms of an effective chain length model. Thus, for monounsaturated acids where the double bond exists closer to either end of the molecule, the acyl moieties behave as if they were saturated chains with an effective chain length identical to the length of the longer of the two chain segments separated by the double bond (Cevec, 1991).

Additional unsaturation further decreases the melting point unless the double bonds are conjugated. Substitution of the alkyl chain also affects the melting point. For saturated FA, hydroxyl groups raise the melting point while methyl groups lower it. *Iso*-branched FA generally have a higher melting point than the corresponding *anteiso*-branched FA; for example, the melting points of 15:0, i15:0, and a15:0 are 52.3, 51.8, and 23.0°C, respectively.

3.6.2.7 Refractive Index

Refractive index (RI) is easily determined (e.g., ISO 6320:2000) and often used as a criterion of purity. The RI of saturated FA reveals a linear increase with increasing chain length when measured at a temperature above 40°C. Below 40°C, intramolecular forces may cause a nonrandom arrangement of molecules, thus affecting the refractive index. The refractive index of unsaturated FA increases with the degree of unsaturation. Conjugated unsaturation results in a marked increase in the refractive index. Some typical values are shown in Table 3.7.

A compound is considered to be optically active, or chiral, if linearly polarized monochromatic light is rotated when the compound is passed through it. The amount of optical activity can be measured using a polarimeter and is determined by the pathlength the light traverses, the concentration of the compound, and the wavelength of the light (normally a sodium light). Specific rotation, $[\alpha]$, is defined as:

$$[\alpha] = \frac{\alpha}{l \times d} \quad (3.1)$$

where α is the measured optical rotation in degrees; l is the sample pathlength in decimeters; and d is the density if the sample is pure liquid, or the concentration if the sample is a solution, expressed as g/cm³. A chiral molecule is one that is not superimposable on its mirror image. Chirality can be caused by a number of factors, such as restricted rotation about a single double bond, but is often indicative of an asymmetrical molecular structure (Figure 3.4).

Most TAG mixtures show little optical activity, but castor oil and those unusual oils containing acylglycerols of cyclopentene acids (e.g., chaulmoogric and hydno-carpic acids present in the seed oils of *Flacourtiaceae*) are notable exceptions. Samples containing sterols will display some optical activity due to the presence of these optically active compounds. Because most edible oils have low optical activity, a method has been proposed to detect the adulteration of edible oils with castor oil based on the optical activity of ricinoleic acid (1,2-dihydroxyoctadecenoic acid), a major constituent of castor oil (Babu et al., 1996).

TAG with FA differing greatly in chain length show small but measurable optical rotation. A saturated TAG with an acyl chain length greater at position *sn*-1 than at position *sn*-3 has a negative optical rotation. However, in complex TAG mixtures, considerable fractionation would be required before analysis because the optical rotations of the different components would partially cancel each other.

3.6.3 ABSORPTION SPECTRA

3.6.3.1 Ultraviolet and Visible Spectra

Ultraviolet (UV) and visible (VIS) spectra of lipids result from electronic excitations and transitions in the lipid molecule. Hence, functionalities with high electron density (e.g., carboxyl groups, double bonds) absorb strongly in the UV or VIS spectrum with characteristic maximum wavelengths ($\lambda_{\max}$) and extinction coefficients ($\epsilon_{\max}$). Values for common UV-absorbing functionalities are shown in Table 3.9.

TABLE 3.9
Ultraviolet Absorption Values for Common Lipid Functionalities

Functionality		Absorption Maximum (nm)	Extinction Coefficient
Name	Structure		
Double bond	-C=C-	177	12600
Conjugated double bond	-C=C-C=C-	217	20900
Carboxyl	-COOH	208–210	32–50
Conjugated carboxyl	-CH=CH-COOH	206	13500

Data compiled from Hemming, F.W. and Hawthorne, J.N. 1996. *Lipid Analysis*, Bios Scientific Publishers, Oxford, Chapter 2.

Conjugation of double bonds brings the absorption bands into the easily accessible region. Extinction coefficients increase with increasing conjugation. With three or more conjugated double bonds, the absorption shows three peaks, a main one and subsidiary maxima on either side. The peaks are spread further apart as the number of double bonds increases. UV spectra are particularly valuable for the analysis of carotenoids.

3.6.3.2 Infrared Spectra

The stretching and bending vibrations of molecular bonds give rise to absorptions in the infrared (IR) region. Absorption bands are expressed as a frequency (wave-number), or the number of waves per centimeter. All FA show a strong absorption in the 2750 to 3000 cm^{-1} range because of the large number of CH_2 and CH_3 groups in the molecules (Table 3.10). Cyclopropane FA give characteristic absorption bands at 1020 and 3050 cm^{-1} due to vibration of the methylene CH_2 and stretching of the C-H bonds in the cyclopropane ring, respectively. Unsaturation can be detected by the C-H stretch near 3020 cm^{-1} . *Cis* and *trans* isomers show little difference, although *trans* double bonds are associated with C-H deformation at 950 to 1000 cm^{-1} . This can be used for quantitative determination of *trans* double bonds as long as there is limited conjugation. Hydroxy FA have a characteristic C-O stretch at 1045 cm^{-1} .

TABLE 3.10
Infrared Absorption Bands of Common Lipid
Functionalities

Functional Group	Frequency (cm^{-1})	Intensity ^a
-CH ₃	2962 and 2872 $\pm$ 10	s
	1450 $\pm$ 20	m
	1375 $\pm$ 5	m
-OCH ₃	1430	m
-CH ₂	2926 and 2853 $\pm$ 10	s
	1465 $\pm$ 10	m
	750–720	m
	3100–3000	m
	1025–1000	m
-C(CH ₃) ₂	1385 and 1365 $\pm$ 5	m
	1170 $\pm$ 5	m
-C(CH ₃) ₃	1395–1385	m
	1365	s
	1250 - 1200	s

^as, strong; m, medium.

Data compiled from Hemming, F.W. and Hawthorne, J.N. 1996. *Lipid Analysis*, Bios Scientific Publishers, Oxford, Chapter 2.

3.7 CONCLUSION

The IUPAC rules of lipid nomenclature have been developed to convey the maximum level of structural information within the name of the lipid. From this information, the reader should be able to reconstruct the component structure in a systematic fashion. For this reason, in most circumstances, the established system of systematic nomenclature should be encouraged rather than the use of trivial or common names. However, exceptions to this generality can be justified in the cases of steroids and carotenoids. In these cases, systematic nomenclature is often unwieldy for everyday usage and common names are well established in the respective fields.

When using structural formulae, care should be taken in the interpretation and representation of isomeric and geometric features. This is particularly true for the stereochemistry of complex lipids such as acylglycerols and the ring systems and substituents of steroids. The development of a solid grounding in lipid nomenclature will stand the researcher or food technologist in good stead.

This chapter has also introduced the major physical properties of lipids and outlined methods by which these parameters can be assessed. These properties are of use in both the description of individual lipid components and natural oil or lipid sources.

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4 Rheological Properties of Lipids

Tadeusz Matuszek

CONTENTS

- 4.1 Introduction
- 4.2 Flow of Lipid Systems
- 4.3 Principles of Measurement of Rheological Properties in Lipid-Based Food Systems
- References

4.1 INTRODUCTION

Lipids, like most food system components, consist of a network of many small particles and macromolecules held together by a wide range of intermolecular and colloidal forces. Their structure, texture, stability, and functionality are strongly influenced by the strength of these interactions. Moreover, the texture of a final food system containing lipids depends strongly on the history of structural changes during processing. Currently, we do not understand the mechanisms by which subtle changes in the interactions of lipids and other components in food systems control the entire structure and mechanical properties of foods. And from a rheological properties perspective, it has been difficult to provide a definition for the class of substances called lipids. Every definition proposed thus far has limitations due to solubility of lipids in organic solvents or in water.

The general strategy in lipid-based food systems is to quantitatively determine the relationships between the interactions, structure, and rheology related to the food functionality. From a structural point of view, this means the complete specifications of the relative distributions of particles in space. From a rheological point of view, it means the frequency-dependent relationship between stress and strain at small deformation rates, as well as dependent behavior at large deformation rates. In general, the lipid-based food functionality can be expressed through the hurdle technological effect, which was achieved at the final stage of food processing. Each particular food product can be assessed by a certain set of hurdles that differs in quality and intensity, depending on the technology used (Gorris, 1995). These hurdles can influence the stability and the sensory, nutritive, technological, and economic properties of a final product.

Most of the textural properties of lipid-based foods are largely rheological in nature, including, for example, creaminess, smoothness, tenderness, and hardness. The stability and appearance of lipid-based foods often depend on the rheological characteristics of their components as well as of the effect of heating on the physicochemical properties of lipids. They are particularly important when lipids are going to be used at high temperatures, for example, during baking or frying. From a rheological standpoint, it means changes between stress and strain rate due to the amount of volatile organic material in oils and fats such as free fatty acids. The rheological properties of lipids as liquid oils are usually characterized in terms of their flow properties as measured by viscosity. Lipids seen as viscoelastic or plastic “solids” are characterized in terms of both their elastic modulus and flow properties. One of the most important rheological characteristics of lipids is their “plasticity.” It is this characteristic that determines the “spreadability” of a lipid. The plasticity of a lipid is due to the fact that fat crystals usually form a three-dimensional network that gives a food product some solid-like characteristics. In this case, below a certain stress, known as the *yield stress* (τ_0 , shown in [Figure 4.1](#)), the product behaves like a solid with an elastic modulus. The yield stress is the stress that must be exceeded before the material will flow. But when the stress overcomes the crystal network forces, the product behaves like a liquid because the crystal network is continually being disrupted. This behavior is similar to some types of Bingham’s liquids, as well as thixotropy, and dilatation and rheopexy liquid flow ([Figure 4.1](#)).

4.2 FLOW OF LIPID SYSTEMS

Flow is an essential step in the production and fabrication of many lipid-based foods. Therefore, the flow properties of lipid solutions constitute an information set that is important in the economical design of food processing equipment and operations.

There are three significant concepts in lipid flow:

- The principle of conservation of mass, from which the equation of continuity is developed
- The principle of kinetic energy, from which certain flow equations are derived
- The principle of momentum, from which equations evaluating dynamic forces exerted by lipid-based food systems can be established

Lipid-based foods are, for the most part, nonhomogeneous; and structure formation implies that various states of aggregation occur during processing. The rheological properties of lipids are critical for attaining accuracy and quality in designing food processing machines and food industry facilities. They are also vital in modeling processing operations. Various energy and heat treatments of lipid-based foods include thermal conductivity, thermal diffusivity, density, consistency and concentration changes, specific enthalpy, specific heat, texture, and subsequently their mechanical and rheological properties. All these factors are related to the theory of flow for predicting the rates of molecular transport and their relationships to the molecular and, consequently, food microstructure properties. This molecular flow

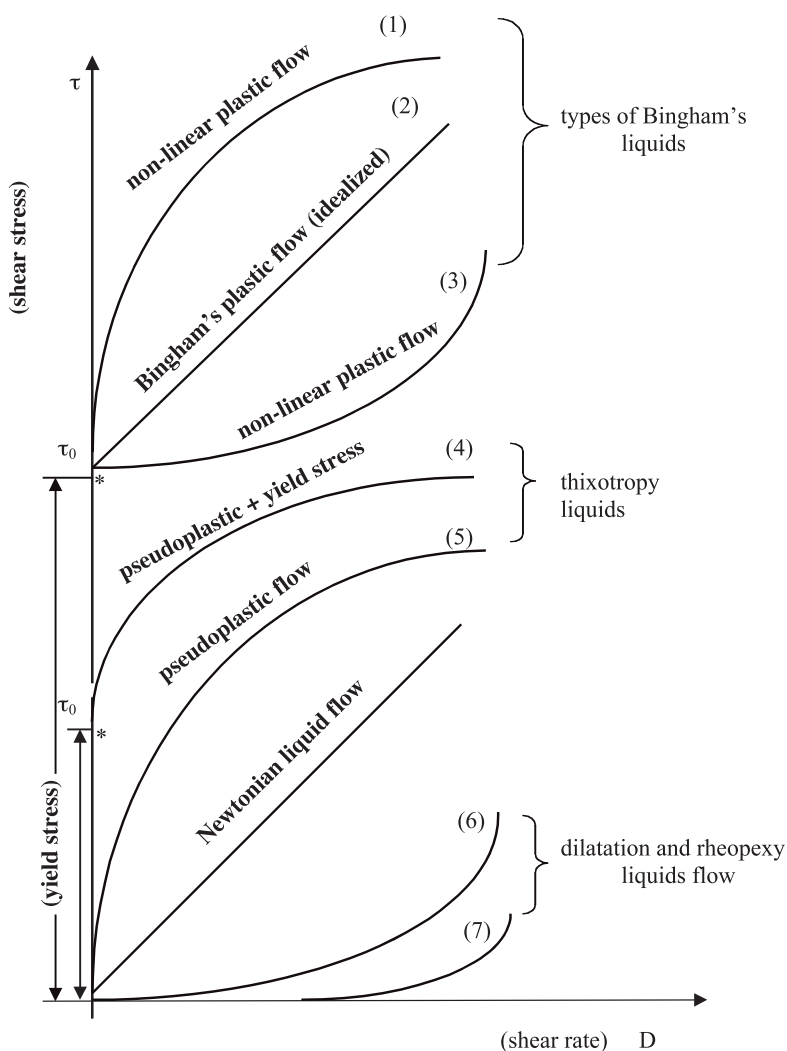


FIGURE 4.1 Relationship between shear stress and shear rate for different lipid flow characteristics. (From Matuszek, T.S. (2001). *Chemical and Functional Properties of Food Components*, 2nd ed., Sikorski, Z.E., Ed., CRC Press, Boca Raton, FL.)

information leads to a better understanding of surface rheological factors and to the organization and motions, together with intermolecular interactions, of molecules at interfaces.

Many examples have been given (Baird, 1982; Hernqvist, 1983; Princen, 1983; Hermansson, 1994; Morrison, 1994; Breitschuh and Windhab, 1997) that illustrate the complexity of the rheological behavior of lipids-based foods. The rheology of lipids, as in other food systems, is the science of deformation and flow of *real* materials, in terms of stress, strain, and time effects, not merely those of ideal

materials such as the Hookean solid or the Newtonian liquid. The real lipid material has both solid-like and a liquid-like properties, and usually exhibits a combination of rheological behaviors. Typically, there are three types of stress: compressive stress, which is directed toward the material; tensile stress, in which the stress is directed away from the material; and shearing, in which the stress is directed tangentially to the material. Shear stress predominates in lipid fluids. Correspondingly, there are three types of strain: compressive, tensile, and shear. As a consequence, lipid-based foods can rheologically deform in three ways: elastic, plastic, and viscous. In an ideal elastic deformation, the strain is directly proportional to stress, and it disappears instantly and completely after the stress is removed. In an ideal plastic deformation, the strain appears after a certain value of stress, called yield stress, is reached. This deformation is permanent, and there is no recovery when the stress is removed. For an ideal viscous deformation, the shear strain occurs instantly when the stress is applied, and its value is proportional to the rate of strain. In contrast to an elastic deformation, there is no recovery when the stress is removed.

Many lipid-based foods show time-dependent rheological behavior and a combination of these, for example, viscous–elastic and viscous–plastic. In general, lipids, from a rheological point of view, can be classified as Newtonian and non-Newtonian, as depicted in [Figure 4.1](#). To characterize lipid properties, such as Newtonian and non-Newtonian behavior, several approaches can be taken and the stress–strain relationships obtained.

One of the most important textural and rheological properties of lipid solutions is viscosity, or consistency. The evaluation of viscosity can also be demonstrated by reference to the evaluation of creaminess, spreadability, and pour-ability characteristics. These characteristics primarily depend on the shear rate and are affected by viscosity and different flow conditions. If related to steady flow, this means that at any point, the velocity of successive fluid particles is the same at successive periods for the entire food system. Thus, the velocity is constant with respect to time, but may vary at different points with respect to distance. Flow is unsteady when conditions at any point in a fluid food system change with time. Lipid flow can be either uniform or nonuniform; laminar or turbulent; one-, two-, or three-dimensional; and rotational or irrotational.

Most practical food engineering problems involving steady flow conditions are based on the Newton suggestion. The thin layer of liquid between two small planes 1 and 2, each of area A (shown schematically in [Figure 4.2](#)) is considered to be part of a laminar flow, or simple shear flow. The middle layer of liquid with a greater velocity is pulled forward with an equal force F . The layer below, which has less velocity, pulls the middle layer back with an equal force F . These two equal but opposite and parallel forces form a shear couple, and produce a shear stress of magnitude F/A . Newton suggested that the shear stress was directly proportional to the velocity gradient; that is:

$$F/A \sim dV/dz \quad (4.1)$$

$$\therefore F/A = \eta dV/dz \quad (4.2)$$

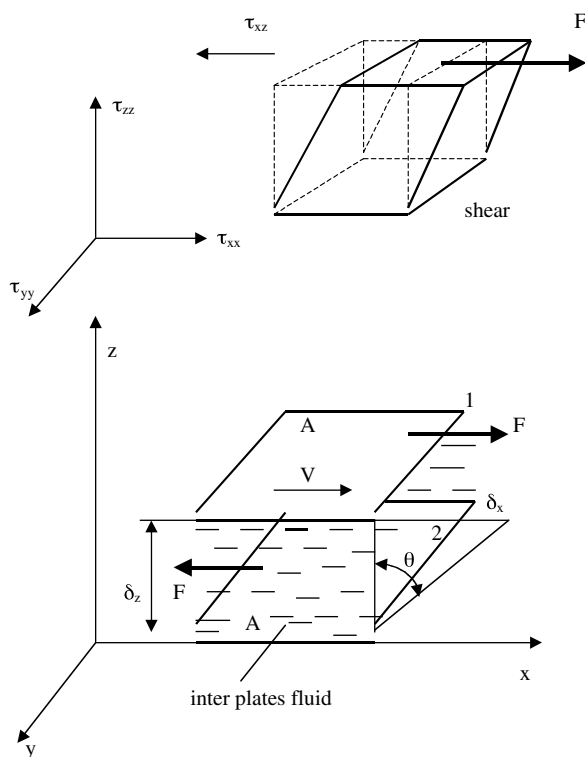


FIGURE 4.2 Simple shear flow: definition of shear stress, strain, and shear rate; τ_{xx} , τ_{yy} , and τ_{zz} are the Cartesian coordinates and the direction of normal stresses in shear flow.

or

$$\tau = \eta \cdot D \quad (4.3)$$

where η is the constant of proportionality and is called the dynamic viscosity, and $dV/dz = D$ is called the velocity gradient, or shear rate. The two planes are separated by a distance ∂z , and the shear strain $\partial\theta = \partial x/\partial z$. Because this has taken place in time ∂t , the rate of change of shear strain is $\partial\theta/\partial t \cong dV/dz = D$, where dV is the velocity of the upper layer relative to the lower layer.

The term “non-Newtonian” is applied to all materials that do not obey the direct proportionality between shear stress and rate of shear. For a non-Newtonian fluid, the viscosity has no meaning, unless the shear rate is specified and the apparent viscosity is not constant. Apparent viscosity (η_{app}) can be used for easy comparison between Newtonian and non-Newtonian fluids at a particular shear rate. It is usually defined as the ratio of the shear stress over the rate of shear.

In general, there are three main types of non-Newtonian lipid liquids and semi-solids:

- *Time-independent*, for which the rate of shear depends only on the shear stress
- *Time-dependent*, for which the relationship between the rate of shear and shear stress depends on the time of shear
- *Viscoelastic*, which has characteristics of both elastic solids and viscous liquids

During food engineering operations, many lipid fluids deviate from laminar flow when subjected to high shear rates. In laminar lipid flow (i.e., in O/W or W/O food structure systems), break-up will take place when the Weber number, which describes the balance between viscous and interfacial forces, exceeds a critical value. The resulting turbulent flow gives rise to an apparent increase in viscosity as the shear rate increases in laminar flow; that is, shear stress = viscosity $\times$ shear rate. In turbulent flow, it would appear that: total shear stress = (laminar stress + turbulent stress) $\times$ shear rate. The most important part of turbulent stress is related to “eddies” diffusivity of momentum. In turbulent flow conditions, droplets can be formed that are no bigger than the radius of the smallest eddies. However, when viscous forces are strong enough to overcome interfacial forces, break-up can also occur to droplets. And under certain conditions in lipid-based food systems, the droplets are smaller than the Kolmogoroff radius. This can be recognized as the atomic-scale mechanism of energy conversion and its redistribution to the dynamics of mass transport, which are responsible for the spatial and temporal evolution of lipids in lipid-based food systems.

In many cases, it is useful to have a simple mathematical model of the shear stress–shear rate relationship. Equation (4.3) defines Newtonian fluids. For non-Newtonian fluids, with data over a wide range of shear, a good fit gives the Ellis model (Middleman, 1975):

$$\tau = \eta_0 \cdot D/1 + (\tau/\tau_{1/2})^{(1 - n/n)} \quad (4.4)$$

where η_0 is called the zero-shear viscosity, $\tau_{1/2}$ is a material constant that represents the shear stress at which the viscosity has fallen to half its zero-shear value, and n is the power law index. For many lipid-based foods, the power law as given in Equation (4.5) is also often used (Holdsworth, 1971),

$$\tau = K \cdot D^n \quad (4.5)$$

where K is called the consistency index. There are other popular equations used for lipid-based foods that expose the yield stress, including (Casson, 1959; Szczesniak, 1963, 1983):

$$\tau^{1/2} = \tau_0^{1/2} + K_1 \cdot D^{1/2} \quad (4.6)$$

and (Charm, 1960):

$$\tau = \tau_0 + K \cdot D^n \quad (4.7)$$

When put into Equation (4.7), the value for $n = 1$ and $K = \eta_0$, and the Bingham plastic flow can be mathematically described.

4.3 PRINCIPLES OF MEASUREMENT OF RHEOLOGICAL PROPERTIES IN LIPID-BASED FOOD SYSTEMS

Through the principles and methods of measurement, specific information regarding the rheological properties of lipids can be obtained, including:

- Important information regarding lipid processing behavior
- Lipid fluid dynamics calculations, including the development of processing theory
- Computer simulations based on lipid characterization, on continuum mechanics approaches, and on molecular theories.

Over the years, many different types of instruments have been developed for measuring certain aspects of the rheological properties of food systems. Most focus on the economic importance of engineering design, based on an accurate and complete understanding of rheological phenomena. The central problem in rheology, as well as in the measurement properties of lipid-based food systems, can be stated as follows: given the deformation undergone by a material, what are the internal stresses experienced by components of the lipid fractions? On the other hand, it is also equally important to know how the lipid-based food system deforms under any given forces acting on the material.

A knowledge of measurement principles of the rheological properties of lipid-based food systems is of utmost importance in determining the nature and design of processing methods and equipment. These measurement principles are primarily concerned with forces and deformation, while time and temperature are other important variables. During measurement, when a force acts externally on a body, several cases must be distinguished. For example, bending involves tension and compression, torque involves shear, and hydrostatic compression involves all three. In all other cases, one of these factors, or a combination thereof, can be observed. In addition, the inertia or weight of a measured food system, as well as moving components of measuring devices, may constitute a force leading to deformation.

There are general measurement principles. When the dimensions of a food system change, we speak of *deformation*. This parameter can be measured as linear deformation, for example, in a tensile test when a body of original length L is subjected to a tensile stress. The linear deformation ΔL can be expressed as strain $\epsilon = \Delta L/L$ and calculated as a ratio or percent. In hydrostatic testing, the deformation is measured by a volumetric strain as the ratio $\Delta V/V$. In many lipid systems, either liquid-like or solid-like, the deformation resulting from an applied force can be very large. In such cases, one is dealing with the rate of deformation during the measurement, that is, the time derivative of the deformation:

$$D = d\epsilon/dt \quad (4.8)$$

where deformation D is equal to the velocity difference divided by the distance between moving elements of the measuring devices (e.g., plates, cones, cylinders), and these remain motionless. The unit of rate of shear is the reciprocal second, sec^{-1} . According to the Newtonian suggestion as given in Equation (4.3), the proportionality constant η is the viscosity of the liquid, the units of which are $\text{dynes} \cdot \text{sec} \cdot \text{cm}^{-2}$, or poise. Some viscometers measure the kinematic viscosity, which is expressed as the ratio of η to the density ρ , and $\eta/\rho = \nu$ is given in units of stokes.

For Newtonian lipid-based food systems, it is sufficient to measure the ratio of shearing stress to the rate of shear, from which the viscosity can be calculated. Such a simple shear flow forms the basis for many rheological measurement techniques. The rheological properties resulting from steady shear flow for variety of food systems have been studied by many laboratories (Charm, 1960; Holdsworth, 1971; Middleman, 1975; Elson, 1977; Harris, 1977; Birkett, 1983; Princen, 1983; Shoemaker and Figoni, 1984; Hermansson, 1994; Kokini et al., 1994, 1995; Morrison, 1994; Pinthus and Saguy, 1994; and Meissner, 1997).

All measurements can be performed in a rheometer in which the experimental geometry for achieving steady shear flow can be one of various types, including capillary flow (Figure 4.3), cylindrical Couette flow (Figure 4.4), torsional flow (Figure 4.5), and cone and plate torsion flow (Figure 4.6). These several configurations, as examples of simple shear flow, can be found in most commercial rheometers, and the principles of measurements are based on the flow geometries discussed herein. For example, the principle of capillary rheometry is that, in capillary flow, shear stresses and shear rates are calculated from measured volumetric rates and pressure drops, which reveals the true shear stress at the capillary wall. Despite two important limitations (i.e., entrance effects and wall effects), capillary rheometry has important advantages. Specifically, capillary rheometers are commercially available; only this method allows one to conduct experiments with high shear rates, and any onset of flow instabilities can easily be observed (Meissner, 1997). In all of these geometries for measurement of the rheological properties of lipid-based foods, there are some end and flow effects. Such limitations should be kept in mind during measurement due to finite geometrical restrictions and the point of departure from the assumed laminar steady flow. The quantitative use of these flows requires precise

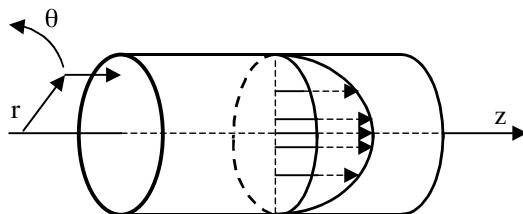


FIGURE 4.3 Geometry of capillary shear flow. (Adapted from Middleman, S. (1975). *Advances in polymer science and engineering: applications to food rheology*, in *Theory, Determination and Control of Physical Properties of Food Materials*, Rha, C., Ed., Reidel D. Publishing Company, Dordrecht, Holland.)

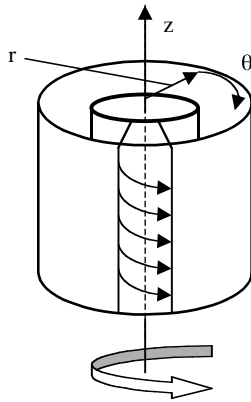


FIGURE 4.4 Geometry of cylindrical shear flow. (Adapted from Middleman, S. (1975). *Advances in polymer science and engineering: applications to food rheology*, in *Theory, Determination and Control of Physical Properties of Food Materials*, Rha, C., Ed., Reidel D. Publishing Company, Dordrecht, Holland.)

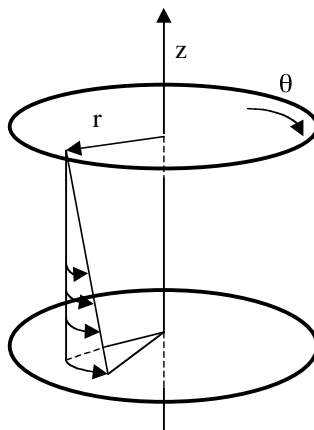


FIGURE 4.5 Geometry of torsional shear flow. (Adapted from Middleman, S. (1975). *Advances in polymer science and engineering: applications to food rheology*, in *Theory, Determination and Control of Physical Properties of Food Materials*, Rha, C., Ed., Reidel D. Publishing Company, Dordrecht, Holland.)

knowledge of their limitations. For example, in the cone and plate configuration, if the liquid is Newtonian, centrifugal effects are a major limitation in the use of this measurement technique.

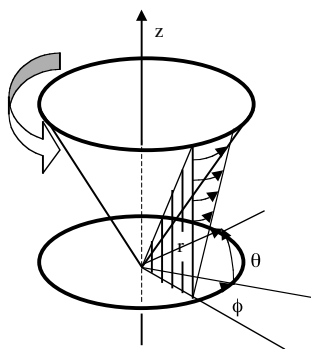


FIGURE 4.6 Geometry of cone and plate shear flow. (Adapted from Middleman, S. (1975). *Advances in polymer science and engineering: applications to food rheology*, in *Theory, Determination and Control of Physical Properties of Food Materials*, Rha, C., Ed., Reidel D. Publishing Company, Dordrecht, Holland.)

In many cases when the structure (e.g., W/O or O/W) of lipid-based food systems is fabricated, the break-up of droplets strongly depends on the type of flow in the processing equipment. Primarily, there is shear flow and elongation flow. Both are laminar flows, but elongational flow is the secondary flow. This is a type of flow that, except at the finite boundaries, is completely free of shear. The measurement of the value of elongation flow is very important in the manipulation of the macroscopic properties of emulsions, such as the effect of many variations in drop size. It is particularly valuable in the use of homogenizers with shear flow in the colloid mill or with elongational flow achieved when the lipid emulsion is forced to flow through a constricted valve. When measuring the elongational flow, the elongational viscosity as a relevant material property to such a flow can be described (Figure 4.7). Elongational viscosity is defined as the ratio of the stress to strain rate; at constant strain rate, the axial stress is associated with this elongational deformation. It can be assessed by the following equations:

$$L/l \cdot dl/dt = D' = \text{constant} \quad (4.9)$$

$$\eta_e = \tau_z/D' \quad (4.10)$$

where D' is the strain rate of elongational flow, τ_z is the axial stress, and η_e is the elongational viscosity. Elongational flow is always more effective than shear flow in the manipulation of lipid structures through processing, particularly when the viscosity of the dispersed phase is higher than that of the continuous phase. It is known from theory, and also confirmed by experiments, that Newtonian fluids exhibit an elongational viscosity that is three times the shear viscosity. Measurement of the elongational viscosity is very difficult. The point is that elongational viscosity is not an elastic parameter of a material, but can be exhibited by inelastic Newtonian materials. A supposition has been made that the manner in which elongational viscosity depends on strain rate may be strongly affected by the elastic nature of the measured structural properties of the food (Middleman, 1975).

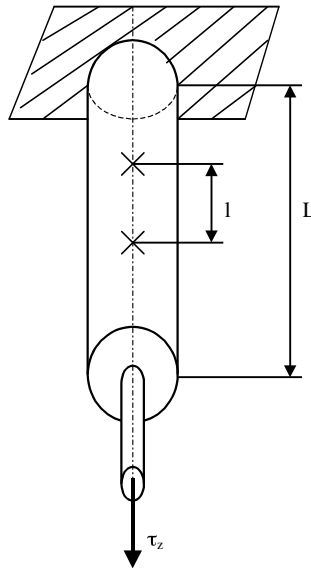


FIGURE 4.7 Elongational viscosity measurement principles.

The measurement of rheological properties for non-Newtonian, lipid-based food systems, such as dilatant, pseudoplastic, and plastic, as depicted in [Figure 4.1](#), are much more difficult. There are several measurement methods that may involve the ratio of shear stress and rate of shear, and also the relationship of stress to time under constant strain (i.e., relaxation) and the relationship of strain to time under constant stress (i.e., creep). In relaxation measurements, a material, by principle, is subjected to a sudden deformation, which is held constant; and in many food systems structure, the stress will decay with time. The point at which the stress has decayed to some percentage of the original value is called the relaxation time. When the strain is removed at time t_0 , the stress returns to zero ([Figure 4.8](#)). In creep experi-

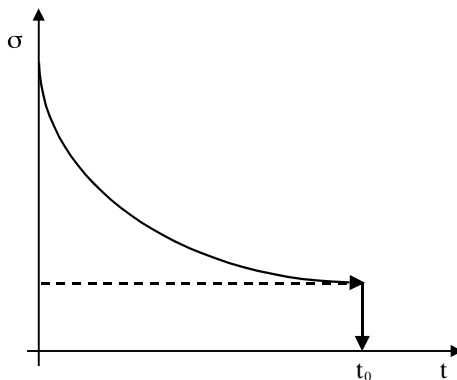


FIGURE 4.8 Relaxation curve stress-time under constant strain.

ments, a material, by principle, is subjected to a constant load or stress, and the strain is measured as a function of time. At time zero, the applied load results in a strain ϵ_0 that increases with time. When the load is removed at time t_0 , the strain immediately decreases, as indicated by the vertical straight part (a) of the curve at t_0 ; the strain continues to decrease thereafter with time, and a permanent deformation ϵ_p results (Figure 4.9).

A uniaxial stress is usually designated by the symbol σ , and a shearing stress by τ . For certain lipids that behave like solid food systems, the relationship between stress and strain is represented by a straight line through the origin, up to the so-called limit of elasticity. The proportionality factor E for uniaxial stress is called Young's modulus, or the modulus of elasticity. For a shear stress, the modulus is called Coulomb modulus, or the tensile modulus G .

There are some lipid food systems that, during measurement, exhibit so-called retarded elasticity. Such deformation is a function of time as well as stress (Figure 4.10). In this case, there is no permanent deformation. The area (A) in Figure 4.10 results from the upward part of the curve with increasing values of stress, and the downward part of the curve when the stress is reduced; the corresponding strains are greater. When the stress reaches zero, the strain has a finite value (ϵ_1), which from this point will slowly return to zero. There are also lipids that present the combined properties of elastic and viscous parameters, and the former may be partially retarded elasticity. Under the influence of a small stress, viscoelastic lipids may flow slowly and nonreversibly. Under larger stresses, the elastic part becomes apparent.

One of the most important rheological properties of lipids is their plastic behavior. A plastic material is defined as one that does not undergo a permanent deformation until a certain yield-stress has been exceeded. The example of an ideal stress-strain curve is depicted in Figure 4.11. Under influence of a small stress from zero to τ_0 , no deformation occurs. At the point τ_0 , stress increases, and the flow will appear from zero to ϵ_1 with stress τ_0 constant. The area (A) in Figure 4.11 is the value of total

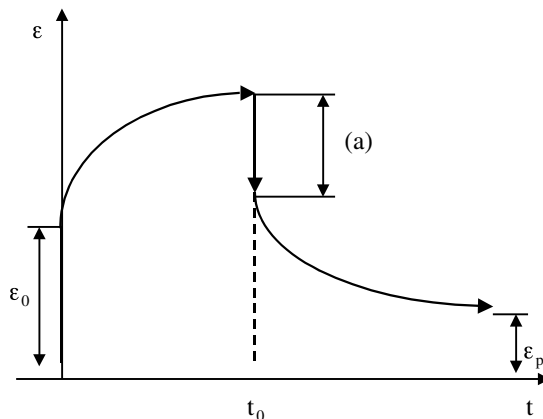


FIGURE 4.9 Creep curve strain-time under constant stress.

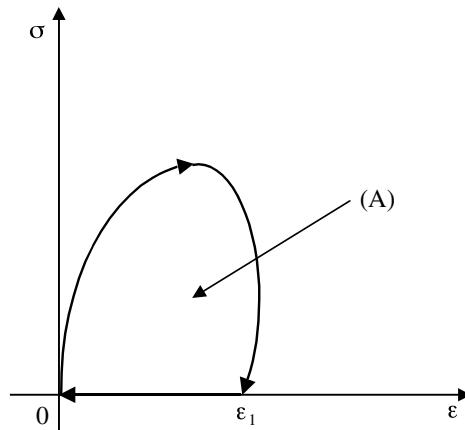


FIGURE 4.10 Stress-strain curve of a retarded elastic food system.

material deformation. In practice, there are no perfectly plastic lipid systems. Instead, there are plasto-elastic and plasto-viscoelastic lipid-based food systems.

For a variety of reasons, it is necessary to measure the thixotropic behavior of lipids. Thixotropy is defined as an isothermal, reversible food structure transformation, and is a feature common to many food systems (Figure 4.12). The measurement of thixotropy results in a lowering of the apparent viscosity; when the experiment is ongoing, with sufficient time, the apparent viscosity will return to its original value.

Rheological measurement principles are also concerned with the methods used for small and large deformations. For large deformations, the test measurement is the one performed through the stress rate–strain rate relationships under tension, compression, and shear. Small deformation tests probe the viscoelastic parameters of lipid-based food systems. These parameters are usually derived from dynamic oscillatory testing, which is on the linear viscoelastic region of the food structure being tested. Two independent parameters can be obtained from the dynamic measurements: modulus G' and modulus G'' . The storage modulus (G') describes

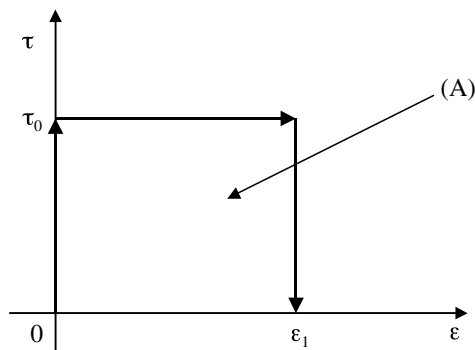


FIGURE 4.11 Stress-strain curve of an ideal plastic food system.

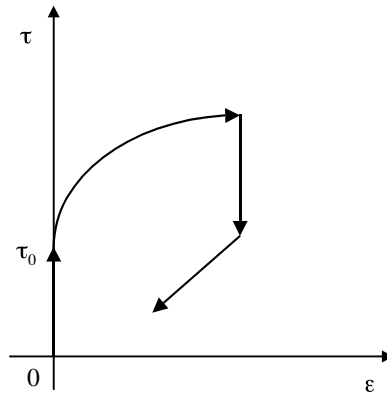


FIGURE 4.12 Shear stress-rate of diagram of a thixotropic food system.

the amount of energy stored elastically in the tested food structure, whereas the so-called loss modulus (G'') describes the amount of energy lost or the viscous response of the tested system. The relationship between modulus G' and modulus G'' is given by:

$$\tan \theta = G''/G' \quad (4.11)$$

where the phase angle θ is a measure of how much the stress and strain are out of phase with each other. The phase angle is equal to 0° for completely elastic material and 90° for a purely viscous liquid (Hermansson, 1994). There are some values of the phase angle θ between 0° and 90° for viscoelastic lipids that exhibit both viscous and elastic properties. These two material properties, (G') and (G''), can be defined as follows:

$$G' = \tau_0 \cdot \cos \theta / D_0 \quad (4.12)$$

$$G'' = \tau_0 \cdot \sin \theta / D_0 \quad (4.13)$$

where τ_0 and D_0 are the amplitudes of the stress and strain waves, respectively, under a periodic oscillatory strain or strain rate applied to the tested food structure system.

These examples of small and large deformations illustrate the complexity of the rheological parameters of lipid-based food systems. Small deformation testing can provide completely different information from large deformation testing. Therefore, information from more than one test is necessary if one wants to understand how the structure relates to the rheological behavior of food system components.

In many lipid-based food systems, in addition to performing rheological experiments to determine global structure, the measurement of surface rheological properties can play a crucial role in determining the quality of the final product. Therefore, the measurement of interfacial tension is sometimes a matter of utmost importance. It is particularly valuable for dispersed lipid food systems, primarily for W/O or

O/W emulsions. The measurement method in this case is different because such a system has an enormous surface area per unit volume, which can drastically change the physical properties of either phase. Within such phases, some non-equal interaction and equal interaction can be found. The force acting perpendicularly to the unit length along a surface is called the interfacial tension (σ_s), and the work required to bring molecules from the interior to the interface in order to expand the surface by a unit area is called the interfacial free energy. The interfacial tension net force results in contraction of the interface minimization of the interfacial area, and hence spherical droplets. Subsequently, these resulting forces act toward the interior of the droplet, causing the droplet to contract and increasing the internal pressure (Figure 4.13). The value of that internal pressure is described by Young–Laplace equation (Wilde, 1996):

$$\Delta P = P'' - P' = 2 \sigma_s / r \quad (4.14)$$

where P' and P'' are the pressures of the air or oil phase, and water phase, respectively. The surface or interfacial active molecules are usually amphipathic, that is, they contain distinct hydrophobic and hydrophilic regions (Figure 4.14). The measurement of interfacial shear rheology is important for lipid food products, for instance, as far as spreadability or wetting parameters are concerned. Among the many measurement principles, the following can be distinguished: the Wilhelmy Plate and Du

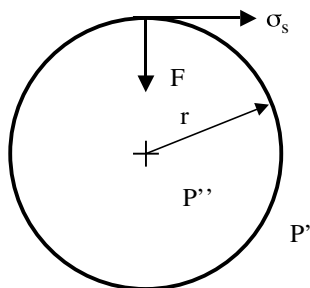


FIGURE 4.13 Interfacial tension and resulting net force (F). (Modified from Wilde, P. 1996. *Tensiometry, Lectures at the Tempus Course*, JEP 09917-96.)

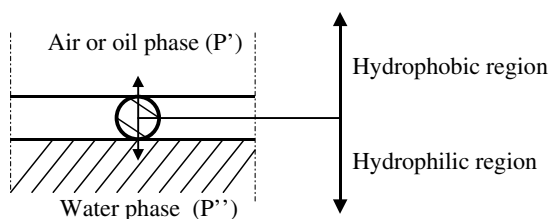


FIGURE 4.14 Surface and interfacial active molecules and their amphipathic regions.

Noüy Ring methods, pendant and spinning drop methods, for surface and interfacial dilational elasticity, thin-film techniques, and surface lateral diffusion when using fluorescence recovery after photobleaching (FRAP) methods.

The measurement principles and methods presented indicate how different responses to small and large deformations can be achieved. It is, therefore, not sufficient to rely on just one type of rheological test when evaluating the behavior of lipid-based food systems. It would be worthwhile to develop new measurement techniques where rheological experiments allow for and make it possible to measure volume changes during structural spatial arrangements in complex food systems.

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5 Phospholipids

Jan Pokorný

CONTENTS

- 5.1 Introduction
- 5.2 Chemical Structures of Phospholipids, Glycolipids, and Gangliosides
 - 5.2.1 The Chemical Structure of Phospholipids
 - 5.2.2 The Chemical Structure of Glycolipids
 - 5.2.3 Gangliosides
- 5.3 The Composition of Natural Phospholipids
 - 5.3.1 Occurrence of Primary Phospholipid Classes in Total Phospholipids
 - 5.3.2 The Fatty Acid Composition of Phospholipids
- 5.4 Occurrence of Phospholipids in Foods
 - 5.4.1 Phospholipid Content in Foods
 - 5.4.2 The Function of Phospholipids in Original Food Materials
- 5.5 Properties and Applications of Phospholipids
 - 5.5.1 The Manufacture of Phospholipid Concentrates
 - 5.5.2 Modifications of Technical Phospholipid Concentrates
 - 5.5.3 Applications of Phospholipids in the Food Industry
- 5.6 The Role of Phospholipids in Food Quality
- 5.7 Changes in Phospholipids during Food Processing
- References

5.1 INTRODUCTION

In addition to the rather nonpolar triacylglycerols (TAG; [Chapter 2](#)), several classes of relatively polar lipids are also present in food materials. The most important are the phospholipids, containing a phosphate or (rarely) a phosphonate group. Another class includes the glycolipids, which contain bound sugar. A large, physiologically important group contains sialic acid. Lipids containing sulfur are less frequent. Phenolic acids are bound in another group of polar lipids. Lipids containing sugars, sialic acid, or sulfates may also contain a phospho group. As their chemical structures are rather complicated, they are usually called by their trivial or semisystematic names, and very rarely by their systematic names.

If possible, the IUPAC/IUB terminology is used in this chapter.

5.2 CHEMICAL STRUCTURES OF PHOSPHOLIPIDS, GLYCOLIPIDS, AND GANGLIOSIDES

5.2.1 THE CHEMICAL STRUCTURE OF PHOSPHOLIPIDS

Major phospholipids are derivatives of glycerol, where the 1- and 2-positions are acylated by fatty acids while the 3-position is esterified with phosphoric acid. This complex group is called phosphatidyl (Figure 5.1.1). If only 1-position is acylated and the 2-position remains free, a lysophosphatidyl (Figure 5.1.2) is formed. The phosphate group can remain free (phosphatidic acid, Figure 5.1.3) or it can be substituted with an alcoholic derivative, most often an amino alcohol derivative, such as choline (phosphatidylcholine, Figure 5.1.4), serine (phosphatidylserine, Figure 5.1.5), and ethanolamine (phosphatidylethanolamine, Figure 5.1.6). Phosphatidylethanolamine can be acylated at the amino group with the formation of an amide, such as 1,2-diacyl-*sn*-glycero-3-phospho-(*N*-acyl)-1'-ethanolamine in oats (Holmbäck et al., 2001). In food materials, they often exist as inner salts (e.g., phosphatidylcholine, Figure 5.1.7).

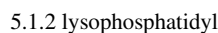
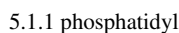
Other glycerophospholipids contain no nitrogen. The most common alcohol bound to the phospho group in these glycerophospholipids is *myo*inositol (phosphatidylinositol, Figure 5.1.8). The inositol moiety is often phosphorylated on the inositol residue so that phosphatidylinositol phosphates or diphosphates are formed. Because they are acidic, they usually exist in the form of salts. In another class of phospholipids of this group, the phospho group is substituted with glycerol (phosphatidylglycerol, Figure 5.1.9) or with 1,2-diacylglycerol (1,1'-*bis*phosphatidic acid). They occur both in microorganisms, in foods of animal origin such as fish or even mammals (Schmid et al., 1990), and in plant food materials; for example, 1,2-diacyl-*sn*-glycero-3-phospho-(3'-acyl)-1'-*sn*-glycerol (Figure 5.1.10) was identified in oat lipids (Holmbäck et al., 2001). Two phosphatidyl groups can be bound to the 1- and 3-positions of a single glycerol molecule, forming cardiolipin (Figure 5.1.11). All the previous derivatives can also occur in the lysophosphatidyl form.

Plasmenolipids are closely related to glycerophospholipids; however, the 1-position is not esterified with a fatty acid, but forms an ether with 1-alken-1-ol (Figure 5.2.1). Plasmenic acid (Figure 5.2.2) and plasmenylcholine (Figure 5.2.3) are examples of this group.

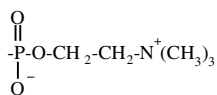
Instead of a phospho group, there may be a diphospho group, such as in 1,2-diacyl-*sn*-glycero-3-diphosphocytidine.

Sphingophospholipids are a different group of phospholipids, containing no bound glycerol, but a long-chain amino alcohol, which is derived from sphinganine (Figure 5.3.1). Its monounsaturated derivative is called sphingosine (Figure 5.3.2), and is present mainly in animal tissues. A hydroxy derivative of sphinganine is called phytosphingosine (Figure 5.3.3); it was detected chiefly in plant materials.

A fatty acid is bound in sphingophospholipids to the amine group, forming an amide. Such an amide is called ceramide (Figure 5.3.4). The 1-hydroxyl group of a sphinganine derivative can be phosphorylated to form a ceramide phosphate (Figure 5.3.5); the choline derivative of a ceramide phosphate is called sphingomyelin (Figure 5.3.6). Sphingolipids, ceramides, and sphingomyelins may also contain bound sugars (see Section 5.2.2).



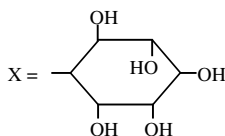
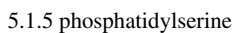
5.1.3 phosphatidic acid



5.1.7 inner salt of phosphatidylcholine

$$X = -CH_2-CH_2-N(CH_3)_3OH$$

5.1.4 phosphatidylcholine



5.1.8 phosphatidylinositol

$$X = -\text{CH}_2-\text{CH}_2-\text{NH}_2$$

5.1.6 phosphatidylethanolamine

$$X = -CH_2-CHOH-CH_2OH$$

5.1.9 phosphatidylglycerol

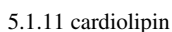
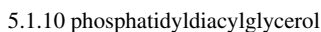
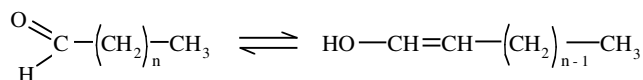


FIGURE 5.1 Chemical structures of glycerophospholipids.



5.2.1 1-alken-1-ol

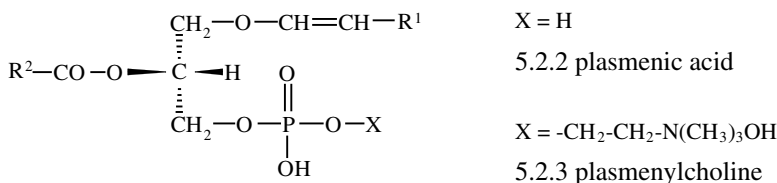


FIGURE 5.2 Chemical structures of plasmenyl derivatives.

5.2.2 THE CHEMICAL STRUCTURE OF GLYCOLIPIDS

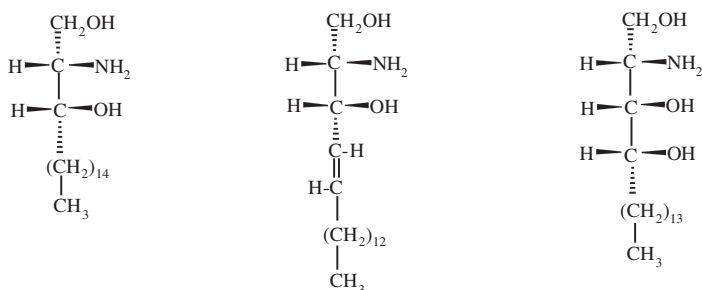
Glycolipids or, more correctly, glycosyllipids, contain both a lipid moiety and bound sugars, most often D-galactose, arabinose, or fucose. They often also contain a phospho group. A simple glycolipid is psychosin (Figure 5.4.1), which is a group of sphingosine glycosides; their more correct name would be 1-monoglycosylsphingolipids. Glycosides of ceramides are called cerebrosides (Figure 5.4.2). In this group of glycosphingolipids, several sugar units may be bound one to another, for example, as a tetragalactoside.

Glycoglycerolipids contain bound glycerol, such as in 1,2-diacyl-3-β-D-galactosyl-*sn*-glycerol (Figure 5.4.3) or the respective digalactoside, which were detected in plant materials. For example, monogalactosyl diacylglycerols and digalactosyl diacylglycerols were detected as major lipids in extracts from spinach leaves. Glycolipids may also contain bound phenolic acids, which are active as antioxidants.

5.2.3 GANGLIOSIDES

An important group of glycolipids are acidic glycosphingolipids, which contain either bound sialic acid or sulfuric acid. They were previously called mucolipids. The sugar moiety is sulfated in sulfoglycosphingolipids; for example, in glycosylceramide sulfates (Figure 5.5.1).

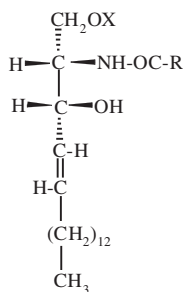
Glycolipids containing bound sialic acid residues (one or more) are called gangliosides. Sialic acid is a common name for *N*-acetyl (Figure 5.5.2) or *N*-glycoloyl (Figure 5.5.3) neuraminic acid (5-amino-3,5-dideoxy-D-glycero-D-galactonulosonic acid) bound as ceramides (Figure 5.5.4). Because of complicated chemical structures, they are most often expressed by a sequence of code, not using chemical structures.



5.3.1 sphinganine

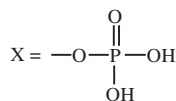
5.3.2 (2S,3S,4E)-2-amino-4-octadecene-1,3-diol, sphingosine

5.3.3 D-hydroxysphinganine, (2S,3S,4R)-2-amino-1,3,4-octadecanetriol

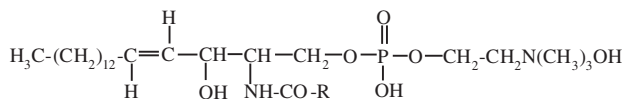


X = H

5.3.4 ceramide



5.3.5 ceramide phosphate



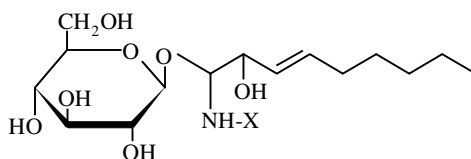
5.3.6 N-acyl-4-D-hydroxysphingene-1-phosphocholine, sphingomyelin

FIGURE 5.3 Chemical structures of sphingolipids.

5.3 THE COMPOSITION OF NATURAL PHOSPHOLIPIDS

5.3.1 OCCURRENCE OF PRIMARY PHOSPHOLIPID CLASSES IN TOTAL PHOSPHOLIPIDS

The composition of the phospholipid fraction in a lipid extract is rather variable, not only between two species, but also within the same species (Table 5.1). Phosphatidylcholine is the main phospholipid fraction in most phospholipid concentrates. In egg yolk, the phosphatidylcholine content is particularly high in comparison with



X = H

5.4.1 psychosins, 1-monoglycosylsphingolipids

X = -OC-R

5.4.2 glycosylceramides, cerebroside

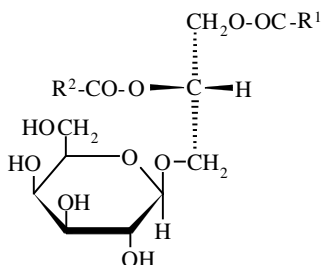
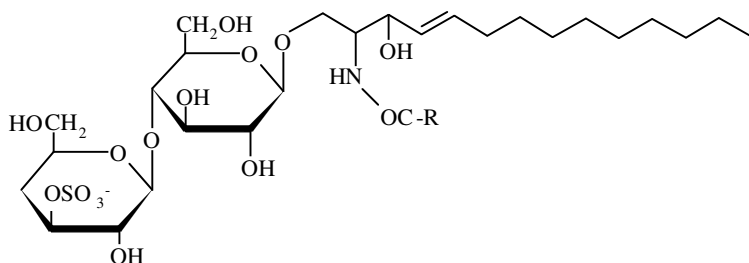


FIGURE 5.4 Chemical structures of cerebroside and glyceroglycolipids.

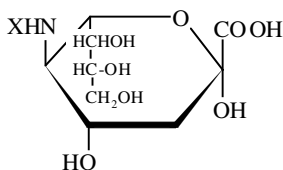
egg white, cereals, vegetables, and legumes. In spinach leaves, it is relatively high, but still about 30 to 40 times lower than in egg yolk. In potatoes, it is still several times lower. In the case of animal phospholipids, the differences are smaller but still noticeable; for example, the phosphatidylcholine content was 27% in total phospholipids in whole milk, about 16% in nonfat skim milk, about 20% in cream, and 26% in milk serum. In chloroform–methanol extracts, cream-bound lipids contained 90% phospholipids, while extracts from skim milk contained only 44%. Phosphatidylethanolamine is selectively extracted from lecithin by supercritical carbon dioxide and ethanol.

5.3.2 THE FATTY ACID COMPOSITION OF PHOSPHOLIPIDS

The fatty acid composition of phospholipids is not the same as that of TAG from the same material. The content of saturated fatty acids is usually higher, and the content of polyunsaturated fatty acids (PUFA) is often also high (Table 5.2), but differences exist even between phospholipid classes from the same material; for example, in pig adipose tissue, phosphatidylethanolamines contain less saturated acids and more PUFA than phosphatidylcholines (Body, 1988); the content of eicosatetraenoic acid is par-



5.5.1 sulpholipid



X = OC-CH₃

5.5.2 sialic acid

X = OC-CH₂OH

5.5.3 sialic acid

X = H

5.5.4 neuraminic acid

FIGURE 5.5 Acidic glycolipids (ganglioside).

TABLE 5.1

Distribution of Lipid Classes in Different Phospholipid Concentrates

Phospholipid Class	Egg Yolk	Milk	Liver	Muscle	Soybean	Rapeseed
Phosphatidylcholine	68–86	10–26	46–52	47–63	18–32	18–26
Phosphatidylethanolamine	8–24	25–45	24–28	18–24	6–17	14–31
Phosphatidylserine	tr-2	tr-6	2–5	2–5	tr-2	1–2
Phosphatidylinositol	tr	tr-4	6–9	4–9	17–24	6–14
Sphingomyelins	1–5	10–30	3–5	tr	tr	tr
Lysophospholipids	2–6	tr-1	tr-1	tr-1	tr	2–12
Phosphatidic acid	tr-1	tr-1	2–4	tr-1	tr-6	tr-5

Note: tr = traces

Data taken from various sources and the author's own unpublished results.

ticularly high in the phosphatidylethanolamine fraction, and fish phosphatidylethanolamines are also rich in docosahexaenoic acid (Bandarra et al., 2001).

TABLE 5.2
Fatty Acid Composition of Phospholipid Classes

Material	Phospholipid Class	Saturated Acids (%)	Monounsaturated Acids (%)	Polyunsaturated Acids (%)
Egg yolk	Phosphatidylcholine	53–54	27–28	18–19
	Phosphatidylethanolamine	49–50	19–20	30–31
Rapeseed	Phosphatidylcholine	8–12	60–66	22–30
	Phosphatidylethanolamine	11–12	51–56	32–38
	Phosphatidylinositol	23–29	42–48	26–32
Palm oil	Phospholipid	40	37	32
	Glycolipid	36	27	26
Spinach leaves	Phosphatidylcholine	20	11	69
	Phosphatidylethanolamine	47	2	51
	Phosphatidylinositol	42	6	52

Data compiled from Amate et al., 1999; Lange et al., 1994; Allen et al., 1964; Ghop et al., 1982; and other sources.

The composition of fatty acids in position-1 is also somewhat different from that in position-2 (Table 5.3); in the position-1, saturated acids prevail, while position-2 is primarily occupied by unsaturated fatty acids. The unsaturated fatty acid content bound in position-2 was 97.3%, 91.4%, and 95.9% in egg, cottonseed, and groundnut phospholipids, respectively (Vijayalakshmi and Rao, 1972).

5.4 OCCURRENCE OF PHOSPHOLIPIDS IN FOODS

5.4.1 PHOSPHOLIPID CONTENT IN FOODS

Phospholipid contents are very similar (about 1 to 2% dry matter) in microbial, plant, and animal tissues. If the content of neutral lipids is low, phospholipids may account for 20 to 40% of lipid extracts (e.g., in marine invertebrates). In egg yolk, 23% of the total lipids are phospholipids and other polar lipids (Kuksis, 1985). On the contrary, in adipose tissue or in oilseeds, the content of phospholipids is between 1 and 3% of total lipids. In oilseeds rich in oil (such as in rapeseed), it is lower than in oilseed with lower oil content (such as soybeans) when the results are expressed in % oil content, but much the same if the content is expressed in terms of total dry matter of the oilseed. Phospholipids are mainly extracted by nonpolar solvents, together with other lipids, and are obtained in the crude oil. However, in the original material, phospholipids are primarily bound to proteins (e.g., in membranes) or may be bound to other tissue components; for example, phospholipids interact with chlorophyll pigments, where they may form complexes between the magnesium ion of the chlorophyll molecule and the phospho group of the phospholipids.

If the material is extracted by nonpolar solvents, the yield of phospholipids is relatively low; but if the bonds between phospholipids and proteins are destroyed

TABLE 5.3**Differences in Composition of Major Fatty Acids (% of total fatty acids) in 1- and 2-Positions in Different Animal Phosphatidylcholines**

Fatty Acids in 1-position	Fatty Acids in 2-position	Egg Yolk Phosphatidylcholine	Milk Serum	Pig Liver	Chicken Muscle	Egg Yolk Phosphatidyl ethanolamine
16:0	18:1	38.2	21.2	15.8	30.2	11.5
18:0	18:1	9.3	5.3	7.8	1.1	10.0
18:1	18:1	3.0	14.8	0.6	1.0	2.4
16:0	18:2	21.8	5.1	12.8	20.0	6.2
18:0	18:2	11.2	2.3	20.8	2.0	12.0
18:1	18:2	1.5	3.8	—	2.0	1.8
16:0	20:4	2.5	1.3	4.1	20.0	5.3
18:0	20:4	3.4	—	12.1	4.0	28.5
16:0	22:6	1.8	—	—	1.5	6.3
18:0	22:6	1.6	—	—	—	4.3

Data from Kuksis, A. 1985. Animal lecithin, in *Lecithin*, Szuhaj, B.F. and List, G.R., Eds., AOCS Press, Champaign, IL, 105–162.

by heat denaturation of the protein moiety or if they are disrupted by the action of alcohols, the yield of phospholipids becomes much higher.

5.4.2 THE FUNCTION OF PHOSPHOLIPIDS IN ORIGINAL FOOD MATERIALS

In both animal and plant tissues, phospholipids are primarily bound in cellular membranes (see [Chapters 2](#) and [4](#)). Phospholipids are very important emulsifiers in living tissues, where they are bound in lipoproteins, and help to transport nonpolar lipids in blood and other intercellular fluids (see [Chapter 11](#)).

In foods, phospholipids are often transformed by processing so that they are often present at the interface of emulsions or cooperate in forming films on the surface of solid particles. The best emulsion stability is achieved when neutral phospholipids (such as phosphatidylcholine) are added to negatively charged lipids, which is sometimes difficult because the major lipidic fraction is neutral. In practice, it is suitable to select common phospholipid concentrates in which a certain amount of negatively charged phospholipid is present (Rydhag, 1979).

5.5 PROPERTIES AND APPLICATIONS OF PHOSPHOLIPIDS

5.5.1 THE MANUFACTURE OF PHOSPHOLIPID CONCENTRATES

The most important phospholipid concentrates are those resulting from oilseed processing. Oilseeds are heated to high temperatures with application of steam. Lipoproteins

and cell membranes are damaged in this operation, and phospholipids are liberated from complexes with proteins and other substances. They are then expeller pressed to obtain crude oil; however, during this operation, only small amounts of phospholipids are extracted into the crude oil. The next operation is the extraction of expeller cakes, which still contain between 5 and 10% oil, usually with hydrocarbon solvents. Pentane gives the lowest yields of phospholipids; larger yields are obtained with hexane (which is the most common solvent), but the highest yields are obtained with heptane (0.06%, 0.22%, and 0.31%, respectively, for pentane, hexane, and heptane) (Seher and Moon, 1978). High yields of phosphatidylethanolamine and phosphatidic acid were obtained with hexane, and high contents of phosphatidylcholine and phosphatidylinositol with chloroform and methanol (Seher and Moon, 1978).

Crude oils contain 1 to 3% phospholipids, which belong to minor lipids and other accompanying substances unsuitable for human consumption. Therefore, crude oils are processed to remove most of the undesirable substances. Phospholipids are removed in the first step, called degumming. They are treated with hot water, but unhydratable phospholipids, consisting mainly of salts, are not precipitated. Therefore, concentrated phosphoric acid or concentrated citric acid solutions are usually applied to reduce the phospholipid content remaining in degummed oil. Most phospholipids are hydrated so that they become insoluble in rather nonpolar neutral lipids. The phospholipid concentrates are then washed, water is removed by distillation, and a preparation containing between 50 and 70% phospholipids is obtained; this is called lecithin.

Most lecithins obtained during degumming of edible oils are not suitable for human consumption. Therefore, they are added back to the extracted oilseed meal and used as feed. Only soybean lecithin is suitable for human and industrial uses. Properties required for soybean lecithin are shown in [Table 5.4](#) (adapted after Procise, 1985). The phospholipid fraction of soybean lecithin contains about 30 to 32% phosphatidylcholine, 22 to 28% phosphatidylethanolamine, 18 to 20% phosphatidylinositol, 3 to 4% phosphatidylserine, and 20 to 29% other substances. The fatty acid composition of soybean lecithin is shown in [Table 5.5](#). The technical soybean lecithin obtained by extraction with a hydrocarbon solvent is very different from the phospholipid fraction obtained by extraction with chloroform–methanol (after Folch).

5.5.2 MODIFICATIONS OF TECHNICAL PHOSPHOLIPID CONCENTRATES

For many purposes, technical soybean lecithin must be modified (van Nieuwenhuyzen, 1981). The dark brown color is improved by bleaching with hydrogen peroxide. Lecithin treated in this way contains additional hydroxyl groups; therefore, it is more active as an emulsifier. Fluid lecithins are obtained by adding free fatty acids or by one of several other methods. Lecithins prepared from soybean oil refining contain about 30% neutral lipids. For some purposes, the material is fractionated with acetone because phospholipids are insoluble in acetone, while TAGs are soluble. They are then obtained in powder form, which should be protected against moisture. They can be fractionated with ethanol as well. Another method is hydrogenation under pressure.

TABLE 5.4
Specifications of Commercial Lecithin

Type of Lecithin	Plastic Lecithin	Fluid Lecithin	Food Grade
Acetone insolubles (%)	≥65	≥62	≥50
Moisture (%)	Max. 1	Max. 1	Max. 0.3
Benzene insolubles (%)	Max. 0.3	Max. 0.3	Max. 1.5
Acid value (mg/g)	Max. 30	Max. 32	Max. 36

TABLE 5.5
Fatty Acid Composition of Soybean Lecithin

Phospholipid Class	Hexane Extract (% Total Phospholipids)	Chloroform–Methanol Extract
Phosphatidylcholine	14.5	36.7
Phosphatidylethanolamine	20.1	13.3
Phosphatidylserine	3.2	2.9
Phosphatidylinositol	11.9	19.3
Phosphatidic acid	30.2	10.6

Phosphatidylcholines are the most important fraction of soybean lecithin. Their content may be increased by transesterification with choline hydrochloride, catalyzed by phospholipase D. Phosphatidylcholine content may thus be increased from 30% up to 60 or 70%, or from 75 or 80% to more than 90% (Juneja et al., 1989). Similarly, phosphatidylserine can be produced from phosphatidylcholine by enzyme-catalyzed inter-esterification (Yaqoob et al., 2001). Another modification of lecithins is the inter-esterification of lysophosphatidylcholine with fish PUFA under catalytic action of phospholipase A2 (Na et al., 1990).

5.5.3 APPLICATIONS OF PHOSPHOLIPIDS IN THE FOOD INDUSTRY

Phospholipids from animal sources, mainly egg yolk phospholipids, are very expensive; thus, they are used only for pharmaceutical purposes or for biochemical research.

Phospholipids from plant sources are obtained on the industrial scale almost exclusively as a by-product of crude oil refining (see [Section 5.4.2](#)). Naturally, the production of high-quality soy lecithin is much lower than needed; therefore, it is sometimes mixed with other raw materials. The most suitable lecithin substitute is produced by phosphorylation of a mixture of diacylglycerols and monoacylglycerols. It is still better to use diacylglycerols, obtained by removal of monoacylglycerols, and the neutralization of the phosphorylated product with ammonia (Ranný et al., 1976).

The applications of phospholipids in the food industry are manifold (van Nieuwenhuyzen, 1981). In the bakery industry, phospholipids improve the volume of bakery goods, improve the fat dispersion, and possess anti-staling properties. They

also improve the nutritional value (Schäfer, 1998). In the chocolate industry, lecithin increases the fluidity of the chocolate mass and prevents the crystallization of high-melting TAG. The addition of lecithin to margarines prevents spattering and promotes browning during frying. Large quantities of lecithin are used for the production of instant foods (Pardun, 1985). Particles of some foods, such as dried milk or dry soups, are covered with a thin film of fat, which prevents rapid contact with water. Phospholipids act as wetting and dispersion agents so that they enable fast dissolution in the aqueous phase or rapid dispersion. There are numerous other uses of lecithins for edible purposes.

Most lecithin is utilized for nonfood purposes, as emulsifiers, dispersion agents, adhesives, or lubricants. It is added to coatings, such as paints, waxes, and polishes. Its use in the cosmetic and pharmaceutical industries is also important (Wendel, 2001).

5.6 THE ROLE OF PHOSPHOLIPIDS IN FOOD QUALITY

The main positive functions of phospholipids in foods are their surface-active properties. They act as emulsifiers and stabilizers of emulsions (van Nieuwenhuyzen and Szuhaj, 1998), facilitate the dispersion of solid particles in the water phase, and improve the texture of multiphase food materials. Phospholipids make the texture smooth and improve the pleasantness by increasing the viscosity. The food tastes full and homogeneous. The bitter taste is also suppressed, but other tastes are not affected.

Phospholipids can increase the nutritional value of food (see [Chapter 11](#)) and can be applied to functional foods (Schneider, 2001).

Phospholipids increase the oxidative stability of fats and oils and fatty foods, in that they act as synergists of tocopherols (Khan and Shahidi, 2001) and other natural phenolic antioxidants, such as flavonoids. They stabilize even polyunsaturated edible oils (Kouřimská et al., 1994) and fish oils. Phosphatidylcholine reacts with peroxy radicals to yield trimethylammonium oxides. Phosphatidylamines react with lipid hydroperoxides in the non-radical way to form imines. Phospholipids can also bind heavy metals, which act as prooxidants, to produce inactive, undissociated salts.

Bulk phospholipids are relatively stable on storage (Rěblová et al., 1991), but are less stable when dissolved in oils. They slowly change during storage of lipid foods. In unheated food materials, phospholipases are active so that hydrolysis occurs, accompanied by formation of phosphatidic acids. In addition to hydrolytic reactions, oxidation reactions also occur. The oxidation of PUFA, detected in phospholipids, proceeds in a way similar to the oxidation of neutral lipids, only the peroxy free radicals or the hydroperoxides formed from them can react with amine groups in a non-radical way.

5.7 CHANGES IN PHOSPHOLIPIDS DURING FOOD PROCESSING

One process that is very important from the standpoint of industrial production of phospholipid concentrates is the removal of phospholipids from crude oils (see [Section 5.4.1](#)). Refined oils contain only traces of phospholipids.

During the storage of raw materials or of unheated foods, phospholipids become partially hydrolyzed. The reaction is catalyzed by different phospholipases. The hydrolyzed products affect food texture, mainly because of increased surface activity. Lysophospholipids are formed during cheese ripening.

Acidic phospholipids easily form salts with free amino acids, especially with free or bound basic amino acids (e.g., lysine) or with metal ions always present in foods (most easily with calcium and magnesium ions).

Glycerophospholipids contain PUFA in the position-2. They are oxidized by similar mechanisms as PUFA bound in TAG.

Amino groups of phospholipids, particularly of phosphatidylethanolamine or serine, react with reducing sugars, osones, and other products of sugar degradation to form brown melanoidins. The pathway via Amadori compounds is similar to the case of amino acids (Utzmann and Lederer, 2001). They thus contribute to the darkening of phospholipid concentrates on storage. Melanoidins are partially bleached by lipid hydroperoxides.

Changes during cooking, baking, or frying are similar to those of TAG; browning reactions are more intensive. Phosphatidylethanolamines are decomposed more easily during microwave heating than other phospholipids because of the presence of a primary amine group (Yoshida et al., 2001). Similar degradation was observed during microwave heating of pork meat. Phospholipids were decomposed during microwave heating of soybeans following the order: phosphatidylethanolamine > phosphatidylcholine > phosphatidylinositol (Takagi and Yoshida, 1999).

Because phospholipids enhance the decomposition of lipid hydroperoxides, they stimulate the formation of flavor-active volatiles in meat and meat products (Mottram, 1999).

Among other interaction reactions, binding of phospholipids to phytin or other phospho derivatives via calcium or magnesium salts is very important. Amine groups of phospholipids may also form complexes with polyphenolic compounds and their oxidation products, particularly with quinones.

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6 Cholesterol and Phytosterols

Erwin Wąsowicz

CONTENTS

- 6.1 Introduction
- 6.2 Cholesterol
 - 6.2.1 Structure and Occurrence
 - 6.2.2 Methods of Reducing Cholesterol in Foods
 - 6.2.3 Cholesterol and Its Relationship to Atherosclerosis
 - 6.2.4 Oxidation of Cholesterol
 - 6.2.5 Biological Effects of Oxysterols
- 6.3 Phytosterols
 - 6.3.1 Structure and Occurrence
 - 6.3.2 Phytosterols and the Stability of Frying Oils
 - 6.3.3 Phytosterol Esters: Lowering Blood Cholesterol
 - 6.3.4 Oxidized Phytosterols in Foods

References

6.1 INTRODUCTION

Sterols are minor constituents present in the fat of the human diet. They comprise the major portion of the unsaponifiable fraction of most edible fats and oils. Sterols can be classified according to their origin as animal sterols or as plant sterols. The latter can be subdivided into phytosterols (higher plant sterols) and mycosterols (lower plant sterols present in the lipid fraction of yeast and fungi). Cholesterol is the main animal sterol, while sitosterol, campesterol, and stigmasterol are the major plant sterols. These phytosterols and cholesterol are all 4-desmethylsterols that share identical ring structures. The various sterols differ only in their side chains. Surprisingly, these minor differences result in major changes in their biological functions. They are widely distributed in nature, and occur both in the free form or combined, most frequently as esters of higher aliphatic acids and glycosides. Sterols, sterol esters, and sterol glycosides are, to different degrees, soluble in fats but completely insoluble in water.

Since the discovery of cholesterol, sterols have continued to be the focus of the research activities of many chemists, biochemists, and clinicians, as attested to by the

fact that 13 Nobel Prizes awarded between 1910 and 1985 were associated with work on sterols. The continued interest of food scientists in dietary cholesterol is connected to its relation to atherosclerosis. The current “hot” topic is phytosterol esters in the lowering of blood cholesterol. Another functional property of phytosterols is their antipolymerization effect on frying fats. Cholesterol oxidation has been of great interest due to its implication in heart-related diseases. Specifically, cholesterol oxidation products have been shown to have potential as cytotoxic and mutagenic compounds. Less data is available on phytosterol oxides and their biological activity.

6.2 CHOLESTEROL

6.2.1 STRUCTURE AND OCCURRENCE

Cholesterol, with a C_{27} carbon skeleton ([Figure 6.1](#)), is a sterol characteristic for higher animals. It is a steroid that is present in all animal tissues as a major structural component of cellular membranes. It is the precursor of bile acids, provitamin B, and the steroid hormones. Cholesterol can be present in the free form or esterified at the hydroxyl group with fatty acids of various chain length and saturation. Cholesterol also occurs in plants, usually in very small quantities, and marine algae.

The relationship between dietary cholesterol and total serum cholesterol has been extensively investigated, along with the suggestion that dietary cholesterol contributes a risk factor in the development of coronary heart disease (Khachadurian, 1990). Therefore, a lower intake of high-cholesterol foods has been suggested as an effective method for lowering serum cholesterol levels. The content of cholesterol in some foods is illustrated in [Table 6.1](#).

6.2.2 METHODS OF REDUCING CHOLESTEROL IN FOODS

Various physical, chemical, and biological methods have been proposed for reducing cholesterol in foods. These include blending with vegetable oils, extraction with

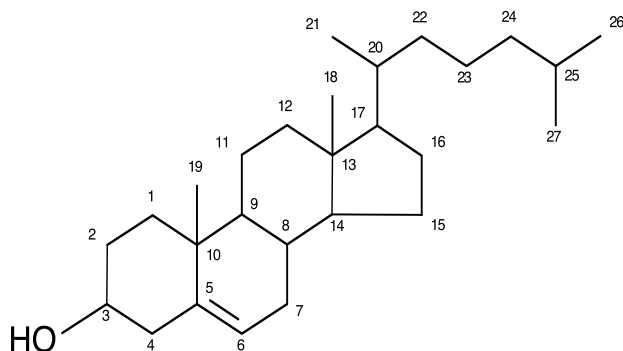


FIGURE 6.1 Structure of cholesterol showing the carbon atom numbering.

TABLE 6.1**Cholesterol and Phytosterol Content in Selected Food Products**

Product	Cholesterol (mg/100g)	Product	Phytosterols (mg/100g)
Skim milk	1.8	Refined oils:	
Whole milk	13.6	- Corn	768
Curd cheeses	5–37	- Olive	185
Process and hard cheeses	51–99	- Soybean	419
Cream and sweet cream	35–106	- Rapeseed	725
Butter	183–248	- Sunflower	349
Pork	72–100	Flax oil	311
Lard	92	Rice bran oil	1190
Beef	65–82	Peanuts	714
Tallow	109	Sesame seeds	221
Polish sausage (different types)	27–83	Almonds	158
Chicken, whole	75	Pecans	143
Turkey, light meat	60	Kidney beans	161
Liver	300–360	Soybeans, mature	108
Raw whole egg	450	Wheat grain	120
Raw egg yolk	1260	Corn kernels	30
Tuna	38	Bananas	31
Cod	73	Carrots	12
Lobster	95	Tomatoes	7
Shrimp	152	Potatoes	5

Adapted from Moreau, R.A. et al. (1999); Kiswa, J. and Jusiewicz, M. (1998); Rudzińska, M. et al. (2001); Zaborowska, Z. et al. (1999); Wąsowicz, E. (1991).

organic solvent, adsorption with saponin to form cholesterol complexes, vacuum distillation, degradation by cholesterol oxidase, and removal by supercritical carbon dioxide. It has been shown that more than 90% of cholesterol in fractionated bleached lard is effectively removed by stirring with 4% β -cyclodextrin (Yen and Chen, 2000). The application of these methods on an industrial scale is not frequent because some methods have significant operations cost and tend to be relatively nonselective, removing flavor and nutritional components along with the cholesterol. On an industrial scale, frying fat, trade name “Appetize,” was introduced in the United States (Haumann, 1996). Appetize has neither the cholesterol nor the *trans* fatty acids with good fried flavor associated with frying in beef tallow. Appetize is obtained by blending cholesterol-stripped animal fats (80%) with vegetable oil.

Another possible way of lowering cholesterol in food is the application of special diets for feeding animals. Many experiments with varying feeding conditions have been performed. Precht (2001) has shown that the cholesterol content in milk fat can be lowered by 8 to 13% with special feeding conditions using rapeseed. An extraordinary lowering of up to 50% can be achieved by dry fractionation of milk fat (stearin “hard” fraction).

The egg yolk of chickens fed the biomass of the red algae *Porphyridicum* sp., which constitutes a unique combination of soluble sulfated polysaccharide and polyunsaturated fatty acids (arachidonic acid and eicosapentanoic acid), tended to have reduced cholesterol (by 10%) (Ginzberg et al., 2000).

6.2.3 CHOLESTEROL AND ITS RELATIONSHIP TO ATHEROSCLEROSIS

Atherosclerosis in humans can have severe clinical sequelae, including heart attack, stroke, and peripheral vascular disease. In developed countries, atherosclerosis is responsible for more morbidity and mortality than any other single degenerative disease. Research over the past few decades has led to a new consensus on the sequence of events that initiate atherosclerotic lesions. Many of these events, including the accumulation of cholesterol in macrophages (“foam cells”), are accelerated by low-density lipoprotein (LDL) that has undergone oxidative modification. The accumulation of lipoprotein cholesterol is clearly central to the initiation of the “fatty streak” — the first anatomically defined lesion in atherosclerosis.

The causal relationship between elevated serum cholesterol and atherosclerosis has been established beyond doubt by several lines of evidence (McMillan, 1995; Steinberg, 1995). Approximately 20% of adults between the ages of 20 and 74 years have serum total cholesterol levels in the “high risk” category; that is, total cholesterol greater than 240 mg/dL and LDL cholesterol greater than 160 mg/dL (Sempos et al., 1993).

The principal nutritional focus in atherosclerosis has been dietary fat and cholesterol. It was concluded that: (1) saturated fatty acids increase serum cholesterol and are its primary determinants; (2) polyunsaturated fatty acids lower serum cholesterol; and (3) monounsaturated fatty acids have no independent effect. Concerning cholesterol, it has been recommended to reduce dietary cholesterol intake to less than 300 mg/day.

6.2.4 OXIDATION OF CHOLESTEROL

The expression “cholesterol oxidation products” (COP) or “oxysterols” refers to a group of sterols similar in structure to cholesterol but containing an additional hydroxyl, ketone, or epoxide group, on the sterol nucleus or a hydroxyl group on the side chain of the molecule. Table 6.2 presents the names of most prominent COP formed in foods, plasma, and tissues.

Foods of animal origin are suspected to contain some amount of COP formed by autooxidation. Cholesterol autooxidation is a well-established free radical process that involves the same chemistry that occurs for the oxidation of unsaturated lipids. Cholesterol contains one double bond at the carbon-5 position; therefore, the weakest points in its structure are at the carbon-7 and carbon-4 positions. However, due to the possible influence of the hydroxyl group at carbon-3 and the tertiary carbon atom at carbon-5, the carbon-4 position is rarely attacked by molecular oxygen, and therefore the abstraction of an allylic hydrogen predominantly occurs at carbon-7 and gives rise to a series of A and B ring oxidation products. In the chain reaction,

TABLE 6.2**Nomenclature of Some Cholesterol Oxidation Products (COP)**

Systematic Name	Common Name	Abbreviated Name
Cholest-5-en-3 β ,7 α -diol	7 α -Hydroxycholesterol	7 α -HC
Cholest-5-en-3 β ,7 β -diol	7 β -Hydroxycholesterol	7 β -HC
5-Cholestane-3 β ,5 α ,6 β -triol	Cholestanetriol	CT
Cholest-5-en-3 β -ol-7-one	7-Ketocholesterol	7-kC
5,6 α -Epoxy-5 β -cholestan-3 β -ol	Cholesterol- α -epoxide	α -CE
5,6 β -Epoxy-5 β -cholestan-3 β -ol	Cholesterol- β -epoxide	β -CE
Cholest-5-en-3 β ,20 α -diol	20 α -Hydroxycholesterol	20-HC
Cholest-5-en-3 β ,22-diols	22-Hydroxycholesterol	22-HC
Cholest-5-en-3 β ,25-diols	25-Hydroxycholesterol	25-HC
Cholest-5-en-3 β ,26-diols	26-Hydroxycholesterol	26-HC

usually initiated by free radicals, epimeric hydroperoxides of cholesterol and cholesterol epoxides are formed. The presence of tertiary atoms at C-20 and C-25 in side chain adds to the center's sensitivity to oxidation, forming oxysterols (usually called side-chain oxysterols) (Figure 6.2) (Smith, 1981, 1996).

In animal and human tissues, COP can be formed either enzymatically or by nonenzymatic oxidation. Enzymatic oxidation of cholesterol occurs by a number of cytochrome P-450 enzymes of the liver. Most prominent, cholesterol-7 α -hydroxylase (EC1.14.13.17) and 26-hydroxylase (EC1.14.13.15) are implicated in bile acid biosynthesis. Notably, 24- and 25-hydroxylase activities were also found in mammalian liver. Outside the liver, 26-hydroxylase activity is detectable in a variety of cells, including fibroblasts, vascular endothelial cells, macrophages, and brain and kidney cells. Biosynthesis of C21-steroid hormones requires 20- and 22-hydroxylation of cholesterol, forming 20 α -HC, 22-HC, and cholest-5-ene-3 β ,20,22-triol. Further, the enzymatic origin of COP is also possible through the action of dehydrogenases and dioxygenases, but its significance for COP formation in humans is not well documented (Linseisen and Wolfram, 1998a).

Nonenzymatic formation of COP from cholesterol *in vivo* can be directly induced by the action of reactive oxygen species like $^1\text{O}_2$, $\cdot\text{O}_2^-$, ROO^* , RO^* , and HO^* (Smith, 1996). However, much more attention has been paid to another mechanism, one in which polyunsaturated fatty acyl esters of cholesterol are subject to oxidation, initially yielding cholesterol esters of fatty acid hydroperoxides with further reaction to 7-hydroperoxycholesterol acyl esters. The epimeric forms of 7-hydroperoxycholesterol are reduced to give 7 α -HC and 7 β -HC, or are dehydrated to give 7-kC. During cholesterol ester autooxidation, the Δ^5 double is also involved, forming α -CE and β -CE and further reacting to give CT. In addition, non-enzymatic side-chain oxidation of cholesterol occurs, yielding, for example, 25-HC.

A number of studies have attempted to determine the levels of oxysterols in various food products. Reviews of the occurrence of oxysterols have recently been published (Paniangvait et al., 1995; Karpiński et al., 1997; Grandgirard, 2000). The common oxysterols formed in food — 7 α HC, 7 β HC, α CE, β CE,

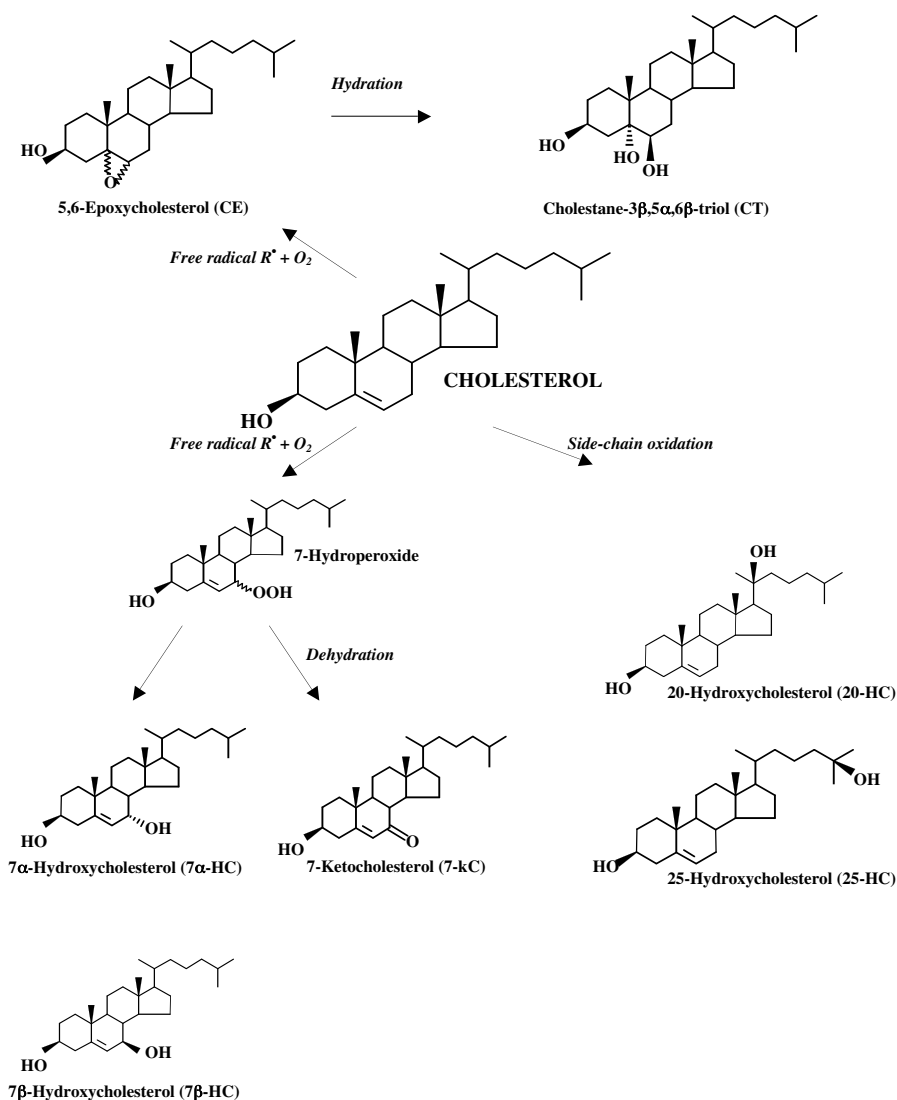


FIGURE 6.2 Cholesterol oxidation pathways.

CT, 7kC, 20HC, and 25HC — are almost the same as in plasma. The extent of cholesterol oxidation in food depends on the composition of the food itself as well the production technology used. It is known that the presence of oxygen, processing temperatures, heating time, and storage conditions affect oxide formation. For example, very high amounts of COP were formed in commercially dried whole egg powder up to 200 ppm (Pie et al., 1990), in powdered milk up to 30 ppm (Chan et al., 1993), and in fried pork loin up to 11 ppm (Echarte et al., 2001); while in fresh eggs, fresh milk, and fresh loin, only trace amounts of COP were detectable. In fried pork loin, 7-kC and 7 β -HC were the main COP.

Pangiangvait et al. (1995) have stated that in many studies the COP content in food reached 1% of total cholesterol and occasionally even more. The results from Zaborowska's laboratory indicate that COP contents in Polish sausage were between 4.4 and 36.5 ppm, and correlated with fat content ($R^2 = 0.394$, $\alpha = 0.05$) (Zaborowska et al., 1999). The content of oxysterol in fresh infant formulas ranged from 5.4 to 10.6 $\mu\text{g/g}$ lipid extract (Przygoński et al., 2000). The storage up to one year of model infant formulas in aluminum foil in a nitrogen atmosphere or vacuum at temperatures lower than 18°C does not increase the COP content. Oxidation was observed in the sample stored at 38°C in air, and there was a high correlation ($r = 0.9478$, $P = 0.05$) between the volatile aldehyde and COP content. (Przygoński et al., unpublished).

There are, as yet, no standard methods for determining oxysterols in food. As a consequence, considerable variations in cholesterol oxide levels in similar kinds of foods have been reported by various laboratories (Dutta et al., 1999). For quantification of oxysterols in samples, the use of an internal standard technique is recommended. For oxysterol separation, capillary columns with a nonpolar stationary phase are most often used. For confirmation of oxysterol structure, the use of gas chromatography/mass spectrometry (GC/MS) is highly recommended. During analytical procedures, serious attention must be paid to the possibility of artifacts of oxysterols from cholesterol. In 1992, Wasilchuk et al. developed a procedure for detecting artifactual generation of oxysterols using [$^2\text{H}_9$] cholesterol as an internal standard.

6.2.5 BIOLOGICAL EFFECTS OF OXYSTEROLS

Oxysterols have several *in vitro* and *in vivo* biological effects at the cellular level including cytotoxicity, atherogenesis, mutagenesis, carcinogenesis, changes in cellular membrane properties, and inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase. The review of Schroeffer (2000) includes a detailed and critical assessment of current knowledge regarding the formation, occurrence, metabolism, regulatory properties, and other activities of oxysterols in mammalian systems. A considerable amount of research has focused on the possible involvement of oxysterols in the pathogenesis of atherosclerosis. The oxidative changes in the modified LDL are associated with significant changes in the levels of oxysterols.

COP from foods are absorbed in the human intestinal tract but differences in the bioavailability of the single COP compounds are found. The rise in plasma total COP concentration begins 6 h after the meal, with a maximum at 8 h, and is statistically significant for $7\alpha\text{-HC}$, $7\beta\text{-HC}$, and 7-kC (Linseisen and Wolfram, 1998a). Several studies have provided direct evidence that a high dietary COP intake enhances atherogenesis in animals. Feeding rabbits a diet enriched in oxidized cholesterol resulted in a 100% increase in fatty streak lesions in the aorta (Staprans et al, 1998). However, it is actually impossible to say if food oxysterols or *in vivo* formed oxysterols are responsible for atherosclerosis. While waiting for more results in this field, the prudent path to follow involves the use of technological treatments allowing a decrease in the formation of these oxysterols in food.

6.3 PHYTOSTEROLS

6.3.1 STRUCTURE AND OCCURRENCE

Plant sterols are present in minor amounts in vegetable oils, nuts and seeds, cereals, and beans. They are classified into three groups:

1. 4-Desmethylsterols (cholestane series), that is, normal phytosterols
2. 4-Monomethylsterols (4 α -methylcholestane series)
3. 4,4'-Dimethylsterols (lanostane series, also known as triterpene alcohols)

In plants, more than 40 sterols have been identified, of which β -sitosterol, stigmasterol, and campesterol are the most abundant. These phytosterols and cholesterol are all 4-desmethylsterols that share identical ring structures and differ only in their side chain. The structures of these plant sterols are similar to that of cholesterol with an extra methyl or ethyl group and a double bond in the side chain (Figure 6.3). Campesterol contains only an additional methyl group at C₂₄; sitosterol is characterized by an ethyl group at C₂₄; and stigmasterol has an ethyl group at C₂₄ and an ethylenic bond at C₂₂. These compounds contain 28 and 29 carbon atoms, compared with the 27 carbon atoms found in cholesterol, which tend to make the compounds more hydrophobic than cholesterol. They occur in the free form, but also esterified to fatty acids, sugar moieties, or phenolic acids. These minor differences result in major changes in their biological functions.

Saturated plant sterols, referred to as stanols, occur in nature only in trace amounts. 4-Methylsterols are intermediates in sterol biosynthesis and are always present in fats and oils accompanying 4-desmethylsterols. The predominant 4-methylsterols are citrostadienol, obtusifoliol, gramisterol, and cycloeucalenol. 4,4'-Dim-

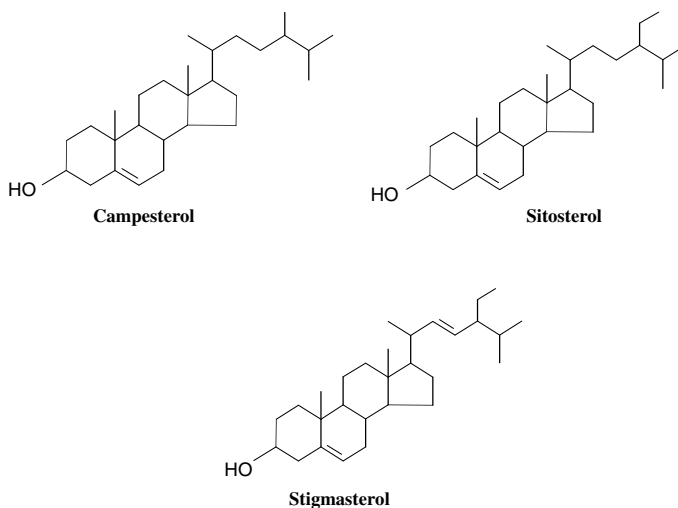


FIGURE 6.3 Common free phytosterols.

ethylsterols are present in vegetable oils in minor amounts (Blekas and Boskou, 1999; Dutta et al., 1997). Phytosterols are not synthesized by the human body, are poorly absorbed (4.2 to 12.5%), and are effectively excreted by the liver (Heineman et al., 1993; Ling and Jones, 1995). Therefore, serum levels are very low, usually below 1.0 mg/dL.

The sterol contents of some common and typical foods and vegetable oils are listed in Table 6.1. Fruits and vegetables typically contain only small amounts of phytosterols (less than 0.05%, wet basis). However, nuts and vegetable oils can contain 1% or more phytosterols. Corn and rice bran oils have the highest levels of sterols of the listed oils. Refining vegetable oils decreases the sterol content by 20 to 60%, and hydrogenation decreases it by an additional 20 to 40%. Dietary intake of phytosterols in northern Europe has been estimated to be between 200 and 300 mg/day (Morton et al., 1995), with intakes of about 500 mg/day by vegetarians (Ling and Jones, 1995). The consumption of stanols, derived primarily from corn, wheat, rye, and rice is much lower (about 25 mg/day).

The aleurone layers of corn, wheat, rye, and triticale have a high percentage of saturated phytosterols (i.e., sitostanol and campestanol), which are esterified to ferulic acid (Figure 6.3), and to *p*-coumaric acid to a much lesser extent in corn. Rice bran differs from most other cereals in having mostly 30- and 31-carbon sterols (triterpenoids) esterified to ferulic acid and small amounts of typical Δ^5 sterols (Moreau et al., 1996).

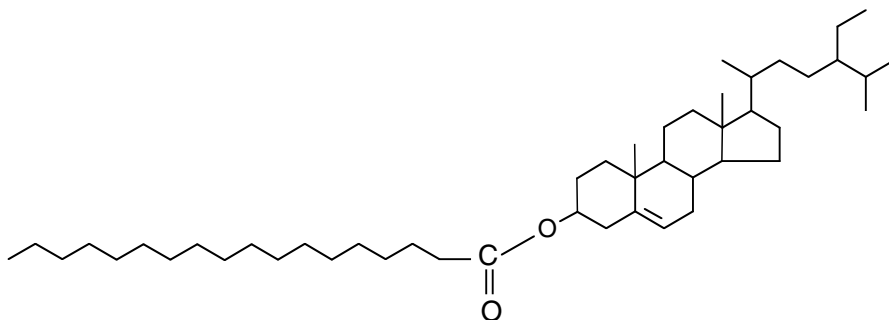
6.3.2 PHYTOSTEROLS AND THE STABILITY OF FRYING OILS

The effect of free and esterified sterols on the stability of heated oils has been often described and proven. 4-Desmethylsterols and 4-methylsterols with an ethylidene group in the side chain, as well as their esters, have been found to be effective in stabilizing oils at temperatures similar to those reached in deep-frying (Boskou, 1998). To these compounds belong Δ^5 -avenasterol and citrostadienol. Virgin olive oil during repeated frying operations was more stable in comparison to other vegetable oils. The content of Δ^5 -avenasterol in olive oil from certain areas in the Mediterranean region was up to 36%. The presence of sesame seeds and rice bran oil in frying oils provides additional stability, which is due to the high content of antipolymerization sterols (Δ^5 -avenasterol, Δ^7 -avenasterol, and citrostadienol). The content of Δ^5 -avenasterol in rice bran oil was approximately 168 mg/kg, Δ^7 -avenasterol — 360 mg/kg, and citrostadienol — 1680 mg/kg. Another group of compounds naturally occurring in rice bran oil is α -oryzanols. These are esters of ferulic acid with 4-desmethylsterols and 4,4'-dimethylsterols (Blekas and Boskou, 1999).

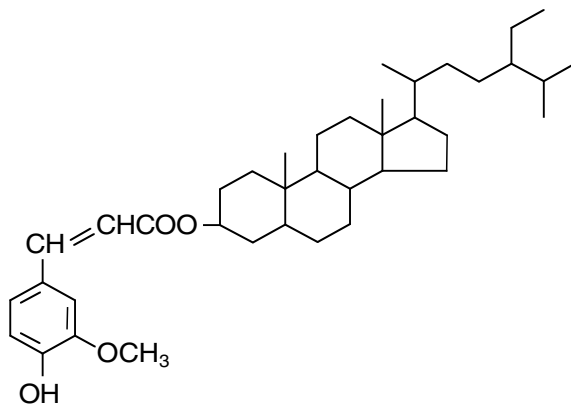
Gertz and Kochhar (2001) have shown that phytosterols from rapeseed and sunflower enhance the stability of refined oils at higher temperatures. The authors suggested that a radical peroxidation mechanism predominates at lower temperature, and acid-catalyzed chemical reactions occur predominantly at frying temperatures during actual frying operations. The probability of two different mechanisms may provide an answer to the different autooxidative activity of sterols. Sterols are almost inactive at temperatures lower than 120°C.

6.3.3 PHYTOSTEROL ESTERS: LOWERING BLOOD CHOLESTEROL

Phytosterols and phytostanols inhibit the uptake of dietary and endogenous cholesterol from the gut, causing a decrease in serum cholesterol levels (Ling and Jones, 1995). However, at least 3 g of free sitosterol a day are needed to lower LDL cholesterol content to a significant extent. Such amounts can never be provided by normal foods. The crystalline nature and poor solubility of free phytosterols limit their application in foods. The solubility of free sterols in oil is only 2%, but the solubility of sterol esters in oil exceeds 20%. Since discovering that phytostanol fatty acyl ester derivatives could be readily incorporated into fatty foods, phytosterol esters are being investigated for use as a novel food ingredient, primarily for use in margarine and spreads as a functional component with blood cholesterol-lowering properties (Miettinen et al., 1996) (Figure 6.4). Stanol esters are approximately 90% hydrolyzed after passage through the small intestine, yielding active free stanol.



β -Sitosterol stearate



Sitostanol ferulate

FIGURE 6.4 The basic structures of phytosterol and phytostanol esters.

They lower LDL cholesterol levels up to about 14% in adult humans. Levels of high-density lipoprotein (HDL) — cholesterol and triacylglycerols — were unaffected (Hicks and Moreau, 2001).

Stanols esters are often derived from “tall oil,” the phytosterol-rich by-products from the pulping of pine and other trees (Miettinen et al., 1996). In that process, tall oil phytosterols are refined and purified, chemically hydrogenated to phytostanols, and esterified with fatty acids. A pine wood-based stanol ester mixture is composed of sitostanol and campestenol in the ratio 92:8. It was recently shown that vegetable oil-based stanol ester mixtures with 68% sitostanol and 32% campestanol have similar LDL cholesterol-lowering effects in a non-hypercholesterolemic population (Plat and Mensink, 2000). It was also shown that esterified unsaturated sterols from soybean had serum cholesterol-lowering effects similar to the β -sitostanol ester (Weststrate and Meijer, 1998). The soy sterol ester and β -sitostanol ester inhibited cholesterol absorption equally, despite the different structures of the plant sterols (Normen et al., 2000). This finding is important because hydrogenation of plant sterols is a labor-intensive process that adds to the cost of manufacturing plant sterol mixtures for functional food use. In addition, hydrogenation of plant sterols for use in margarine leads to an increase of undesirable compounds (Jones and Ntanos, 1998).

Several recent studies indicate that the consumption of 2 g/day of sterols or stanols could result in a reduction in the risk of heart disease by about 25% (Law, 2000; Jones and Raeini-Sarjaz, 2001; Hicks and Moreau, 2001). Also, a lack of toxicity was demonstrated in studies with phytosterols and stanols, except for individuals with the extremely rare genetic condition known as sitosterolemia. Some concerns remain regarding the impact of phytosterol consumption on other lipid-soluble nutrients. Plant sterols and stanols lower blood concentrations of β -carotene by about 25%, the concentration of α -carotene by 10%, and the concentration of vitamin E by 8%.

The growing recognition of the health benefits associated with phytosterol esters leads to the marketing of new products. Several companies have marketed stanol and sterol ester products. In 1995/1996, Raisio Company from Finland introduced the margarine Benecol™ with 9% of sitostanol ester. Benecol was also launched in the United States in May 1999. In 1999, Unilever launched its product, Take Control™, which contained vegetable oil derived sterol esters. In addition to existing products on the market in 2000, Phytrol™, unesterified tall oil phytosterols, was announced (Hicks and Moreau, 2001).

A unique oil with the highest content of natural stanols and stanols ester was discovered in corn fiber (Moreau et al., 1996). This corn fiber oil, called Amazing Oil, was shown to lower cholesterol levels in animal models (Moreau, 1998). The levels of total phytosterols in corn fiber oil range from about 15 to 50%, depending on extraction and fiber pretreatment. Most of the phytosterols in corn fiber oil are naturally esterified with either fatty acids or phenolic acids, such as ferulic acid, a powerful antioxidant (Figure 6.4). Scientists have worked to move the oil toward use in commercial products (Hicks and Moreau, 2001).

6.3.4 OXIDIZED PHYTOSTEROLS IN FOODS

Owing to the structural similarities between cholesterol and phytosterols, the latter also form oxidation products similar to those formed from cholesterol (Dutta and

Appelqvist, 1997). The occurrence of oxyphytosterols in foods was much less studied than that of oxysterols. Different oxidation products of phytosterols — 7 α and 7 β hydroxy-sito- and campesterol, 7-ketosito- and 7-ketocampesterol, 5 α ,6 α -epoxy-sito- and campesterol, 5 β ,6 β -epoxy-sito- and campesterol, dihydroxysitosterol and dihydroxycampesterol — were identified and quantitated in plant oils, chips, and French fries (Dutta and Appelqvist, 1997; Dutta, 1997). A rapeseed oil/palm oil blend, sunflower oil, and high-oleic sunflower oil contained 41, 40, and 46 ppm total sterol oxides, respectively. After two days of frying operations, these levels increased to 60, 57, and 56 ppm, respectively. Total sterol oxides in lipids of French fries fried at 200°C in the rapeseed oil/palm oil blend, sunflower oil, and high-oleic sunflower oil were 32, 37, and 54 ppm, respectively. Potato chips fried in palm oil had the lowest level of sterol oxides (5 ppm) in the lipids. The level of total sterol oxides in chip samples fried in sunflower oil was 46 ppm, and the lipid content in samples fried in high-oleic sunflower oil was 35 ppm. During 25 weeks of storage, no considerable increase in sterol oxides was observed in the samples of chips fried in palm oil and sunflower oil.

Cold-pressed plant oils and refined plant oils from Polish markets were assessed for contents of sterol oxidation products. The content of oxyphytosterols in refined oils was 2 to 2.5 times higher than in cold-pressed oils. The 7-hydroxy-phytosterols and 7-keto-phytosterols epimers were the predominant compounds in cold-pressed oil, whereas epoxy derivatives were predominant compounds in refined oils. Percentage composition of oxyphytosterol fraction differed from the phytosterol percentage fraction (Figure 6.5) (Rudzińska et al., 2001).

7-Keto-sitosterol and 7-keto-cholesterol were selected as markers for the oxidation of phytosterols and cholesterol in infant milk formulas and infant milk cereals (Zunin et al., 1998). The data indicated no significant differences in the extent of oxidation of sitosterol and cholesterol. The content of 7-keto-sitosterol was found to be less than 5 ppm of extracted lipid.

There is lack of literature data concerning intestinal absorption of phytosterol oxides in humans. It is well-known that nonoxidized phytosterols are absorbed to a lesser extent by the human intestine than cholesterol. It is assumed that the reduced

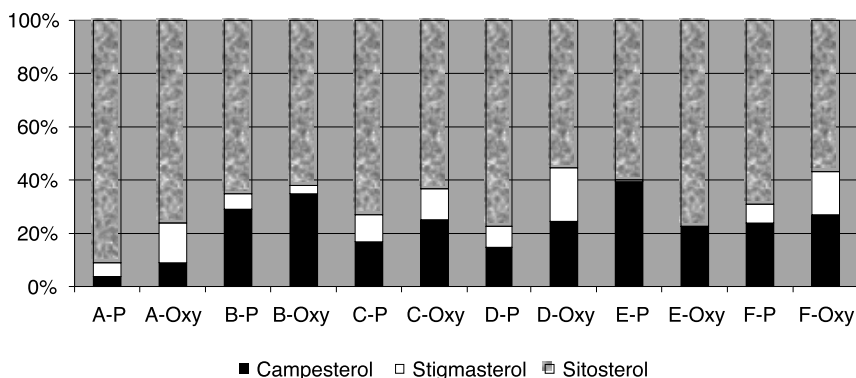


FIGURE 6.5 Phytosterol (P) and oxyphytosterol (Oxy) compositions in cold pressed plant oils (A—olive oil, B—flax oil, C—soybean oil, D—sunflower oil, E—rapeseed oil, F—corn oil).

absorption of phytosterols could lead to a low absorption of the oxyphytosterols. The lymphatic recovery of phytosterol oxides in adult male rats was low: 4.7% of the given dose for epoxy derivatives and 1.5% for 7-keto compounds. The campesterol oxides were better absorbed than the sitosterol oxides (Grandgirard et al., 1999).

The metabolic fate and physiological effects of phytosterol oxides in humans are not known. However, the cytotoxicity of oxides β -sitosterol/campesterol in a culture-derived macrophage cell line was shown (Adcox et al., 2001). The toxic effects of these oxides were similar to those of cholesterol oxides, although less severe. These results would suggest that foods high in phytosterols should be monitored for their potential contribution to dietary risk factors associated with the consumption of their oxidation products.

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7 Fat-Soluble Vitamins

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CONTENTS

- 7.1 Introduction
 - 7.2 Vitamin A and Carotenoids
 - 7.2.1 Structure
 - 7.2.2 Metabolism and Functions
 - 7.2.3 Deficiency and Excess
 - 7.2.4 Antioxidant Concurrency
 - 7.3 Vitamin D
 - 7.3.1 Structure
 - 7.3.2 Metabolism and Functions
 - 7.3.3 Deficiency and Excess
 - 7.4 Vitamin E
 - 7.4.1 Structure
 - 7.4.2 Metabolism and Functions
 - 7.4.3 Deficiency and Excess
 - 7.5 Vitamin K
 - 7.5.1 Structure
 - 7.5.2 Metabolism and Functions
 - 7.5.3 Deficiency and Excess
 - 7.6 Natural Sources of Fat-Soluble Vitamins and Food Processing
 - 7.6.1 Introduction
 - 7.6.2 Occurrence
 - 7.6.3 Food Processing
- References

7.1 INTRODUCTION

The first fat-soluble vitamin was discovered in 1909; it was vitamin A. Ten years later, vitamin D₃ was identified; and in 1922, Evans and Bishop discovered vitamin E. The last of the fat-soluble vitamins, vitamin K₁, was identified in the 1930s by Dam and Doisy, who achieved worldwide recognition of their studies on vitamins and received the Nobel Prize for these efforts.

Vitamins do not constitute any source of energy for the human body, nor are they considered to be structural material. But in cells and tissues, they have various

regulatory functions that determine the development, physical efficiency, and health status of humans.

The main characteristic shared by fat-soluble vitamins is the similarity in their occurrence in the human body and the effect exerted upon biological processes. Some vitamins can be generated in the human organism to a certain extent, but such a biosynthesis is usually not sufficient to cover the physiological demand. This includes vitamin A originating in the human body from β -carotene (provitamin A) and some other carotenoids as a result of bioconversion, to vitamin D₃ being synthesized in the skin under the influence of the UV rays, to vitamin K, which can be produced in the gut by intestinal microflora. On the other hand, vitamin E is totally exogenous for humans and animals. The fat-soluble vitamins (A, D, E, and K) differ from other vitamins by lacking nitrogen in their molecular structures.

These vitamins can accumulate in some human tissues, and thus it is not necessary to consume their required quantity every day. Their bioavailability depends on the presence of fat in foods, and on the extent to which they are released in the gastrointestinal tract and then absorbed and distributed to tissues and organs. The biological activity of vitamins is an accompanying notion, important from the point of view of human physiology. It determines the way in which a compound is being assimilated from food, and transformed into an active form of a vitamin, and prevents the signs of deficiency. Numerous intra- and extrasomatic factors affect the biological activity of vitamins, for example, their chemical structures, source and intake, as well as the efficiency of the mechanisms of intestinal absorption. In the case of vitamins A, D, E, and K, the presence of fat is indispensable, as is the appropriate level of biliary and pancreatic secretion and also the presence of appropriate enzymes (e.g., carboxylic esterase), bringing about decomposition of the vitamins' ester forms. The nutritional status of the organism and the related somatic reserves are of great significance. From a nutritional point of view, vitamins A and E are of particular importance because they are supplied solely by consumption of the appropriate foodstuffs. Their biological activities can be affected by metabolic disorders connected with various diseases and by the administration of drugs. Drugs can interact with vitamins and act synergistically or antagonistically, thus drastically affecting the vitamin level. It was calculated that a properly composed daily diet supplies sufficient quantities of fat-soluble vitamins. Considering the physiological possibilities of their storage in some organs, supplementation is not always necessary because their excess can lead to acute and chronic symptoms of toxicity, which was proved and described in case of vitamins A and D.

7.2 VITAMIN A AND CAROTENOIDS

7.2.1 STRUCTURE

A group of compounds described as vitamin A (retinol) includes those that, within their molecules, possess the characteristic β -ionone ring and the isoprene chain. They are capable of forming molecules of polyene alcohol, aldehyde, acid, and ester (retinal, retinoic acid, and retinyl palmitate, respectively) (Figure 7.1). They can occur in many isomeric forms as groups of compounds possessing diversified prop-

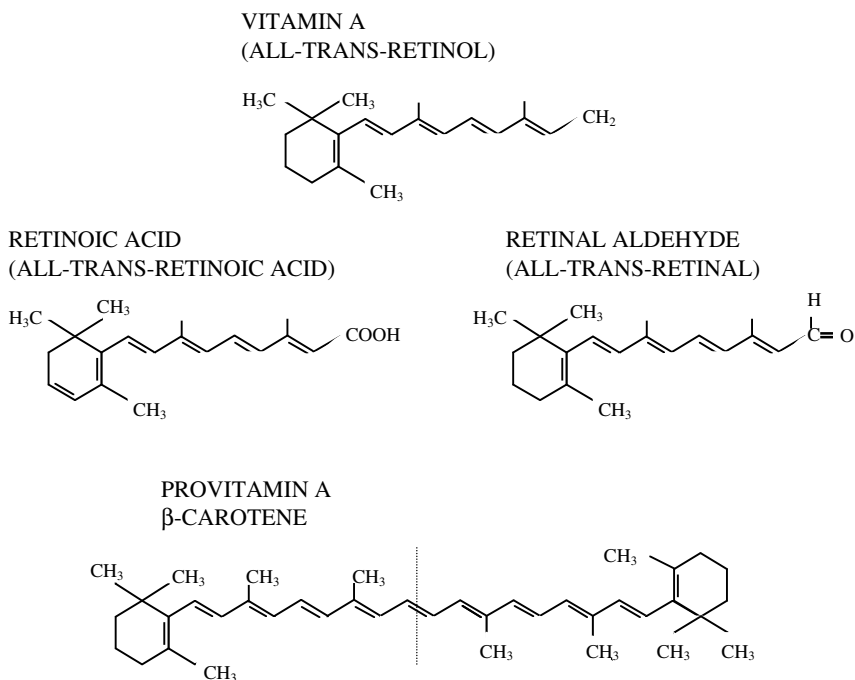


FIGURE 7.1 Vitamin A: chemical structures.

erties that are revealed by their various functions in metabolic and physiological processes. The vitamin A family includes several plant pigments identified in nature — carotenoids — described as provitamin A because they are converted to vitamin A in human and animal organisms. These compounds are composed of two β -ionone rings and most often they have α , β , and/or γ isomeric forms. The most valuable form is β -carotene, which displays the highest biological activity. It is composed of two β -ionone rings linked by the isoprene chain. Bioconversion consists of splitting the symmetric double bond between C_{15} - $C_{15'}$, causing simultaneous formation of two molecules of retinal which is then reduced to retinol. The α and γ isomers form only one molecule of retinal. The rate of conversion of β -carotene to vitamin A depends on vitamin C, zinc, and the level of thyroid hormones.

7.2.2 METABOLISM AND FUNCTIONS

Vitamin A in foods is present in its ester form and, after enzyme-catalyzed hydrolysis, it is absorbed into the gastrointestinal tract. After forming an emulsion in the presence of bile salts and pancreatic juices, it is incorporated into molecules of chylomicrons (Olson, 1996). Other forms of carotenoids are oxidatively converted to retinal in the intestinal mucosa. Then, via lymphatic vessels, it is transported from the bloodstream to the liver (Cortner et al., 1987). Vitamin A is primarily stored in the liver, and is also found in ester form in lipocytes. Transport from the liver depends on the level of retinol binding proteins (RBP) (Wolf and Phil, 1991). Vitamin A is well absorbed

from the alimentary tract — its absorption efficiency is assessed as 80%. Retinol levels should exceed 300 $\mu\text{g/L}$ blood plasma (Gerstner, 1997).

The oldest and best-defined function of vitamin A in humans is its participation in vision. As a light-sensitive factor, it is a component of rhodopsin. In the eye, light induces the decomposition of rhodopsin with simultaneous splitting off of 11-*cis*-retinal and opsin. During a series of conformational reactions in the dark, regeneration of visual purple takes place. Long-lasting vitamin A deficiency leads to disturbed functioning of the retina (i.e., night blindness, xerophthalmia); and at a later stage, keratomalacia and necrosis resulting in loss of sight. The second function of vitamin A is cell differentiation; its presence allows regulation of new cell generation processes, particularly those in epithelium, mucous membranes, and bone tissue. Vitamin A participates in the synthesis of the adrenocortical and thyroid hormones and also in the metabolism of the steroid hormones. Retinol controls ubiquinone formation in the liver and probably serves as a coenzyme in the synthesis of glycoproteins and mucopolysaccharides. Vitamin A also plays a role in normal growth regulations, especially in young organisms (Gerstner, 1997). It affects spermatogenesis, and it has been discovered — although not yet fully clarified — that retinol and retinoic acid are essential for embryonic development. It has been suggested that vitamin A plays a significant role in immune response and inhibition of neoplastic cells development (Johnson, 2001), as well as in appetite and taste regulation.

7.2.3 DEFICIENCY AND EXCESS

Vitamin A deficiency may manifest itself by decreased immunity to infections, growth inhibition, and reproduction disorders. Typical pathological signs of vitamin A avitaminosis include lesions of the mucous membranes of the gastrointestinal tract, air passages, and urinary system. Skin keratosis can be seen, especially around outlets of the hair sheaths (phrynoderma).

Vitamin A can be toxic when ingested in overly large doses. The most common sign of excessive vitamin A intake is the so-called dermal paralysis manifested by changes in skin pigmentation and dryness, accompanied by nail brittleness and hair loss. Vitamin A excess may be the reason for joint and bone pain as well as weight loss (Eckoff and Nau, 1990). Enlargement of the liver and spleen usually occurs (hepatosplenomegaly). Teratogenic activity has also been suggested, notably birth defects (Rothman et al., 1995; Hathcock, 1997). Symptoms of acute toxicity include headache, nausea and vomiting, convulsions, and light intolerance.

7.2.4 ANTIOXIDANT CONCURRENCY

Some vitamins also participate in antioxidant defense mechanisms (DiMascio et al., 1991). Vitamin A, carotenoids, and vitamins E and C, when supplied with food, protect against the generation of radicals, reactive oxygen species, and peroxides (Halliwell, 1996). A disturbance in the pro- and antioxidant equilibrium of the organism in favor of oxidation is the cause of many diseases. Dietary antioxidants — vitamin A and especially carotenoids — play an important role in relieving the oxidation damage that occurs in humans (Biesalski et al., 1997). Laboratory studies

on animals, as well as the clinical studies, show that a chronic deficiency of vitamins with antioxidative potential causes numerous biochemical changes and may result in the development of atherosclerosis and carcinogenic processes (Albanes et al., 1995; Omenn et al., 1996). Antioxidative vitamins protect the physiological functions of the arterial endothelium, inhibit thrombocyte aggregation, relieve infections, and reduce the progression of atherosclerotic changes. It is supposed that, at an early stage, they are capable of inhibiting the development of AIDS (Gerstner, 1997; Anstead, 1998).

7.3 VITAMIN D

7.3.1 STRUCTURE

Vitamin D is considered a hormone-like compound. Ergosterol is a vitamin D₂ precursor, a phytosterol that occurs mainly in plants. Ergocalciferol results from UV irradiation of ergosterol (Figure 7.2).

7-Dehydrocholesterol can be converted into the most active form — vitamin D₃ (cholecalciferol). Ergosterol and 7-dehydrocholesterol are clearly identified as provitamin D because, after irradiation of the skin with UV light or exposure to sunlight, they can be converted into the active vitamin D₂ and D₃ forms (Fraser, 1980).

The structures of compounds termed vitamin D are based on a system of three rings called phenanthrene; the fourth ring is cyclopentane. This general structure undergoes transformation by breaking one of the rings, and therefore there are only

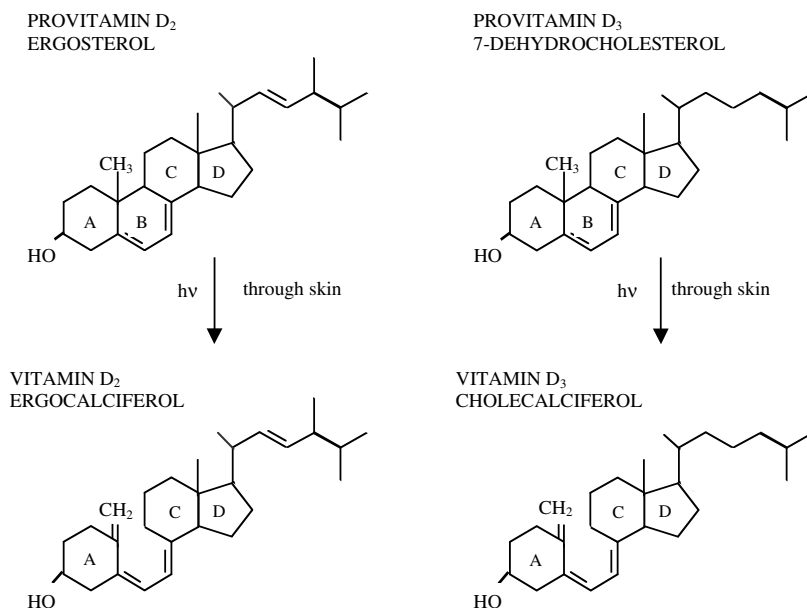


FIGURE 7.2 Vitamin D: chemical structures.

three aromatic rings in the molecules of vitamins D₂ and D₃ (Figure 7.2). Photoisomerization alters the properties of molecules, particularly the biological activity. This alteration depends on the chemical structure of the isomer being generated. Evidence has also suggested the possibility of transforming cholesterol, via enzymatic and chemical dehydrogenation, into the precursor of vitamin D₃. Vitamin D is endogenously produced in human and animal organisms, and also supplied with food (Collins and Norman, 1990).

7.3.2 METABOLISM AND FUNCTIONS

Vitamins synthesized in the skin (D₃) and those supplied with food undergo the same transformations, finally leading to the generation of their active metabolites. They are deposited in the liver, lungs, and kidneys as well as in fatty tissue. The amount of vitamin D in the organism depends on exposure to sunshine and diet, and it ranges from 65 to 165 IU/100 mL blood. All forms of this vitamin are being absorbed in the small intestine with bile salt, fatty acids, and acylglycerols present, and then delivered to the liver bound to a specific vitamin D-binding protein. From the liver the vitamin is transported via chylomicrons to the bloodstream. Molecules of vitamin D₂ and D₃ are activated in the liver by hydroxylation; the OH group appears at carbon-25 to give 25-hydroxy-D₃[25-(OH)D]. Further reaction occurs in the kidneys, bone, cartilage, and/or in the placenta under the influence of the specific D₃-1-hydroxylase. The next two stages produce the most potent and physiologically active molecule, 1,25-dihydroxy vitamin D₃ [1,25-(OH)₂D₃]. According to the most recent nomenclature, it is called calcitriol.

25-(OH)-D is activated under the influence of the specific D₃-24-hydroxylase at carbon-24 [24,25-(OH)₂D₃]. Both circulate in the bloodstream, and their main function as active metabolites is maintaining the homeostasis of calcium and phosphorus, which is vital for many body functions (Henry and Norman, 1984).

Active molecules of vitamin D work in conjunction with parathyroid hormone and calcitonin. The structure and mode of action of vitamin D metabolites resemble those of typical steroid hormones (Wardlaw and Insell, 1993), such as estrogen or testosterone, but their function in the endocrine system remains the subject of numerous studies and discussions (Bouillon et al., 1995). It was also discovered that vitamin D regulates specific gene expression following interaction with its intracellular receptor or target genes in order to stimulate or suppress specific transcriptions (DeLuca, 1986; DeLuca and Zierold, 1998). Vitamin D is involved in normal cell growth and maturation. Clinical studies have revealed that cell growth inhibition may protect the body against some kinds of neoplasms, for example, human leukemia and colon, prostate, and breast cancers (Gann et al., 1996; Boston, 1998).

The action of vitamin D in human and animal organisms is primarily related to calcium metabolism and the mineralization of bones and teeth, although the mechanism of these processes has not yet been unequivocally confirmed. The cooperation of vitamins A and K is necessary for the proper functioning of vitamin D. Vitamin D stimulates differentiation of bone-forming osteoblasts and inhibits the formation of bone-decomposing osteoclasts (Anderson and Toverud, 1994). It has been assumed that vitamin D affects the blood sugar level by appropriate

secretion of insulin by the pancreas (DeLuca and Zierold, 1998). Vitamin D is also thought to regulate the level of phosphorus (P) in the kidneys and facilitate the transition of P from the organic to inorganic form, the junction of the two being necessary for bone tissue generation (Arnaud and Sanchez, 1996). Vitamin D regulates calcium levels for normal nerve impulse transmission and muscle contraction, and also influences immune system regulation (white blood cells — monocytes and lymphocytes).

7.3.3 DEFICIENCY AND EXCESS

In human and animal organisms, vitamin D deficiency can result from its decreased synthesis in the skin or disturbances in its metabolism related to malabsorption in the alimentary tract (Devqun et al., 1981). Certain liver and kidney disorders may result in excessive excretion of vitamin D in the urine and feces, thus leading to vitamin D deficiency. If calcium and phosphorus homeostasis is disturbed, bone changes quickly follow (Suda et al., 1992). The main symptom of vitamin D deficiency in newborn children is rickets; in adults, it is osteomalacia. In children, the disease primarily affects the chest, skull bones, and legs. The development of baby teeth is also slowed. Changes in the bone tissue, becoming more intense, are also related to metabolic disturbances and deficiencies in dietary calcium and phosphorus, and may lead to osteoporosis (bones become lighter, less dense, and prone to fractures) (Nishii et al., 1993; Bikle, 1994). Clinical studies reveal that vitamin D deficiency plays a role in tuberculosis, stroke, high blood pressure, and inflammatory bowel disease. Low vitamin D levels could be a risk factor for arteriosclerosis, rheumatoid arthritis, or osteoarthritis (Thomas et al., 1998).

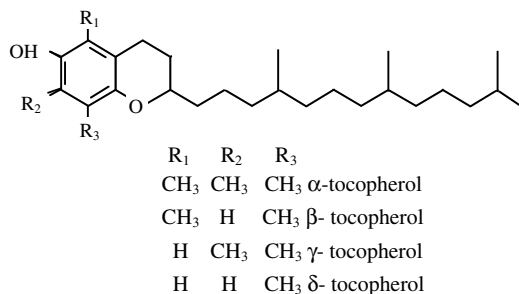
Ingestion of excessive quantities of vitamin D may be toxic, and can result in bone and/or pain, diffuse demineralization of bones, muscle fatigue, loss of appetite, thirst, sore eyes, itchy skin, vomiting, diarrhea, urinary urgency, and abnormal calcium levels in the urine (indicative of kidney stones). High doses of vitamin D cause a build-up of calcium in soft tissues and in organs such as the liver, lungs, heart, kidneys, and muscles (Watson et al., 1997).

7.4 VITAMIN E

7.4.1 STRUCTURE

Vitamin E represents a group of lipids with an isoprene structure and it is the only one possessing eight homologous forms. These are multimolecular phenolic compounds consisting of the ring system 2-methyl-6-chromanol with a hydroxyl group at C-6 and linked at C-2 to a 16-carbon saturated isoprenoid side chain derived from plant diterpenoid alcohol — phytol. Built in this way, a molecule forms tocol structures. If a carbon chain of the same length, but with three double bonds, is linked to the ring system, it generates a tocotrienol structure. Binding a $-\text{CH}_3$ group to the C-5, C-7, and/or C-8 positions of the tocol structure results in the formation of four homologous tocopherols (-T); that is, α -T, β -T, γ -T, and δ -T. If the $-\text{CH}_3$ group is substituted in the tocotrienol structure, then four corresponding tocotrienols

TOCOPHEROL



TOCOTRIENOL

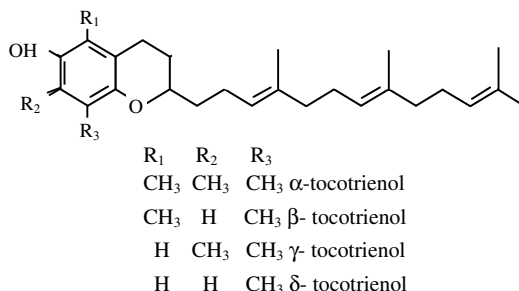


FIGURE 7.3 Vitamin E: chemical structures.

(-T-3) are formed: α-, β-, γ-, and δ-T-3 (Figure 7.3). All these compounds have been given the common name of vitamin E, and they occur as enantiomers (D) and (L); (D) α-T predominates in nature. They can occur in free or bound forms, most often as esters that, before absorption, are hydrolyzed by pancreatic esterases. In comparison to other homologues, α-tocopherol displays full biological activity. The biological activity of the remaining forms was determined: β-T possesses 40% of α-T activity, γ-T — 10%, and δ-T — only slight activity. The greatest activity among the tocotrienols was determined for α-T-3 (it was described as 30% of α-T activity) (Sokol, 1996).

7.4.2 METABOLISM AND FUNCTIONS

Vitamin E, like other lipids, is digested and absorbed only in the presence of bile. Bile functions as the lipid emulgent and makes absorption of lipids in the intestinal epithelium possible. Vitamin E can be absorbed 30 to 90% while its assimilability amounts to about 30%, the rest being excreted from the organism. In adults, the concentration of α-T in plasma should exceed 30 μmol/L (Biesalski, 1997). A diet rich in vitamin E allows its accumulation in the organism, for example, in the liver, pituitary gland, and adrenals, as well as in adipose and muscular tissues. Vitamin E

takes part in various metabolic processes in human and animal organisms. Its primary role is thought to be that of determining the proper functioning of reproductive organs. Research has shown that vitamin E actively participates in tissue respiration and also in the synthesis of some hormones. Vitamin E protects against macrocytic anemia in children and the occurrence of dermal changes. Similar to vitamin A, carotenoids, and vitamin C, it protects against neoplastic lesions (Johnson, 2001). It conditions proper structure and permeability of cell membranes. Being a natural antioxidant, vitamin E displays protective properties in relation to unsaturated fatty acids and β -carotene, reacting with radicals generated in metabolic processes and effectively quenching singlet oxygen and radicals, thus preventing its reaction with other polyenoic fatty acids present in the phospholipids of cell membranes (Diplock, 1991; Bramley et al., 2000).

Tocopherols are considered to be secondary antioxidants; alone, they can quickly react with peroxide radicals, thus inactivating them (Porter et al., 1995; Kamal-Eldin and Appelqvist, 1996). In this way, they influence the inhibition of radical chain reactions. The antioxidant activity is significantly affected by the hydrogen from the hydroxyl group and the related ability to form radicals of tocopherols, which are further capable of reacting among themselves to form dimers and trimers (Figure 7.4). At the end, radicals are being inactivated, and the generated molecules possess antioxidant properties less potent than the mother tocopherols (Niki et al., 1984). Free radical forms of tocopherols can be secondarily regenerated in systems where ascorbic acid and glutathione are present. Interestingly, the regeneration of the α -T radical takes place at the junction of the water and fat phases (Packer and Kagan, 1993).

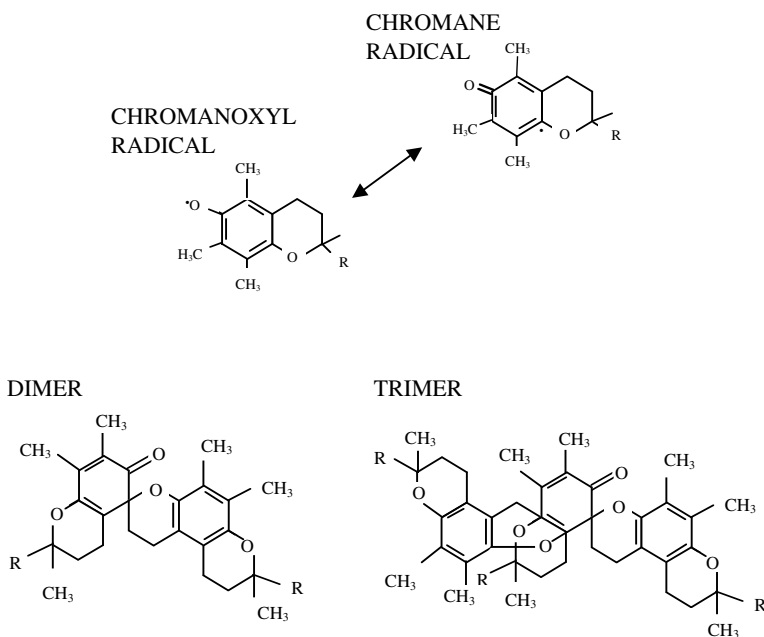


FIGURE 7.4 Tocopherol radicals, dimer, and trimer: chemical structures.

Studies on tocochromanols revealed significant α -T-3 activity in humans. This was explained in terms of their presence in low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL), which are the main means of transport of tocopherols to target cells (Pearce et al., 1992; Theriault et al., 1999). The phenyl side-chain of tocotrienol has been postulated to be responsible for differential membrane distribution and metabolism (Theriault et al., 1999). Studies conducted *in vitro* proved that the antioxidant activity of α -T-3 against lipid peroxides generated from residues of the polyenoic fatty acids is significantly higher than that of α -T (Serbinova et al., 1991). The ability to prevent cardiovascular disease, coronary heart disease, and cancer by arresting radical damage has been also suggested (Meydani, 1995; Hodis et al., 1995). Free radicals may play a role in the pathogenesis of neurological disorders, including Alzheimer's and Parkinson diseases. However, although there is no unequivocal agreement, some scientists believe that in an early stage, α -T can protect neurons from amyloid beta-protein toxicity (Behl et al., 1992, 1999; Fahn, 1992). Vitamin E is also indispensable for immune defense (Beharka, 1997). It has been suggested that tocopherols, acting as hormones or as a secondary transmitter of genetic information, control the expression of some genes.

7.4.3 DEFICIENCY AND EXCESS

An inappropriate diet that does not ensure a proper supply of vitamin E results in a vitamin deficiency manifested by characteristic symptoms. It is very difficult to determine when vitamin E deficiency occurs. In clinical practice, it is the concentration of vitamin E in blood plasma, in which the homologue α -T constitutes 80 to 90% of the total tocopherol content, that is most often determined (Hosomi et al., 1997). Low vitamin E levels usually accompany infections and affect the immune and nervous systems. An insufficient quantity of this vitamin accelerates the breakdown of red blood cells, makes red blood cells susceptible to hemolysis, and may also be the reason for decreased hemoglobin synthesis, which — particularly in infants and children — may result in anemia. The efficiency of the α -T activity depends on the equilibrium between its prooxidant and antioxidant effects. Vitamin E deficiency causes damage to cellular membranes resulting from oxidation of the unsaturated fatty acids in phospholipid tails. Greater permeability of cellular membranes brings about visible skin keratosis and aging. Vitamin E deficiency can also manifest itself as muscular pain and progressing muscular dystrophy. In cooperation with vitamin A, vitamin E can affect vision (Jacques, 1999). It was also demonstrated that with chronic oxidation stress present and the lack of so called co-antioxidants (i.e., the vitamin E-regenerating substances), this vitamin alone can bring about the prooxidant effect (Biesalski et al., 1997).

No signs of toxicity related to the vitamin E hypervitaminosis were observed. However, there are some symptoms linked with its excessive intake. Supplying an adult with a dose over 1000 mg α -T acetate over a long period of time can result in considerable headache, vision disorders, and muscular weakness (Bendich and Machlin, 1993). It was also demonstrated that large doses of α -T act antagonistically to vitamin K, thus causing a decrease in blood clotting capability (Corrigan, 1979, 1982; Elmadfa and Bosse, 1985).

7.5 VITAMIN K

7.5.1 STRUCTURE

Vitamin K comprises derivatives of 1,4-naphthoquinone. Naturally occurring forms are equipped with structures possessing the unsaturated isoprenoid side chain linked to naphthoquinone at carbon-3 (Figure 7.5). Vitamin K₁, phyloquinone (2 methyl-3 phytyl-1,4-naphthoquinone), is one of the natural forms. Similar to compounds belonging to the vitamin E family, those included in the vitamin K group have the isoprenoid side chain consisting of 20 carbon atoms with one double bond. This side chain is derived from plant diterpenoid alcohol (C₂₀H₃₉OH). These compounds have been detected in alfalfa oil.

Vitamin K₂ compounds have about 75% of the potency of K₁ and are synthesized by bacteria in the intestinal tract of humans and various animals. This vitamin can also be found in putrefied fish meat. Compounds of this family differ from phyloquinone in the number of isoprene units in the side chain and also in the degree of their unsaturation. Hence, the general term applied to them is menaquinones (Figure 7.5). Unlike vitamin K₁, they are not capable of optical light rotation; however, maximum activity was observed for the trans configuration of the side-chain double bonds.

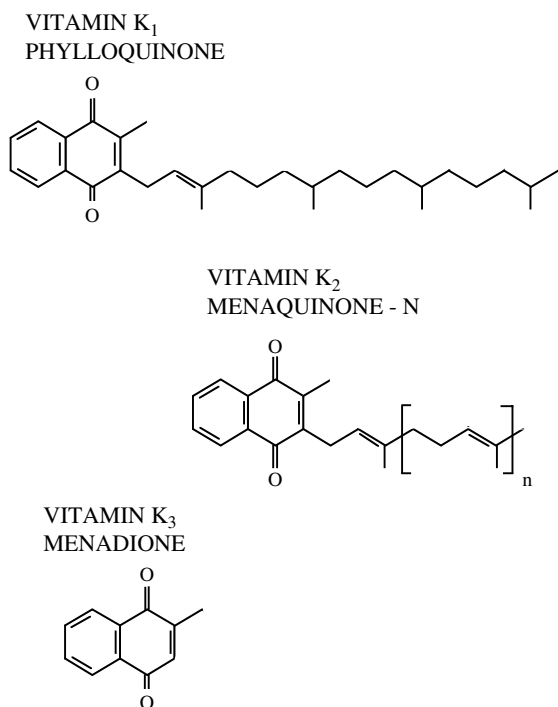


FIGURE 7.5 Vitamin K: chemical structures.

Vitamin K₃ — menadione — is the only form isolated from *Staphylococcus aureus* and also chemically synthesized. It is a synthetic compound that can be converted into K₂ in the gastrointestinal tract. Unlike other fat-soluble vitamins obtained by chemical synthesis, vitamin K₃ is characterized by its high biological activity, just like the naturally occurring ones.

7.5.2 METABOLISM AND FUNCTIONS

The rate of vitamin K absorption in humans depends on the kind of fats included in the diet. Long-chain unsaturated fatty acids facilitate absorption of vitamin K₁ in lymphatic vessels. The efficiency of this process is affected by the presence of bile salt and pancreatic juices, the form of vitamin K, as well as the site in the gastrointestinal tract. Vitamin K₁ is primarily absorbed in the jejunum and ileum; only small amounts are absorbed in the colon. From the lymphatic system, vitamin K is transported to the circulatory system and, in chylomicrons, to the liver from which it is distributed to target tissues.

Intestinal anaerobes such as *Escherichia coli* and *Bacillus fragilis* are capable of synthesizing vitamin K₂ (Holzapfel et al., 1998). The gastrointestinal bacterial flora of human and animal organisms appears to be responsible for a substantial supply of vitamins, especially vitamin K. It was demonstrated that about half of the total vitamin K present in humans is of dietary origin, and the remainder is synthesized by the intestinal bacteria of the large intestine. Conversion of vitamin K₁ to K₂ takes place via the so-called shikimic pathway; however, this refers only to healthy individuals. The greatest concentrations of the various forms of vitamin K were detected in liver. The presence of phyloquinone and menaquinones of varying chain lengths (up to 13 prenyl units, $n = 13$, can occur) confirms their diversified origin, while the heterogeneity of menaquinones depends on the kind of microorganisms capable of their synthesis (Shearer et al., 1974; Shearer, 1992). Several studies of the total vitamin K content in liver, adrenals, kidneys, lungs, and bones prove that the possibility of its absorption is but slight. The concentration of phyloquinone in plasma ranges from 0.3 to 2.6 nmol/l (Sadowski et al., 1989). In liver, the content of phyloquinone was determined as approximately 10% (2 to 20 μg) of the total vitamin K stored, and the remainder is primarily composed of menaquinone-7 and menaquinone-8 (Hodges et al., 1990; Usi et al., 1990; Uchida and Komeno, 1988).

Since its discovery, the antihemorrhagic action of vitamin K has been its best-known function. Vitamin K is located in hepatic microsomes where vitamin K-dependent carboxylation in prothrombin synthesis occur (Bell, 1978). The plasma clotting factors (II, VII, IX, and X) are produced by the liver in precursor forms and are converted to functional proteins by vitamin K-dependent reaction (Corrigan, 1982). They also depend on vitamin K for their synthesis and contain γ -carboxy-glutamic acid (Gla) residues (Stenflo and Suttie, 1977; Suttie, 1992) because Gla is part of a protein that controls calcium binding (Price, 1988; Knapen et al., 1989). Vitamin K participates in the process of carboxylation, which gives the proteins “claws” so they can hold calcium ions. Vitamin K participates in protein carboxylation during which proteins containing three residues of Gla are formed — osteocalcin. Osteocalcin is active in calcium binding, making it possible for both organic

and inorganic matter to unite in the bone tissue. This protein is probably synthesized in cells similar to osteoblasts, and its presence prevents osteoporosis. Many studies have confirmed the activity of vitamin K in inhibiting the generation of interleukin-6 (Feskanich et al., 1999). People with disordered connection between apolipoprotein E and vitamin K levels in the brain are particularly prone to Alzheimer's disease. Scientists have explained this in terms of vitamin K regulating the level of building calcium not only in bones, but also in the cardiovascular system and brain (Jie et al., 1993).

Vitamin K displays antibacterial and antimycotic properties, and it was demonstrated that it also has analgesic and anti-inflammatory activity. Studies of the influence of vitamin K on blood sugar levels are being continued because its presence in pancreas was observed; it has been suggested that it may affect the insulin secretion. Other investigations have shown that vitamin K can be more powerful than vitamin E and coenzyme Q₁₀ in scavenging radicals (Malorni et al., 1993).

7.5.3 DEFICIENCY AND EXCESS

In the elderly, when the absorption of vitamin K and the efficiency of its synthesis decrease, lowered prothrombin levels in blood as well as liver dysfunction may occur (Hodges et al., 1991). Blood clotting time is extended, resulting in hemorrhages from the nose, or urinary or gastrointestinal system. In newborn infants, one symptom of decreased vitamin K levels is the hemorrhagic syndrome (Lane and Hathaway, 1985). Substances such as dicoumarol and its derivatives decrease the utilization of vitamin K-dependent clotting factors because they are its antagonists. Several antibiotics, various intestinal illnesses, mineral oil, and irradiation can inhibit absorption of this vitamin. In large doses, vitamin E can enhance the anticoagulant action of vitamin K (Booth, 2000). Some physicians, by virtue of observations and clinical studies, believe that vitamin K can act like a hormone, but such a role for this vitamin is not clearly understood.

The toxicity associated with excessive amounts of vitamin K in humans has not been unequivocally defined, although it has been suggested that excessive doses of one of its forms, menadione, can contribute to the occurrence of hemolytic anemia, jaundice, kernicterus, and hyperbilirubinemia (Worthington-Roberts, 1988).

Vitamin K belongs to a group of compounds, the properties of which are still under investigation, and its function in humans has yet to be fully determined.

7.6 NATURAL SOURCES OF FAT-SOLUBLE VITAMINS AND FOOD PROCESSING

7.6.1 INTRODUCTION

Fat-soluble vitamins are widespread in raw materials and foodstuffs of plant and animal origin. However, they occur in various compositions and quantities. Therefore, no products rich in all vitamins are encountered in the diet. Becoming familiar with sources of fat-soluble vitamins makes it possible to satisfy the needs of the human organism as far as substances indispensable for its correct functioning are concerned.

7.6.2 OCCURRENCE

Several plant and animal products serve as excellent sources of fat-soluble vitamins (Table 7.1). In products of animal origin, vitamin A occurs as pure retinol and its derivatives, and as carotenoids in products of plant origin. Pure retinol is more easily assimilated than its precursors. For this reason, the content of vitamin A in food products is determined by stating the content of retinol, β -carotene, and other carotenoids and then converting to retinol equivalents (RE). Common dietary sources of vitamins A and D are livers, especially the livers of marine fish, and oils obtained from the livers of shark, cod, halibut, and tuna. Fish livers are used as raw materials to obtain oils with high concentrations of these vitamins. The most efficient method for obtaining concentrates, or vitamins A and D alone, is to apply molecular distillation accompanied by simultaneous refining of the raw oil; this method allows for minimal thermal and oxidative losses of vitamin A. Other rich sources of vitamin A include various dairy products, whereas the main sources of carotenoids are dark-green leafy vegetables in which chlorophyll masks the yellow color of the carotenoids.

For vitamin E, the richest dietary sources are vegetable oils and the products made from these oils, such as margarine, shortenings, and salad dressings. Ample amounts of this vitamin occur in wheat germs, and almost as much in grain meals. Green vegetables, such as broccoli, cauliflower, spinach, and parsley leaves, complement the diet with vitamins E and K because they contain large amounts of phyloquinone. Sufficient quantities of vitamin K are also found in pluck, especially in giblets.

The content of fat-soluble vitamins in foodstuffs is unstable and varies considerably. The biological activity of vitamins is affected by numerous extrinsic and intrinsic factors that influence their assimilation from food products. The variety of compounds displaying varying degrees of the biological activity of vitamins, especially vitamins A and E, has made it necessary to adopt a uniform system of expressing the organism's demand for these vitamins and their content in foods, taking into consideration differences in the bioactivity effect. The action of vitamin preparations in the form of esters or salts is weakened by fragments that do not show any vitamin activity apart from the active vitamin released in the alimentary tract (e.g., retinol). The biological activity of a compound is inversely proportional to participation of such a fragment in its molecule. An international unit (IU) was introduced to express vitamin activity:

IU vitamin A = 0.3 μg *trans* retinol or 0.6 μg β -carotene

IU vitamin D = 0.025 mg cholecalciferol or ergocalciferol

IU vitamin E = 1 mg α -tocopherol acetate

TABLE 7.1
Natural Sources of Vitamin A, D, E, and K^a

Food Category	Content (per 100 g of edible portion)			
	Vitamin A Retinol Equivalent (μg)	Vitamin D (μg)	Vitamin E Tocopherol Equivalent (mg)	Vitamin K (μg)
Animal				
Beef meat, lean	20.0	0.3	0.5	12.5
Beef liver	15.3 mg	0.6	0.7	74.5
Pork meat, lean	6.0	b	0.3	18.0
Pork liver	39.1 mg	1.0	0.2	56.0
Chicken hearts	9.0	0	1.2	720.0
Chicken livers	12.8 mg	1.3	0.4	80.0
Fish Products				
Herring	38.0	26.7	1.5	b
Mackerel	100.0	4.0	1.6	5.0
Sardine	20.2	10.7	b	b
Tuna	450.0	4.5	b	b
Salmon	15.0	16.3	0.9	7 ^b
Oil from cod liver	b	250	b	b
Oil from herring liver	b	3.5 mg	b	b
Dairy Products				
Milk, 5.5% fat	31.0	0.2	0.1	4.0
Powdered milk	253.0	1.2		55.0
Butter	653.0	1.2	2.2	60.0
Cottage cheese, 30% fat d.m.	99.0	0.2	0.3	50.0
Cheddar cheese, 50% fat d.m.	360.0	0.3	1.0	3.0
Eggs				
Egg	190.0	2.9	0.8	47.5
Egg yolk	550.0	5.6	2.1	147.0
Oils				
Soybean	4.0	—	50.0	3.0
Sunflower	b	—	38.8	7.5
Sesame	583.0	—	29.0	10.0
Olive	9.4 mg	—	24.5	49.6
Corn germ oil	608.0	—	13.6	60.0
Cereals				
Wheat germs	10.0	—	24.7	131.0
Wheat ground grain	traces	—	2.1	30.0
Wheat brans	traces	—	2.7	81.5
Oat flakes	b	—	1.5	63.0

continued

TABLE 7.1 (CONTINUED)
Natural Sources of Vitamin A, D, E, and K^a

Food Category	Content (per 100 g of edible portion)			
	Vitamin A Retinol Equivalent (μg)	Vitamin D (μg)	Vitamin E Tocopherol Equivalent (mg)	Vitamin K (μg)
Vegetables, Fruits				
Tomato	114	—	0.8	8.0
Cauliflower	2.0	—	0.1	167.0
Broccoli	50.0	—	0.6	174.0
Green peas	60.0	—	—	33.4
Parsley, leaves	1.0 mg	—	3.7	620.0
Lettuce	200.0	—	0.6	130.0
Kale	1.4 mg	—	1.7	817.0
Apple	6.0	—	0.5	20.2
Avocado	7.0	—	1.3	20.3
Kiwi	7.0	—	b	28.5
Orange	3.0	—	0.3	3.7

^aBased on Elmadfa, I. et al. (1997). *Die grosse GU Nährwert — und Kalorien-Tabelle*, Gräfe und Unzer Verlag GmbH, München, 7.

^bNo data available.

For the sake of greater analytic precision and as a result of numerous tests conducted on laboratory animals (e.g., rats and chickens), equivalents (E) have been proposed relating the quantity of a given compound to the form displaying the greatest activity of a given vitamin. For vitamin A, it is the RE:

$$1 \mu\text{g RE} = 1 \mu\text{g retinol} = 6 \mu\text{g } \beta\text{-carotene} \\ = 12 \mu\text{g of other provitamin A carotenoids}$$

For vitamin E, the tocopherol equivalent (TE) is being applied:

$$1 \text{ mg TE} = 1 \text{ mg } \alpha\text{-T} = 2 \text{ mg } \beta\text{-T} = 4 \text{ mg } \gamma\text{-T} = 5 \text{ mg} \\ \alpha\text{-T-3} = 25 \text{ mg } \beta\text{-T-3 or } \gamma\text{-T-3}$$

It is sometimes difficult to calculate the RE because, in order to determine the actual content of vitamin A in food products, meals, or daily food allowance, it is necessary to first determine the content of retinol, β -carotene, and other carotenoids, and only then calculate the RE. Currently, recommended dietary allowances (RDA) are being proposed to ensure that there are appropriate quantities of individual vitamins in the diet to cover the organism's demand. The recommended doses are subject to modification and take into consideration changing life conditions and dietary patterns. In addition, increasing knowledge of human nutrition makes it necessary to update them from time to time (Russell, 1997).

7.6.3 FOOD PROCESSING

The vitamin content in foods, due to the constant demand for vitamins by human and animal organisms, is a significant criterion in the assessment of the effect of technological processes on the quality and biological/nutritive value of food products. The most significant losses of vitamins occur in raw materials during storage and as a consequence of handling, food processing, and extending the shelf-life of nutritive foodstuffs. Decreases in vitamin levels result from physical and chemical, or biochemical, changes and depend on various factors. Fat-soluble vitamins in raw materials and foodstuffs can be affected by physical factors (temperature, sunlight and UV radiation, oxygen/air), chemical factors (radicals, peroxides, metal ions, e.g., Cu^{2+} , Fe^{3+}), and the biochemical factors (enzymes, mainly oxidases, e.g., lipoxygenase). Fat-soluble vitamins are particularly susceptible to oxidation due to all of these factors, and the process is further accelerated by the presence of oxidized fats. These factors, acting jointly, could cause even greater vitamin losses in foodstuffs during technological processes, and afterward during food storage.

Vitamin A, in the retinol form, as well β -carotene in raw materials are sensitive to light, the storage temperature, and atmosphere. Also plant oxidative enzymes could accelerate changes and decomposition of vitamin A (Southon and Faulks, 2001). The factor that also influences decomposition of fat-soluble vitamins is the pH of the environment.

In vegetables and fruits, during storage, β -carotene decays under the influence of sunlight, causing photodegradation of carotenoids as manifested by discoloration, or bleaching. An investigation of the effect of light and temperature on tomato and carrot juices revealed faster decomposition of the α - and β -forms than other carotenoids. Carotenoid losses were higher in blanched than in unblanched freeze-dried carrots. During tomato juice production, a 20% loss of β -carotene was observed; further loss (another 20%) occurred during 7 months of storage at 22°C (Rodriguez-Amaya, 1993). Blanching of vegetables can bring about greater vitamin destruction than further storage of deep-frozen products and dishes (Gross, 1991). Research on the effects of storage of other foodstuffs, such as vacuum-packed rainbow trout fillets stored for up to 6 months at -20°C, confirmed that there were no significant losses of carotenoids (No and Storebakken, 1991). Beta-carotene, added to various plant oils heated at 120°C, had some influence but only in the refined corn oil, in which the addition of 0.05 to 0.2% β -carotene slightly prolonged the oil stability during heating. Addition of 0.01 to 0.2% β -carotene to olive and rapeseed oils displayed prooxidative properties (Wagner and Elmadfa, 1999).

Results showed that canning at 121°C, 30 min destroyed the carotenoids in carrot juice more than HTST at 120°C, 110°C, 30 s, and acidification. Destruction of vitamin A during canning amounted to 55.7%. In other processes, vitamin A content was observed to decrease with increasing temperature and time of heating (Chen et al., 1995). In carotenoid powder obtained from carrot pulp waste, the stability of carotenoid was studied under light and dark storage at various temperatures (ranging from 4 to 45°C) for 12 weeks. The experiment proved that the content of α - and β -carotene decreased with increasing storage time and light exposure; this was confirmed by the high correlation between the change in color and carotenoid content in carrot powder (Chen and Tang, 1998). In model studies of the decomposition of

β -carotene using the Bickoff test and the influence of homologous tocopherols added and exposed to light at 20°C, there was a significant decrease in decomposition dynamics — it was from 4.2 to 5 times slower in the case of α -, γ -, and δ -T than in the control sample (Nogala-Kańska and Zabiński, 1999).

Irradiation, which is primarily applied to reduce the activity of microorganisms, inhibits sprouting and inactivates toxic compounds and significantly affects vitamin losses, depending on the applied kiloGray (kGy) dose and the environment present. Studies of the decomposition of homologous tocopherols after γ -irradiation of various edible oils (soybean, sunflower, palm, linseed, corn, and wheat germ oils) revealed that for 2.5 to 20 kGy irradiation, tocopherol losses did vary. For example, at 20 kGy, tocopherol loss in soybean oil amounted to 37, 61, and 44% for α -, γ -, and δ -T, respectively; and in linseed oil, 30 and 55% for α - and γ -T, respectively. It was also noted that above 2.5 kGy, there was a rapid increase in peroxides, which resulted in considerable acceleration of the autoxidation process (Gogolewski et al., 1996, 1997). A study of the changes in tocopherols in irradiated rice bran during storage confirmed the above results obtained for oils: the 50 to 82% loss of tocopherols took place immediately after irradiation, and further decomposition (amounting to 10 to 35%) was recorded during longer storage (up to 52 weeks) (Shin and Godberg, 1996). Tilapia and Spanish mackerel fillets were subjected to γ -irradiation (range, 1.5 to 10 kGy) and then stored for 20 days. In both fish, the decomposition of homologous tocopherols increased with increasing irradiation dose. The 3.0 kGy dose was optimum for maintaining the tocopherol level, and doses higher than that brought about greater losses of α -T and γ -T. The 20-day storage only slightly influenced further changes in the homologous tocopherol content (Al-Kahtani et al., 1996).

Deep-frying of potato chips in rapeseed oil at 162°C has been investigated. It was discovered that at this temperature, α -T decomposed more rapidly than β -T and γ -T. However, the addition of rosemary extract or ascorbyl palmitate to the oil decreased decomposition of tocopherols during frying (Gordon and Kourimska, 1995). Model studies of the antioxidant properties of tocopherols in various systems (linoleic acid and its methyl esters) proved that at higher temperatures (37 and 47°C), γ -T was a better antioxidant with respect to these substrates than α -T (Gottstein and Grosch, 1990). It is difficult to unequivocally determine the decomposition of, for example, β -carotene, as well as tocopherols, because they can migrate to the oil used for frying or, together with that oil, be absorbed by the product being fried (Pokorný, 1999; Pokorný and Schmidt, 2001).

Application of microwave heating for preparing dishes also results in the loss of vitamin E. It was reported that in microwave processing, the decomposition of tocopherols depended on the kind of oil and duration of heating (Yoshida et al., 1991a,b). The total loss of tocopherols varied, depending on the kind of oil being heated; the order was: olive oil, rapeseed and corn oils, and soybean oil. Other studies of the changes in oxidation stability and tocopherol in soybean, corn, sunflower, rapeseed, and olive oils showed that, after 15 minutes, the decrease in tocopherol content was 5, 14, 43, 32, and 61%, respectively. After 25 minutes of heating (maximum heating time), α -T was completely destroyed in all oil samples, while in the soybean, corn, and rapeseed oils, the presence of γ - and δ -T was noted (Marinova et al., 2001).

In industrial food processing, vitamin E loss is primarily caused by the oil refining process, which consists of several stages in which tocopherols are being partially removed. This significantly influences the change in content of homologous tocopherols. Many studies on various oils (e.g., rapeseed and soybean) confirmed total loss amounting up to 30% tocopherols in the refined oil in comparison with the crude one. The largest amounts of tocopherols, two thirds, are removed during deodorization and one third during degumming and bleaching (Nogala-Kałużka et al., 1993; Shahidi et al., 1997; Cmolik et al., 2000).

Tocopherols were also determined during storage of rapeseed and soybean oils, both crude and refined, at 20°C, with respect to the Lea number. At the Lea number 10, tocopherol decomposition was the following: δ -T > γ -T > α -T for the rapeseed oil and γ -T > α -T for the soybean oil. Generation of tocopherol dimers was noted, the quantities of which were smaller than the respective tocopherols being decomposed. Tocopherol dimers, due to their antioxidant properties, can also inhibit the autoxidation of fats in which they occur. In margarine stored at 4 and 20°C, after 136 days of storage, the greatest decomposition observed concerned α -T, amounting to 12%. Decomposition of γ -T and δ -T amounted to 8% each at the lower temperature, and to 50, 47, and 36% for α -T, γ -T, and δ -T, respectively, at 20°C (Nogala-Kałużka and Gogolewski, 1995, 2000). On the basis of numerous studies of the preservation of oils and other edible plant fats, one can state that tocopherol losses depend primarily on the kind of oil or margarine (especially the composition of fatty acids), storage temperature, packaging, and access of light and air/oxygen.

Not much literature data is devoted to changes in vitamins D and K in raw materials and foodstuffs. Both vitamins are light sensitive and the acidity or alkalinity of the environment can additionally activate their decomposition. The effects of adding menadione (vitamin K₃) to stored oils upon tocopherols and formation of their dimers have been studied. Commercial rapeseed and soybean oils were stored in transparent and brown bottles at 20°C. Addition of vitamin K₃ to oil samples depended on the tocopherol content in the tested oil (0.05 mM vitamin K₃: 0.5 mM tocopherols). It was discovered that in both rapeseed and soybean oils stored in transparent bottles, decomposition of tocopherols was accompanied by the simultaneous formation of their dimers. After 71 and 94 days, respectively, complete destruction of tocopherols and their dimers was noted. In brown bottles, the time of tocopherol decomposition was longer and amounted to 105 days for rapeseed oil and 167 days for soybean oil. In comparison with control samples, the decomposition of tocopherols was enhanced by the addition of vitamin K₃ and exposure to light (transparent bottles) (Kupczyk and Gogolewski, 2001). The same authors, in a model study of irradiation of tocopherols (range, 2.5 to 10 kGy) and storage for 2 months at 4°C, noted that the decomposition of all irradiated tocopherols increased during storage and depended on irradiation doses and the reaction medium (samples were dissolved in benzene and ethanol and were also irradiated “in substantia”), with the highest decomposition taking place in polar solvents. Destruction of δ -T irradiated “in substantia” was smaller than other homologues, which corroborates data concerning the influence of the environment on the decomposition of tocopherols and also the influence of the accompanying substances having, for example, an antagonistic effect (Kupczyk and Gogolewski, 1999).

Nutritional studies carried out in many highly industrialized countries have shown inadequate intake of recommended vitamins by some population groups. Therefore, the primary aim of elaborating new food processing technologies is to maintain the maximum amount of vitamins of which a given product is a natural source.

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8 Lipid Oxidation in Food Systems

Anna Kolakowska

CONTENTS

- 8.1 Introduction
- 8.2 Mechanism
 - 8.2.1 Free Radical Oxidation
 - 8.2.2 Photooxidation
 - 8.2.3 Lipoxygenases (LOX)
 - 8.2.4 Secondary Oxidation Products
- 8.3 Transition Metals in Lipid Oxidation
- 8.4 *In Vivo* Oxidation
- 8.5 Effects of Oxidized Lipids in Nutrition
- 8.6 Antioxidants
 - 8.6.1 Introduction
 - 8.6.2 *In Vivo* Defense System
 - 8.6.3 Food Antioxidants
 - 8.6.3.1 Important Antioxidants Present in Food
 - 8.6.3.2 Antioxidants Allowed in Food
- 8.7 Methods for the Determination of Lipid Oxidation
 - 8.7.1 Introduction
 - 8.7.2 Traditional Methods
 - 8.7.2.1 Peroxide Value (PV)
 - 8.7.2.2 Carbonyl Compounds
 - 8.7.2.3 Sensory Analysis
 - 8.7.3 Volatile Compound Analysis
 - 8.7.4 Instrumental Determination of the Primary Products of Lipid Oxidation
 - 8.7.5 Other Instrumental Methods
 - 8.7.5.1 Ultraviolet (UV) Spectroscopy
 - 8.7.5.2 Infrared (IR) Spectroscopy
 - 8.7.5.3 Luminescence Spectroscopy
- References

8.1 INTRODUCTION

Lipid oxidation in food systems is a detrimental process. It deteriorates the sensory quality and nutritive value of a product, poses a health hazard, and presents a number of analytical problems (Figure 8.1). Lipid oxidation is affected by numerous internal and external factors such as fatty acid (FA) composition, content and activity of pro- and antioxidants, irradiation, temperature, oxygen pressure, surface area in contact with oxygen, and water activity (a_w). Because lipids are only a part of a food product, it is difficult to find a food component that would not be capable of affecting lipid oxidation. The complex process of food lipid oxidative changes is interpreted in terms of an oxidation mechanism derived from model studies, predominantly involving a single FA. Lipid oxidation in foods is assumed to proceed along a free radical route (autoxidation), photooxidation route, and/or lipoxygenase route. The oxidation mechanism is basically explained by invoking free-radical reactions, while the photooxidation and lipoxygenase routes differ from it at the initiation stage only. For this reason, they can be treated as different forms of free radical reaction initiation.

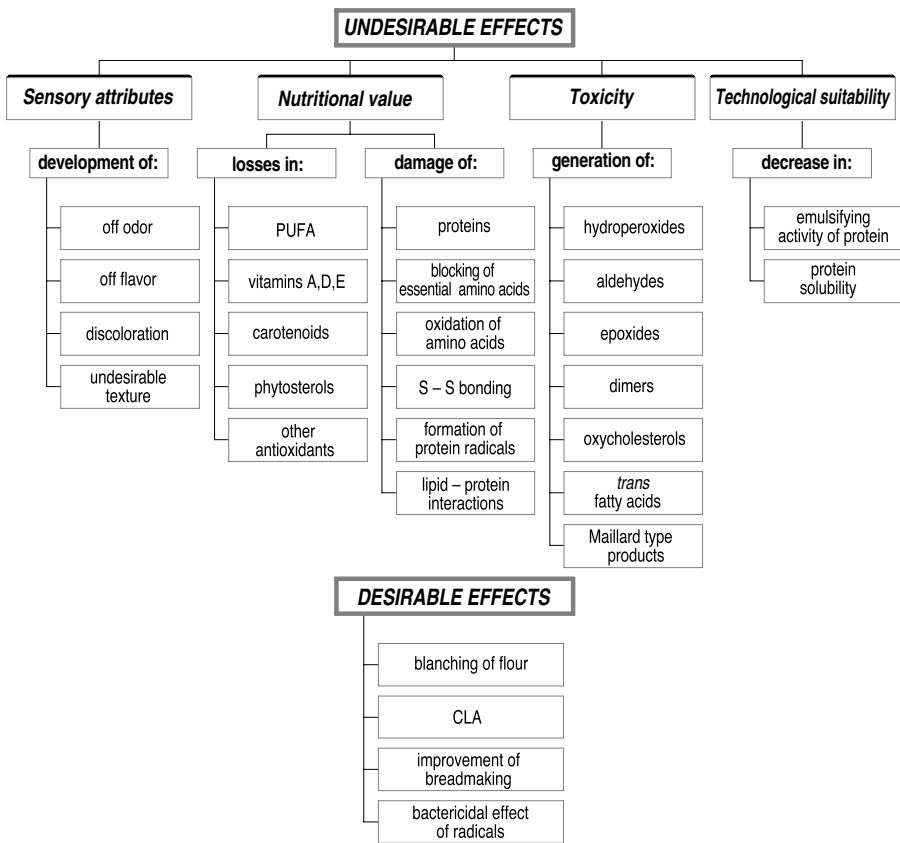


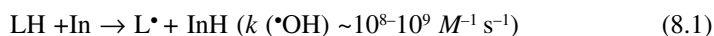
FIGURE 8.1 Effects of lipid oxidation on the quality of food.

8.2 MECHANISM

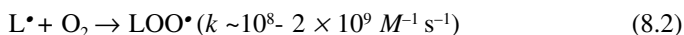
8.2.1 FREE RADICAL OXIDATION

The classical free radical route involves three stages: initiation, propagation, and termination. Their respective mechanisms have been described in numerous papers, including those by St. Angelo (1996), Halliwell et al. (1995), and Frankel (1998). The stages differ in reaction rate (Chaudière, 1994), depending on the substrates and reaction conditions.

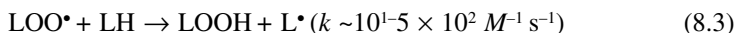
Initiation: In the presence of an initiator (In), unsaturated lipids (LH) lose a hydrogen to form a lipid radical (L•):



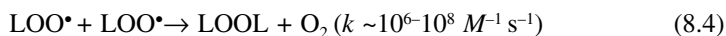
Propagation: The alkyl radical lipid (L•) reacts with molecular oxygen to form peroxy radical (LOO•):



Peroxy radicals abstract hydrogen from another molecule of unsaturated lipid (LH) to form hydroperoxides (LOOH) and a new lipid radical (L•):



Termination: The peroxy radicals react with each other to form non-radical products:



Oxidation is initiated by radicals present in living organisms (e.g., hydroperoxide $\text{HO}_2^\bullet$, hydroxide $\bullet\text{OH}$, peroxide $\text{ROO}^\bullet$, alcoxyl $\text{RO}^\bullet$, alkyl $\text{L}^\bullet$) or by thermal or photochemical homolytic cleavage of an R–H bond.

The oxidation activation energy and reaction rate at this stage depend on the type of initiator and the number of unsaturated bonds in the substrate. The dissociation energy of the C–H bonds in saturated FA does not depend on the length of the FA carbon chain and is similar in FA, their esters, and in triacylglycerols (TAG) (Litwinienko et al., 1999). In unsaturated acids, the weakest C–H bond is found in the bis-allylic position, the activation energy there amounting to 75 kcal/mol versus allylic and methylene hydrogens 88 and 100 kcal/mol, respectively (Simic et al., 1992).

The newly formed alkyl radicals of unsaturated lipids $\text{L}^\bullet$ react very rapidly with molecular oxygen to form peroxides at each end of the allylic system [Equation (8.2)]. Allylperoxy radicals undergo rearrangements with the oxygen atoms and the two components migrate across the allylic backbone. This is the way a mixture of positional *cis* or *trans* isomers is formed, the isomers involved

depending on the type of lipid substrate (Porter, 1995; Frankel, 1998). The number of positional isomers from polyunsaturated FA (PUFA) is determined by the formula $2n-2$, where n is the number of double bonds in the FA (Kitts, 1996).

By abstracting hydrogen from a lipid molecule, the peroxide forms hydroperoxide. Initially, monohydroperoxides are formed in subsequent TAG molecules; they are then followed by bis- and tris-hydroperoxide formation (Frankel, 1998). This stage of propagation proceeds at a low rate because it depends on the C–H bond strength, that is, on the presence of double bonds. The reaction rate is higher in FA richer in bis-allylic methylene groups. However, two bis-allylic methylene groups act independently and are not activated by each other (Frankel, 1998). Linoleate was found to be 40 times more reactive than oleate, while linolenate was 2.4 times more reactive than linoleate. The fact that the autoxidation rate increases with increasing number of double bonds has been confirmed in pure FA and FA esters, but it is not necessarily the case in a mixture of FA and natural lipids. For example, TAG containing docosahexaenoic acid (DHA) and α -linolenic acid as their main PUFA are oxidatively more stable than TAG containing linoleic acid (Miyashita et al., 1999). The FA oxidation rate also depends on the acid location in a TAG or phospholipid molecule. PUFA at the *sn*-2 position are more stable than when located at the *sn*-1 and *sn*-3 positions in TAG (Wada and Koizumi, 1983; Haraldsson et al., 2000). The composition of FA is more important for the profile of oxidation products, particularly volatile compounds (Meynier et al., 1999).

Addition of oxidized oil to fresh oil resulted in a synergistic effect of oxidation (Figure 8.2).

Termination of free radical oxidative reactions occurs when two radical species (peroxyl, alcoxyl, or alkyl) react with each other to form a non-radical adduct as in Equation (8.4).

Free radical reactions can also be terminated when one of the lipid radicals reacts with an antioxidant proper, because hydrogen abstraction by a peroxide radical from the antioxidant molecule produces an inert antioxidant radical.

8.2.2 PHOTOOXIDATION

Photooxidation involves the formation of hydroperoxides in a direct reaction of singlet oxygen addition to unsaturated lipids, without radical formation. The singlet oxygen $^1\text{O}_2$ emerges during a reaction of sensitizers (e.g., chlorophyll, hemoglobin, myoglobin, and riboflavin) with atmospheric oxygen. Photosensitization can also occur *in vivo* (Halliwell et al., 1995).

The singlet oxygen is 1450 times more reactive than molecular oxygen. It is inserted at the end carbon of a double bond, which is shifted to an allylic position in the *trans* configuration. The resulting hydroperoxides have an allylic *trans* double bond, which renders them different from hydroperoxides formed during autoxidation. Hydroperoxides formed during photooxidation are more easily cyclized than hydroperoxy epidioxides (Frankel, 1998).

In addition, light, particularly ultraviolet light, may be involved in initiation of the classical free radical oxidation of lipids and catalyze other stages of the process.

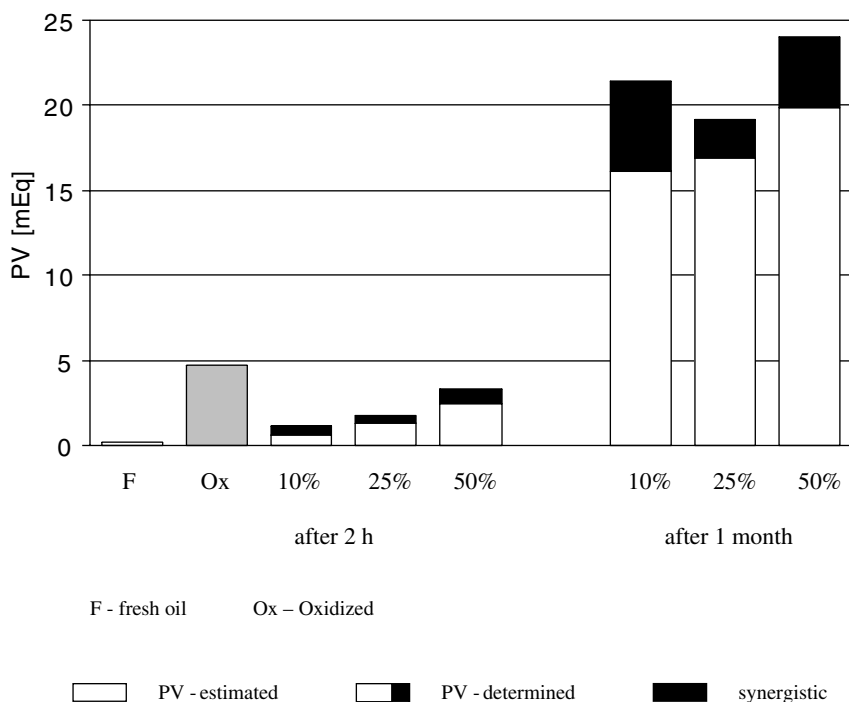


FIGURE 8.2 Synergistic effect of oxidized rapeseed oil on peroxide value (PV) of fresh oil. Mixture composed in 10, 25, and 50% oxidized oil. PV was determined after 2 hours and 1 month storage of the mixture at room temperature. F is fresh oil, and Ox is oxidized oil. (From Kołakowska, A., Domiszewski, Z., and Żych, A., unpublished data.)

In the presence of light energy-activated riboflavin, which is a sensitizer, a lipid radical can form, while oxygen gives rise to superoxide radical anion $O_2^{\bullet-}$.

During UV irradiation of muscle lipids, the quantity of hydroperoxides and the ratio of their formation differ, depending on the origin of lipids (Table 8.1). Significant differences exist, both between the groups and within each group, depending on many biological factors. For example, the photooxidation ratio (slope of hydroperoxide accumulation over time of UV exposure of lipids) of the muscle lipids of fish varied from about 1 to more than 20, both within and between the species (Kołakowska et al., 1992, 2000). No correlations between the photooxidation ratio and monounsaturated fatty acids (MUFA), PUFA, eicosapentaenoic acid (EPA), or DHA content in fish lipids were noted.

8.2.3 LIPOXYGENASES (LOX)

LOX-catalyzed lipid oxidation differs from the free radical reaction by the formation of hydroperoxides at a certain position of the chain of, most often, a free FA. Lipoxygenases use molecular oxygen to catalyze the stereo- and regiospecific oxygenation of PUFA with 1-*cis*,4-*cis*-pentadiene moieties. LOX react enzymat-

TABLE 8.1**Photooxidation Ratio of Lipids and LOX Activity of Muscle Tissue**

Muscle Tissue	Fatty Acid Composition Group (%)			Photooxidation Ratio ^a	Relative Enzymatic Activity
	SFA	MUFA	PUFA		
Pig, fresh	43	50	6	3.5	5
frozen					11
Chicken (leg), fresh	42	45	13	7.5	51
frozen					40
Herring, fresh	34	40	25	6.8	67
frozen					119
Sprat, fresh	34	36	28	14.6	43
frozen					47

^a Slope from linear equation PV versus time (min) during 120-min UV irradiation of lipids.

Note: LOX - lipoxygenase.

SFA = saturated fatty acids.

MUFA = monounsaturated fatty acids.

PUFA = polyunsaturated fatty acids.

From Kołakowska, A., unpublished data.

ically with more than one methylene carbon on the substrate molecule to yield double oxygenation sites (German et al., 1992). The newly formed FA peroxy free radical removes hydrogen from another unsaturated FA molecule to form a conjugated hydroperoxy diene. LOX forms a high-energy (radical) intermediate complex with the substrate; this complex is capable of initiating the oxidation of lipids and other compounds (e.g., carotenoids, chlorophyll, tocopherols, thiol compounds, and protein), which can themselves interact with the enzyme-substrate complex as well (Hammer, 1993; Hultin, 1994). They are responsible for the off-flavor in frozen vegetables (Ganthavorn et al., 1991), lipid oxidation in cereal products, rapeseed, pea, avocado, and for the “beany” and bitter flavor. Soybeans contain LOX-1 with activity optimum at pH 9 and LOX-2 with optimum at pH 6.5 (there is LOX-3 as well). LOX-1 is specific for linoleic acid and, like other LOXs, catalyzes free FA oxidation. LOX-2 requires no prior lipid hydrolysis and catalyzes the oxidation of TAG, carotenoids, and vitamins. Grinding of grains accelerates lipolysis, thus enhancing LOX activity (Frankel, 1998). A change in pH results in a reduction in LOX activity. The activity of soybean LOX having its optimum at pH 7 was observed to drop to 2% at pH 4; an increase in pH brings about a much slower reduction in activity. Heating to 55°C influenced the soybean activity only slightly (Tedjo et al., 2000). Soybean LOX has been used, since 1930, to bleach flour to produce white bread crumbs. It is well known that soybean LOX, by oxidizing free lipids, improves the dough-forming properties and baking performance of wheat flour (Hammer, 1993).

Mammalian LOX are categorized according to the positional specificity of oxygen insertion into arachidonic acid. Four isoform positions of arachidonate LOX have been identified: 5-lipoxygenase (E.C. 1.13.11.34), 8-lipoxygenase, 12-lipoxygenase (E.C. 1.13.11.31), and 15-lipoxygenase (E.C. 1.13.11.33). The lipoxygenase that catalyzes oxidation of linoleate (E.C.1.13.11.12) attacks linoleic acid, both at position 9 and position 13.

In chicken meat arachidonate, 15-lipoxygenase was found to be active during a 12-month storage at -20°C (Grossman et al., 1988). In frozen-stored fish, LOX contributes to oxidative lipid deterioration. However, LOX in fish is also responsible for the formation of desirable fresh fish flavor, the seaweed flavor (Lindsay, 1994). Some species show a higher activity of 12-LOX, while 15-LOX is more active in others; for this reason, the fresh fish flavor spectrum is species dependent. The half-lives of 12- and 15-lipoxygenase at 0°C were less than 3 h and more than 10 h, respectively (German et al., 1992). LOX was observed to be active in cold-stored fish after 48 h of storage (Medina et al., 1999). The storage of herring, 3 weeks at -20°C , resulted in an increase in LOX activity (Table 8.1). During prolonged frozen storage of herring, a decrease in LOX activity was observed (Samson and Stodolnik, 2001). Although the participation of LOX in the *post mortem* animal lipid oxidation is acknowledged, the role of LOX in lipid oxidation is much more important in plant than in animal food products.

8.2.4 SECONDARY OXIDATION PRODUCTS

These are all products formed from lipids during their oxidation, except for radicals and hydroperoxides, regarded as primary oxidation products. The composition of a mixture of compounds found in oxidized lipids differs, both qualitatively and quantitatively, depending on the kind of lipids, the presence of pro- and antioxidants, and conditions of oxidation. Transformation of hydroperoxides gives rise to ketones, aldehydes, and hydroxides with a functional group situated at different positions, depending on the unsaturated FA, epoxides, dimers, and oligomers (Figure 8.3). Even under mild conditions ($25\text{--}40^{\circ}\text{C}$), oxidized unsaturated FA may produce various dimers: peroxy-linked, carbon-carbon, carbon-ether, conjugated diene-triene-containing ones, dihydroperoxides, or hydroperoxy epidioxide (Frankel, 1998). Volatile compounds are formed as a result of homolytic β -scission of FA hydroperoxides. This is the way short-chain saturated and unsaturated *cis* and *trans* aldehydes, alcohols, and hydrocarbons are formed (Figure 8.2). Unsaturated aldehydes and ketones undergo autooxidation and supply further volatile compounds. Oxidation of a single pure FA gives rise to several tens of volatile compounds. Propanal and hepta-2,4-dienal characterize the oxidative decomposition of n-3 PUFA, while hexanal and pentane are typical for oxidative decomposition of n-6 PUFA (Frankel, 1993). The rancid lipid odor profile is made up of a mixture of several volatile compounds. Among them, the *trans*, *cis*-alkadienals, and vinyl ketones have the lowest flavor threshold in oils, while the threshold of hydrocarbons (alkanes and alkenes) is the highest (Min, 1998). The sensory effects depend on the composition of the participating compounds and on the composition of the food matrix, while the rancid off-odors and off-flavors of foods emanate from the interactions between lipids and other components, especially proteins.

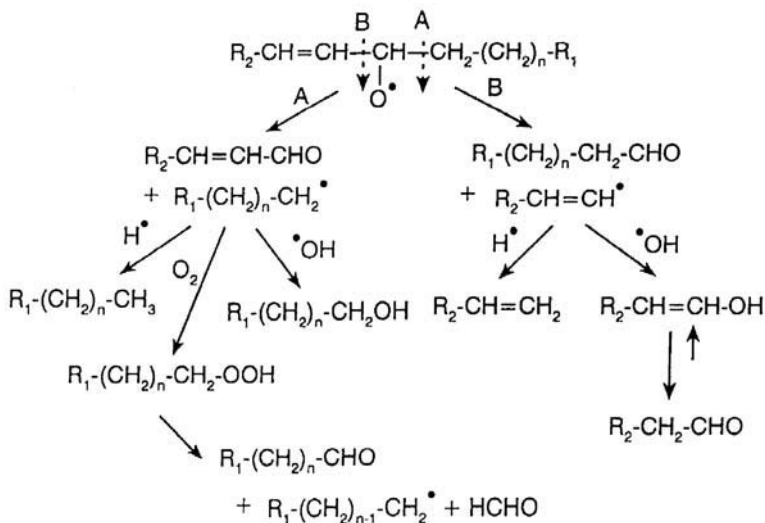
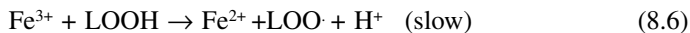


FIGURE 8.3 Formation of secondary products by hydroperoxide decomposition. (From Frankel, E.N. 1998. *Lipid Oxidation*, The Oily Press, Dundee, U.K., [Chapter 4](#). With permission.)

8.3 TRANSITION METALS IN LIPID OXIDATION

Hydroperoxides formed at the propagation stage of the free radical oxidation, as well as those produced by photooxidation and enzyme-catalyzed oxidation, can disintegrate and yield alcoxyl, alkyl, and peroxy radicals, which reinitiate the oxidation of unsaturated FA. Hydroperoxide decomposition may be triggered by temperature and/or light, but most important in this respect is the activity of transition metals, mainly iron and copper [see Equations (8.5) and (8.6)].



The Fe^{2+} ions are more reactive than Fe^{3+} ions and decompose hydrogen peroxide over 100 times faster (Girotti, 1998).

Iron occurs in human and animal bodies, in up to 90% in a bound form in: hemoglobin, myoglobin, cytochromes, the storage protein ferritin and hemosiderin, the iron transport proteins, transferrins, and as prosthetic groups of enzymes. A small amount of iron occurs in a “free” form, that is, primarily as low-molecular-weight (LMW) iron. It complexes with organic phosphates, inorganic phosphates, amino acids (histidine, glycine, cysteine), and organic acids (citric acid) (Decker and Hultin, 1992). LMW iron contributes between 2.5 and 3.8% to the total iron content in muscle tissue of lamb, pork, and chicken. Dark muscles of chicken,

turkey, and mackerel contain twice as much LMW iron and more ferritin than light muscles (Kanner, 1992).

LMW iron acts as a catalyst. Protein-bound Fe and Cu are minimally catalytic in oxidation. Ascorbate, NAD(P)H, thiol compounds, reduced glutathione, cysteine, and protein thiol groups release iron, which can catalyze the Fenton reaction. This occurs *post mortem* during, for example, the storage of fish or turkey, but the amount of reductants is then also decreased (Kanner, 1992; Hultin, 1994).

Heating results in the release of heme-bound iron and in forming other polymers with proteins; those polymers enhance the catalytic effect of iron. This is also true with respect to the thermal inactivation of enzymes that contain metals acting as prosthetic groups (e.g., lipoxygenases and peroxidases). These enzymes, even after denaturation, are capable of catalyzing oxidation. On the other hand, heating does not release iron from ferritin, but does enhance its reduction (Kanner, 1992). The rate of oxidation in the presence of metals is higher at lower pH than at neutral pH for Fe^{3+} and for Fe^{2+} (Koppenol, 1994; Richards and Hultin, 2000). The effects of pH on the catalytic action of transition metals in emulsion systems are not so clear-cut (Decker, 2001).

Food products contain less copper than iron; copper is mainly bound to protein as in ceruloplasmin. Copper ions are also chelated by albumin; in mammalian and avian skeletal muscles and brain, they are chelated by carnosine, anserine, and other histidine dipeptides. Cu^{1+} ions are more reactive than Fe^{2+} ions and decompose hydrogen peroxide to produce hydroxyl radicals at a rate over 50 times higher than Fe^{2+} (Decker, 2001). However, the mechanism of the prooxidative effects of copper is most likely a mechanism other than that for iron, which is the reason why prevention of that catalysis in food systems requires a different strategy (Hultin, 1994).

8.4 IN VIVO OXIDATION

The human body and other aerobic organisms contain reactive oxygen species (ROS) that can induce *in vivo* lipid oxidation. Those species include singlet oxygen $^1\text{O}_2$, hydroperoxyl radical $\text{HO}\cdot$, superoxide radical anion $\text{O}_2^{\cdot-}$, hydroxyl radical $\cdot\text{OH}$, hydrogen hydroperoxide H_2O_2 , hydroperoxide ROOH , peroxy radical $\text{ROO}\cdot$, alkoxy radical $\text{RO}\cdot$, and hypochlorous acid HOCl . Reactive oxygen species participate in a number of *in vivo* biological processes, are products of enzymatic reactions of oxidases such as xanthin oxidase and NAD(P)H oxidase, and may be produced by various cells. They contribute to antimicrobial action of the phagocytic cells in the arsenal of defense cells (neutrophils, monocytes, macrophages, eosinophils) and participate in removal and destruction of bacteria and damaged cells. ROS induce cell differentiation as, for example, in an immune response to oxidative stress, and affect information transfer to and within a cell:receptor stimulation by inhibiting receptor function. Hydroperoxides stimulate glucose transport to cells by simulating insulin activity and serotonin transport. Lipid oxidation activates phospholipase A_2 , and peroxides further control prostanoid synthesis. They can stimulate or inhibit cyclooxygenase activity (Rice-Evans, 1994; Halliwell et al., 1995).

Lipid peroxides and aldehydes are formed in damaged plant tissues as protection against bacteria and fungi at the place of damage. Ripening and aging of fruit is a process of controlled oxidation. Seed germination is accelerated by very low doses of ionizing radiation.

The ROS level in the body is controlled by the antioxidative system. Disturbing the balance between antioxidative activity and ROS production leads to oxidative stress. Radicals and other reactive oxygen species can become highly destructive to cells and tissues, and damage DNA as well. Whereas massive quantities of oxidized lipid products are thought to cause gross structural or metabolic damage, moderate amounts may initiate a stress signal cascade inducing cellular death programs leading to apoptosis. Oxidative damage to crucial cellular molecules has been implicated as a possible factor in the etiology of aging and several human diseases (Figure 8.4). *In vivo* lipid peroxidation plays an important role in the pathogenesis of atherosclerosis (Shimasaki et al., 1998), tumors (Burdon, 1994), chronic inflammatory disorders such as rheumatoid arthritis (Zahara et al., 1998), diabetes, asthma (Wood et al., 2000), cataracts (Hamre et al., 2001), dementia, and stroke, as well as central nervous system disorders such as Parkinson's and Alzheimer's diseases and Down's syndrome (Neely et al., 2000; Heimli et al., 2001), amyotrophic lateral sclerosis, erythrocyte diseases mainly due to membrane lipid peroxidation, and others (Aruoma, 1998). An *in vivo* link between gastric mucosal injury and lipid peroxidation was demonstrated (Ray et al., 1999). Radicals may also explain the lack of bactericidal effect of NO on *Helicobacter pylori*. These bacteria are likely to produce a superoxide radical that inactivates NO, whereupon ONOO⁻ is formed. Mutagenic ONOO⁻ and hydroxy radicals can explain the genesis of gastric inflammation and cancer (Nishikawa et al., 1998).

Radical production is the essence of the action of certain drugs, some antibiotics, and psoralens; they may be activated by UV light, while the male contraceptive gossipol acts via ROS formation, the ROS damaging the spermatozoa motility (Bartosz, 1995). Because ROS induce apoptosis, they are experimented with in selective killing of cancer cells (Sen et al., 1998; Heimli et al., 2001).

The efficiency of the natural defense system weakens as the organism ages. The aging process is explained by an increased activity of radical reactions and a disturbed oxidant–antioxidant balance in the organism.

8.5 EFFECTS OF OXIDIZED LIPIDS IN NUTRITION

Knowledge of the harmful effects of oxidized lipid consumption comes from direct nutritional studies on laboratory animals, the experiments also involving various ¹⁴C-labeled fractions of oxidized lipids. Research on human subjects has been helpful as well, the studies targeting the effects of *in vivo* oxidation, reduction of antioxidant levels in the body, and increased antioxidant request accompanying diets rich in unsaturated lipids and cholesterol.

The toxicity of dietary oxidized lipids was studied in various animal species and involved highly oxidized fats, particularly thermoxidized frying fats. Among oxidation products contained in such fats, polymers appear to be harmless because they are minimally absorbed and are removed with feces. Nonpolar dimer FA showed digestibility below 5% (Sanchez-Muniz and Sanchez-Montero, 1999). Noncyclic

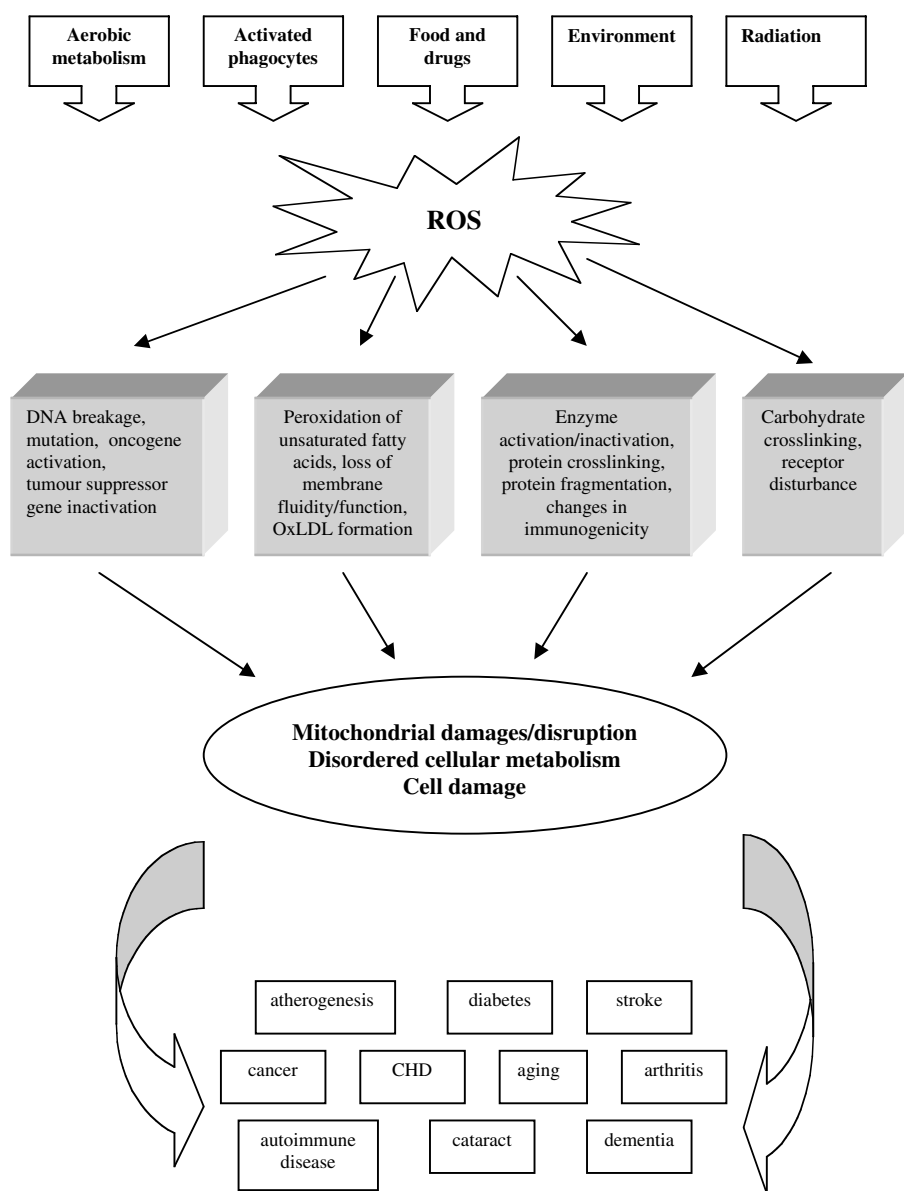


FIGURE 8.4 The role of reactive oxygen species (ROS) in inducing diseases. CHD is coronary heart disease. (Adapted from Strain, J.J. and Benzie, I.F.F. 1999. Diet and antioxidant defense, in *Encyclopedia of Human Nutrition*, Sadler, M.J., Strain, J.J., and Caballero, B., Eds., Academic Press, San Diego, 95.)

dimeric FA were also poorly absorbed (10%). On the other hand, as much as 50% of the total oxidized monomeric acids and 95% of the total cyclic monomeric acids from thermoxidized fats and TAG were recovered in the lymphatic lipids (Mahungu

et al., 1999). Rats fed mackerel fried in coconut oil over the course of 4 days showed initial stages of cell damage in the liver and kidney as well as an increase in total lipids and cholesterol in heart and serum, compared with the control group fed steamed mackerel (Ammu et al., 2000). Eder (1999) showed that the consumption of oxidized lipids (thermally, at 150°C, 6 days) caused a reduction in the desaturation rate of linoleic acid and α -linolenic acid by microsomal $\Delta 4$ -, $\Delta 5$ -, and $\Delta 6$ -desaturases and also a reduction in $\Delta 9$ -desaturation. On the other hand, recent research on humans demonstrated the conversion of *trans*- α -linolenic acid isomers into the following long-chain PUFA: 19-*trans*-C22:5n-3, 19-*trans*-C22:6 n-3, and *trans*-C24:5. These *trans*-LC-PUFA can interfere with platelet and endothelium metabolism. The *trans*- α -linolenic acid isomer-rich diet raised the LDL- to HDL-cholesterol and total cholesterol ratio, which would increase the risk of cardiovascular disease by 8% (Chardigny et al., 2001).

Moderately thermally oxidized soybean oil (130°C, air flow-through) of peroxide value (PV) = 75 mEq O₂/kg diet (compared with the control of 9.5 Eq O₂/kg diet) was fed to rats for 40 days (Eder and Kirchgessner, 1999). The study showed no adverse effects on liver, heart, kidney, or adipose tissue FA composition, and even a reduction in the osmotic fragility of erythrocytes and hepatic lipogenesis. However, the moderately oxidized oil slightly reduced the vitamin E status in the tissues. A slightly increased susceptibility of LDL to lipid peroxidation, and an increased concentration of thiobarbituric acid reactive substances (TBARS) in LDL, were also observed.

The harmful effects in animal fats are closely related to the potential activity of cholesterol oxidation products, particularly in the presence of unsaturated FA and thermal treatment at temperatures exceeding 100°C.

UV-oxidized fish lipids, and a threefold increase in PV and TBA compared to controls, resulted in weight loss, lower body weight increments, increased liver weight, reduced hemoglobin and hematocrit in guinea pigs fed a diet with a 14% addition of those lipids for 12 weeks (Ziemiański et al. 1991). The presence of oxidized lipids in the diet of humans and animals results in an increase in TBARS in plasma and tissues. An increase in the peroxide value could not be detected. There was a dose-dependent increase in conjugated dienes in chylomicrons of rats and humans given oxidized lipids (Hamre et al., 2001).

The research on animals conducted so far, including experiments on mammals, shows aldehydes in free form or conjugated with amino acids to be absorbed from the gastrointestinal tract to plasma, muscles, and liver. The absorbable aldehyde adducts with protein from the diet are less toxic than free aldehydes. With regard to hydroperoxides, they are generally thought to be decomposed in the stomach, from where they are not transported any farther. It is possible that at low doses, FA hydroperoxides are converted to the corresponding hydroxy FA in the mucosal membrane before being transported to the blood. A recent study on structured lipids confirmed that hydroperoxides were absorbed as monohydroxy and monooxy FA. They can influence endothelial dysfunction, promote thrombosis, and induce atherosclerosis (Riemersma, 2001).

Apart from the antioxidant system, an additional mechanism of protection is furnished by diarrhea, induced in rats by high doses of lipid hydroperoxide (Hamre

et al., 2001). The LD₅₀ of the hydroperoxide of highly unsaturated FA methyl ester was between 285 and 545 mg active hydroperoxide oxygen per kilogram body weight (Arai and Kinumaki, 1980). Maximum hydroperoxide content in fatty fish products does not exceed 50 mg/100 g. This hydroperoxide level is accompanied by a 20% decrease in EPA and DHA content. Thus, the effects of oxidized lipids in human diet are rather long-lasting.

8.6 ANTIOXIDANTS

8.6.1 INTRODUCTION

Antioxidants can be divided into two major groups: (1) primary (or chain-breaking) antioxidants that react with lipid radicals to produce stable products; and (2) secondary (or preventive) antioxidants that retard the oxidation process by various mechanisms. Antioxidants have also been classified into five types: primary antioxidants, oxygen scavengers, secondary antioxidants, enzymic antioxidants, and chelating agents (Hands, 1996). Pokorný (2001a) grouped antioxidants into five classes, depending on the mechanism of their activity: antioxidants proper, hydroperoxide stabilizers, synergists, metalchelators, singlet oxygen quenchers, and hydroperoxide reducing substances. The occurrence of antioxidants, their characteristics, mechanisms of activity, and applications are discussed elsewhere (see, for example, Hudson, 1990; Halliwell et al., 1995; Pokorný et al., 2001).

8.6.2 *IN VIVO* DEFENSE SYSTEM

Living organisms have a defense system consisting of intracellular and extracellular antioxidants. These include hydrophobic and hydrophilic substances, as well as intermediate substances that act at interphases and primarily affect cellular membranes (Figure 8.5). Hydrophobic antioxidants include tocopherols, carotenoids, xanthophils, bilirubin, and ubiquinol-10 (coenzyme Q10). α -Tocopherol is the most important hydro-

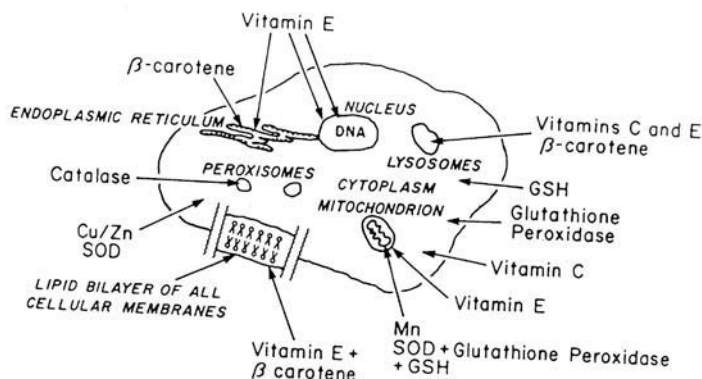
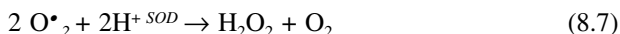


FIGURE 8.5 Antioxidant protection within the cell. (From Bendich, A. 1988. *Antioxidant Nutrients and Immune Functions*, Plenum Press, New York. With permission.)

phobic antioxidant; it protects membrane lipids in living organisms. The major hydrophilic antioxidants in humans and other animals include ascorbate and glutathione. Low-molecular-weight antioxidants can protect lipids contained in both the aqueous and lipid phases. Uric acid, certain amino acids and peptides, creatinine, carnosine, neopterin, bilirubin, glucose, and pyrogronate also exhibit antioxidant properties.

The enzymatic system that controls the ROS level (Figure 8.4) consists of three major enzymes: superoxide dismutase, catalase, and glutathione peroxidase, as well as a number of other enzymes.

Superoxide dismutases (SOD) (E.C. 1.15.1.1.) catalyze the dismutation of superoxide via the following reaction:



SOD occurs in all aerobic organisms. Plant prokaryotes contain two types of SOD: MnSOD and FeSOD. Mammals contain three types of SOD: CuZnSOD in the cytoplasm, MnSOD in the mitochondria, and extracellular EC-SOD. CuZnSOD is an exceptionally stable enzyme, active even after a 1-h incubation at 70°C and also at pH exceeding 10.

Catalases (E.C. 1.11.1.6) catalyze H_2O_2 scavenging reactions:



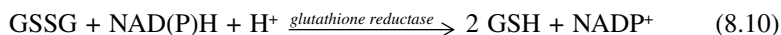
and occur in the liver, erythrocytes, and milk. They are active in the pH range between 5 and 10.5.

Glutathione peroxidase (E.C. 1.11.1.9) contains selenium. It scavenges H_2O_2 within cells by catalyzing reactions between H_2O_2 and glutathione (GSH)



Oxidized glutathione (GSSG) is harmful to a cell because it can form disulfides with proteins or oxidize thiol groups, thus forming disulfide bonds in proteins. GSSG is reduced back to GSH by glutathione reductase.

Glutathione reductase (E.C. 1.6.4.2) re-produces the reduced form of glutathione at the expense of NAD(P)H oxidation:



The major function of SOD, catalase, and glutathione peroxidase is to protect against the Fenton reaction; that is, decomposition of H_2O_2 by Fe^{2+} , resulting in the emergence of the most toxic radical, hydroxyl ($\text{HO}^{\bullet}$). In addition, glutathione peroxidase reduces lipid hydroperoxides to alcohols:



thus stopping the chain reaction of lipid oxidation (Bartosz, 1995). Certain glutathione transferases (E.C. 3.1.2.7) may produce similar effects.

Living organisms are protected from ROS by cytochrome C peroxidase (E.C. 1.11.1.5) in mitochondria, ascorbate peroxidase (E.C. 1.11.1.11) contained in chloroplasts and in the cytoplasm, as well as dehydroxyascorbinate reductase (E.C. 1.8.5.1) and NADH reductase (E.C. 1.6.5.4). Protection against ROS is also provided by ceruloplasmin (E.C. 1.16.3.1) via oxidation of Fe^{2+} to Fe^{3+} , DT-diaphorase (E.C. 1.6.99.2), flavoprotein, and epoxide hydrolases (E.C. 3.3.2.3).

Iron and copper ions in the body are controlled by metal-binding proteins and chelators to prevent the Fenton reaction from occurring. These proteins and chelators maintain the iron in its oxidized form. The proteins include transferrin, which transports Fe^{3+} to cells, and lactoferritin, which is present in blood serum. Ferritin is a protein that stores Fe^{3+} ; its large storage capacity allows a single ferritin molecule to store as many as 4500 Fe atoms. In addition, blood serum contains haptoglobin, which is known to strongly bind hemoglobin. Iron is released from ferritin as a result of reduction by superoxide radical from stimulated phagocytes, ascorbate, reduced flavins, NO, and xenobiotics.

Copper ions are bound by a specific protein — ceruloplasmin — and transferred to a cell to be bound by metallothionein. Ceruloplasmin is also active as a ferroxidase; it oxidizes Fe^{2+} to Fe^{3+} , thus scavenging Fe^{3+} . Albumin binding Cu is also a good scavenger of peroxy radicals and hypochlorous acid (Frankel, 1998; Bartosz, 1995).

The human antioxidative system also includes dietary antioxidants (Figure 8.4). An example of double protection in the defense system is furnished by vitamin E deficiency when the demand for selenium increases and glutathione peroxidase Se takes over the protection of membrane phospholipids (Spallholz, 1988).

Unfortunately, the activity of the natural defense system weakens with age. At the same time, more frequent are situations that enhance *in vivo* oxidation. For example, stress, excessive physical effort; and numerous external factors, such as pollution (xenobiotics generate radicals), ionizing radiation, excessive availability of transition metals, redox cycling drugs, and tobacco, all play a role as well.

The amount of oxidant necessary in a pathological condition and at a constant presence of situations enhancing *in vivo* oxidation may exceed a common dose by a factor of as much as 100 (Bermond, 1990).

8.6.3 FOOD ANTIOXIDANTS

8.6.3.1 Important Antioxidants Present in Food

Other than drugs, food is the sole source of exogenous antioxidants. These antioxidants are supplied by plants and food additives. The most active dietary antioxidants contained in plants are phenolic and polyphenolic compounds. The most important among them are tocopherols and tocotrienols (tocols), as well as flavonoids. Tocopherols retard the formation of hydroperoxides, inhibit rearrangement of *cis*, *trans* peroxy radicals to *trans*, *trans* isomers (Porter et al., 1995), inhibit peroxide decomposition (Hopia et al., 1996; Makinen and Hopia, 2000), and inhibit the β -scission of alkoxy radicals (Frankel, 1998). The ability of tocopherols to inhibit the formation of hydroperoxides decreases in the order: α -tocopherol > γ -tocopherol > δ -tocopherol at a low initial level of addition (100 ppm), a reverse order of activity being revealed when the initial

tocopherol concentration was 500 ppm and higher. For α -tocopherol and γ -tocopherol to reach maximum antioxidant activity, they must be present in amounts of 100 ppm and 500 ppm, respectively; δ -tocopherol increases with concentration (Kulas, 2001; Kulas and Ackman, 2001). The activity of phenolic antioxidants is often lost at high concentrations, whereupon they may become prooxidative. This is due to their involvement in initiation (Gordon, 1990).

Plants appear to have certain qualitative and quantitative strategies for designing their PUFA/tocopherol mixtures: (1) γ -tocopherol appears to be the main antioxidant for the depot lipids of oilseeds and nuts, especially those rich in linolenic acid; (2) both α - and β -tocopherols and tocotrienols are important for the protection of PUFA of cereal grains; and (3) lipids in the green parts of plants are usually protected by low amounts of α -tocopherol synergized by other phenolic and non-phenolic antioxidants (Kamal-Eldin, 1999). The thermal stability of tocopherols increases in the following order: α -tocopherol < β -tocopherol < γ -tocopherol $\approx$ δ -tocopherol (Yoshida and Tagaki, 1999).

Flavonoids, including flavones, flavonols, isoflavones, and chalcones, with about 8000 compounds known to date, are the most abundant group of natural antioxidants. Flavonoids inhibit oxidation by various mechanisms, including radical ($\text{ROO}\cdot$, $\text{RO}\cdot$, $\text{HO}\cdot$, $\text{O}_2\cdot$, $^1\text{O}_2$) scavenging, protein (enzymes and their metal binding sites, apo-b of LDL) complexing, and synergistic effects by reducing oxidized antioxidants and metal chelation (Frankel, 1999). Flavonoids are poorly soluble in lipids but their antioxidative activity is manifested in aqueous lipid systems (Pratt and Hudson, 1990). The radical scavenger capacity of fruits, vegetables, teas, spices, wines, and juices primarily results from the presence of flavonoids.

Not all the dietary flavonoids are absorbed in the digestive tract. The absorption also depends on the flavonoid source; for example, the quercetin from onions is better absorbed than that supplied by apples (Scalbert and Williamson, 2000). The nature of conjugates may influence the bioavailability of flavonoids, although both nonconjugated and conjugated aglycones may be absorbed in the intestine and enter the bloodstream (Hollman and Katan, 1999).

In addition to free radical scavenging, the biological activity of flavonoids involves their action as protein kinase inhibitors and transcriptional regulators. However, the inhibitory effects of flavonoids on DNA modulating enzymes emphasize that the use of these substances as dietary supplements may also increase the risk of disease development (Moskaug, 2001).

Carotenoids and ascorbic acid belong to the most common auxiliary antioxidants, although carotenoids are thought of as both singlet oxygen quenchers and also, reacting with chain-carrying peroxy radicals or alkyl radical intermediates (Matsushita et al., 2000). Taking carotenoid antioxidant capability into account, Beutner et al. (2001) divided them into three classes: (1) those with very low antioxidative capability; (2) those with good antioxidative and also prooxidative properties (β -carotene, lycopene); and (3) those that are strong antioxidants without any prooxidative nature (astaxanthin, canthaxanthin). Antarctic krill is a good source of the latter two carotenoid classes (Kořakowska, 1991). The bioavailability of carotenoids is discussed by Southen and Faulks (2001).

Ascorbate is a strong reductant with respect to singlet oxygen, superoxide, H_2O_2 , hydroperoxyl radicals, hydroxyl radicals, and hypochlorous acid when occurring at

low concentrations and in the presence of transition metal ions (Fe, Cu, Co), especially in aqueous lipid systems (e.g., milk, mayonnaise, sauces, margarine, and butter). However, it may also act as a prooxidant, in reducing metals (Heinonen et al., 2000).

Taking into account their antioxidative mechanism, Beutner et al. (2001) differentiated between three main categories of antioxidants: (1) *excellent antioxidants* that perfectly quench excited states as well as ground-state radicals (e.g., actinioerythrol, astaxanthin); (2) *good antioxidants* that strongly inhibit peroxide formation but are less efficient in quenching excited states (e.g., flavonols, tocopherols) or lead to considerable degradation of the antioxidant itself (e.g., β -carotene, lycopene); and (3) *moderate antioxidants* that fail to excel in both reactivities (e.g., ζ -carotene, flavone). Hall (2001) and Yanishlieva-Maslarova (2001) have discussed vegetables, fruits, herbs, spices, teas, oilseeds, nuts, cereals, legumes as rich sources of natural antioxidants.

Compared with that in plants, the *post mortem* activity of the defense system in muscles of animals consumed by man is not even capable of preventing oxidation of the muscles' own lipids. However, muscles may show a *post mortem* capability of accumulating antioxidative substances such as products of proteolysis and phospholipid hydrolysis, which, coupled with the simultaneously proceeding changes in prooxidants, may form a new anti-prooxidative system. When subjected to, for example, freezing or cooking, that system may turn out to protect lipids from oxidation (Kořakowska and Szczygielski, 1994; Kořakowska et al., 1995). Mail-lard products formed during heating were found to be active as oxidation inhibitors in several food products (Pokorný, 2001b).

8.6.3.2 Antioxidants Allowed in Food

The lists of antioxidants allowed in food by both the EU and the FDA are similar (see Table 8.2). The major difference involves *t*-butylhydroquinone (TBHQ), which is not permitted in food according to EU regulations and those of several other countries. In general, the regulations of the EU are more restrictive than those of the FDA. Other differences relating to antioxidant applications involve the amounts of antioxidants used and the type of food product that can be protected.

An antioxidant added to food must be effective at low concentrations. Synthetic antioxidants are more efficient than the natural ones. To increase effectiveness, combinations of antioxidants are used, whereby oxidation is inhibited by various mechanisms and at various stages. A synergistic effect is then achieved. In a food product, a synergy is obtained by combining added and inherent antioxidants. However, antioxidants are often applied without sufficient recognition as to the contents of pro- and antioxidants in the matrix to be protected.

The behavior of antioxidants in food and their antioxidative ability can vary markedly, depending on the lipid-containing systems. Food products are predominantly multiphase systems in which the activity of the antioxidants depends on the lipid composition, pH and ionic strength, non-lipid constituents, additives such as emulsifiers, and the presence of metal ions. These factors may affect several important physicochemical properties of the antioxidant, such as interphase transport,

TABLE 8.2
Antioxidants Permitted in Foods

Compound	ADI (mg/kg b.w.)	Maximum Level (mg/kg)	
		EU	FDA
Butylated hydroxyanisole (BHA)	0–0.5	100 E 320	200/500
Butylated hydroxytoluene (BHT)	0–0.125	200 E 321	200/500
Gallates: propyl-, octyl-, dodecyl-	0–2.5	200 E 310-312	200
Tertiarybutylhydroquinone (TBHQ)	0–0.2	Not permitted	200
Tocopherols	0.15–2.0	300 E 306-309	300/ <i>Qs</i>
Erythorbic acid, sodium erythorbate	Not limited	500 E 315-316	<i>Qs</i>
Ascorbic acid, sodium, calcium salts	Not limited	<i>Qs</i> E 301-302	<i>Qs</i>
Ascorbyl palmitate	0–1.25	<i>Qs</i>	<i>Qs</i>
Ascorbyl stearate		E 304	
Ethylenediaminetetraacetic acid (EDTA) and calcium disodium salts	2.5	250 E 385	340

Note: ADI = Acceptable daily intake; b.w. — body weight.

UE = European Union (Directives 95/2/EC, 98/72/EC).

FDA = Food and Drug Administration (Office of Regulatory Affairs Inspectional References: IOM-2001).

Qs = *quantum satis*.

surface or interface accessibility, redox potential, hydroxylic hydrogen abstraction kinetics, and/or the formation of hydrogen bonding (Schwarz, 2001). For this reason, paradoxically, polar antioxidants may be more effective in bulk lipids, whereas nonpolar antioxidants may be more active in emulsified media (Frankel, 1998; Cuvelier et al., 2000).

In vitro antioxidant activity is not identical to that *in vivo*, and is insufficiently known in all the systems. The antioxidant “carry-through” concept should not be limited to the technological process only, but ought to be extended to the human body. Knowledge pertaining to the persistence of feed antioxidants in meat is insufficient as well (He and Ackman, 2000).

Signals such as a change in the body tocopherols composition at a high vitamin E consumption or a reduction of the necrotic effect induced by EPA in lymphoma cell, brought about by antioxidants (Heimli et al., 2001) suggest that antioxidants be applied with caution in view of their potential effect on the organism’s pro- and antioxidative balance.

8.7 METHODS FOR THE DETERMINATION OF LIPID OXIDATION

8.7.1 INTRODUCTION

The methods used to determine lipid oxidation can be divided into:

1. Oxidation product (amount and composition) assay techniques
2. Indirect methods to determine the loss of unsaturated FA, vitamins, and carotenoids
3. Noninvasive instrumental methods

In the assessment of lipid oxidation in foods, when the amount of lipids is small and/or they are strongly bound to non-lipid components, extraction is the key analytical stage. To minimize lipid changes during separation, a compromise must be reached instead of striving for a full lipid extraction using drastic conditions. Usually, extraction is performed with a mixture of polar and nonpolar solvents, such as, for example, in the Folch, Bligh–Dyer, and other methods. However, it excludes from the analysis those lipids that form — via oxidation products — strong bonds (including covalent ones) with proteins and starch. For this reason, noninvasive, requiring no lipid extraction, instrumental methods are being explored. However, these should be specific and quantitative, and should provide more information than traditional techniques.

Due to the complexity of the lipid oxidation mechanism in foods, more than one method is usually applied. Traditionally, the initial oxidation products are assayed and the secondary products, characterizing sensory changes in lipids, are included in the analysis as well.

8.7.2 TRADITIONAL METHODS

8.7.2.1 Peroxide Value (PV)

Determination of PV is the oldest method of lipid oxidation evaluation; it is carried out using the iodometric technique. The result depends on the amount of oxygen, availability of light, and the number of double bonds in the substrate. For this reason, it is mandatory to adhere to the assay timing conditions of individual operations and the specific glassware required. To improve the sensitivity of the method, several iodine spectrophotometric and potentiometric techniques have been developed (Oishi et al., 1992; Lovaas, 1992). The thiocyanate technique of peroxide determination is ten times more sensitive than the iodometric technique; it involves $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ oxidation. The red Fe^{3+} complexes formed are determined spectrophotometrically at 470 nm. The assay can be performed directly in the extract, e.g., chloroform, obtained from the Bligh–Dyer lipid extraction. Fish lipid oxidation assay was found to require no more than 20 mg, with a detection limit of less than 3 μg oxygen. The oxidation of Fe^{2+} to Fe^{3+} by peroxides involving the formation of a color complex, using the dye xylenol orange is also applied. (Wolff, 1994; Grau et al., 2000).

8.7.2.2 Carbonyl Compounds

Traditionally, the extent of oxidation is determined by the peroxide value and some of the carbonyl compound assays. Most involve the formation of color products that result from the reaction of the carbonyl group with the amine group of a cyclic leuco-compound. The most popular old method involves determination of carbonyl compounds in reaction with 2,4-dinitrophenylhydrazine (carbonyl value). The reaction with benzidine (benzidine value) was used for the first time by Wode in 1957; Soviet authors referred to it as the Lyubavina technique. This technique was abandoned due to carcinogenicity of benzidine.

The most popular method involves 2-thiobarbituric acid (TBA); two molecules of 2-thiobarbituric acid are condensed with malonaldehyde. The emergent chromogen — the two tautomeric structures of the red TBA-malonaldehyde adduct — is determined at 532 nm, and also often at 450 nm, to determine alkenals and alkanals, respectively. The qualitative Kreis test was based on a similar principle; it involved detection of the epihydrine aldehyde — a tautomeric malondialdehyde — in a color reaction with resorcline or phloroglucinol. The popularity of the TBA test stems from a correlation between the results and sensory evaluations. Paradoxically, this is related to the most important drawback of the TBA technique — its lack of specificity. In addition to the reaction with malonaldehyde, TBA forms compounds of identical color with other aldehydes and ketones, products of aldehyde interaction with nitrogen compounds, and also with saccharides, ascorbic acid, creatine, creatinine, trimethylamine oxide, trimethylamine, proteins, and amino acids. For this reason, the TBA test may even be treated as a proteolysis indicator (Kořakowska and Deutry, 1983). Recently, TBA-reactive substances (TBARS) were introduced, primarily to stress that the reaction involves hydroperoxides in addition to aldehydes. Due to the nonspecificity of the TBARS test, its results reflect the rancidity of food better than other conventional methods, especially off-flavor, which is caused by volatiles from lipids as well as being affected by products of lipids' interaction with nitrogenous compounds.

There have been numerous modifications made to the TBA test, which was originally developed by Sinhuber in 1958. The modifications provide evidence of both the insufficiency and attractiveness of this test. The reaction proceeds by heating in an acidic medium to release malonaldehyde from its complexes with protein, although alkaline medium is used as well. The following techniques can be used: (1) direct heating of a sample with TBA and measurement of the distillate absorbance; (2) distillation, most frequently with water vapor, of volatile compounds, which are then subjected to hot reaction with TBA; (3) reaction in trichloroacetic acid extract; and (4) techniques based on single phase (e.g., with isopropanol) or in a two-phase system, followed by spectrophotometric assessment of the TBA reaction products in the aqueous layer. High-performance liquid chromatography (HPLC) is used as a more specific means of determining malonaldehyde (MDA) or the MDA-TBA complex (Whang, 1999; Whang and Kim, 2000). Park (1995) discussed in detail TBA determination by HPLC.

Among the simple methods for carbonyl compound determination, the anisidine value (AsV) is quite popular. The method involves a color reaction between carbonyl compounds and *p*-anisidine. The anisidine value is a combined measurement of

mostly 2-alkenals, 2,4-dienals, and less saturated aldehydes. The pink-red color is measured at 350 nm. The technique is convenient when applied to oils and edible fats; however, when lipids are extracted from a product, a correction for the product's own absorbance may be a source of error in AsV determination.

PV and AsV allow one to calculate total oxidation, determined as $\text{Totox} = 2 \times \text{PV} + \text{AsV}$.

8.7.2.3 Sensory Analysis

Rancidity is the sensory evidence of lipid oxidation. During the sensory analysis of food, rancidity may be signaled by a change in color to colorless, yellowing, or browning, as well as off-odor. Rancid off-flavors are detected at the second stage of chemosensory analysis, in the mouth. The product, diminished, mixed with the saliva, having an increased surface area, and exposed to increasing temperature, gradually releases volatile compounds, particularly polar ones. These volatiles are carried up into the nose and stimulate the olfactory receptor cells. Thus, volatile compounds play an important role in the off-flavor as well, because the off-flavor is caused by a complex mixtures of volatiles and nonvolatiles.

The sensory effects of volatile compounds depend on the chain length, type of functional group, and positional and geometric isomerism. The same aldehyde can produce different odors and flavors in aqueous and oil solutions, the threshold being much lower in the former medium. In oil-in-water emulsions such as mayonnaise, the oil-water partition coefficient ($P_{o/w}$) of a given compound affects its sensory impact on the emulsion (Jacobsen, 1999).

In addition to lipid oxidation and hydrolysis reactions, the rancid off-flavor results from interactions, particularly those between aldehydes and nitrogen-containing compounds. However, for some of these interactions, it is impossible to evaluate the off-flavor.

Flavors associated with oxidized lipids are described as rancid, metallic, soapy, tallowy, oily, painty, fishy, grassy, cucumber-like, beany, fried, or pungent. Combining the gas chromatography/mass spectrometry (GC/MS) assay of volatile compounds with sensory analysis of compounds released from the column has made it possible to ascribe them to individual flavors. Having used direct chromatography involving trap and purge methods, St. Angelo (1996) was able to describe volatile compound profiles formed during lipid oxidation in different groups of food products.

For all practical purposes, the sensory analysis of rancidity of food products includes, in addition to using the rancidity intensity scale, a profile of off-flavor with the attributes listed above and/or more characteristic of the product. In the case of fish products, for example, it is necessary to carry out separate profile analyses for off-odors and off-flavors.

8.7.3 VOLATILE COMPOUND ANALYSIS

The simple AsV and TBA tests are frequently replaced by a qualitative-quantitative analysis of volatile compounds using a GC/MS detector. Headspace GC/MS

and a variant of the method in the form of electronic nose provide more information on the progress of the oxidation (e.g., during storage of fish lipids) than do classical methods such as PV, AsV, and TBARS. However, a correlation was found between some specific volatile compounds and the results of classical analysis (Vogt et al., 2001).

Volatile compound analysis is treated as an objective off-flavor determination. The critical analytical problem is the extraction of these compounds from a lipid matrix and concentration to detectable levels such that no excessive change in their composition is induced. Analyses of odor-producing compounds require a high-resolution chromatographic column. The “purge and trap” technique, as well as static and dynamic headspace techniques, are used. A headspace solid-phase microextraction (SPME) technique is being increasingly applied. This technique has given satisfactory detection limits (less than 1 µg/l) for a number of aldehydes, ketones, hydrocarbons, and alcohols (Keszler et al., 1998; Jeleń et al., 2000). For the determination of hexanal and pentanal, this technique was used to follow changes during the storage of cooked turkey; the results obtained were in good agreement with those obtained using the TBA test (Brunton et al., 2000). The technique, when compared with traditional static headspace techniques gave reduced recovery and higher detection limits for compounds with higher volatility, but made the measurement of high-molecular-weight aldehydes possible (Dole-schall et al., 2001).

The separation of aldehydes and ketones can also be performed using an HPLC system. For structural identification, the configuration with an MS system is recommended.

Smith et al. (1999) proposed determination of hexanal, with a detection limit of 0.7 ng hexanal/mL in the form of hexanal–lysine adducts with carrier protein, by ELISA.

8.7.4 INSTRUMENTAL DETERMINATION OF THE PRIMARY PRODUCTS OF LIPID OXIDATION

Radicals can be detected with electron spin resonance (ESR) spectroscopy, which involves the detection of unpaired electrons. The short half-life of radicals requires a complex sample preparation that consequently has an effect on the analysis. The method was proposed by the CEC Community Bureau of Reference for the detection of irradiated food (Raffi et al., 1992).

Hydroperoxides can be analyzed by HPLC with detection at 240 nm and with MS; GC/MS can also be used. However, these methods — particularly when used without a suitable internal standard — result in poor reproducibility and semiquantitative data. Catalytic hydrogenation is used for stabilization. As a result, the hydroperoxy groups are reduced to the corresponding hydroxy groups and double bonds are removed; subsequently, GC/MS can be applied. Wilson et al. (1997) have refined the method by subjecting the resultant monohydroxy FA to on-column methyl ester derivatization with tetramethylammonium hydroxide. The resulting methoxy FA methyl esters were analyzed by GC/MS with detection limits of 10 ng and 0.2 ng for a single isomer.

8.7.5 OTHER INSTRUMENTAL METHODS

8.7.5.1 Ultraviolet (UV) Spectroscopy

The UV lipid spectrum (Figure 8.6) is primarily used to determine conjugated bonds. The absorption peaks of dienes and trienes appear at 233 and 268 nm, respectively. The absorption maxima of peroxide and hydroxyl groups in FA occur at the same wavelengths. The presence of a carbonyl group in the immediate vicinity of a conjugated bond results in a shift in the conjugated acid maximum because of the additional double bond. Assays at 233 and 268 nm can be a combined measure of FA with conjugated bonds and products of oxidation. Conjugated FA contribute only partially to the absorbance observed (Tynek and Drozdowski, 1998). The method is frequently applied due to its facility and, despite ambiguities in the interpretation of results, can be useful in monitoring changes in lipids under different conditions (Figure 8.6). Conjugated dienes and trienes were found to correlate well with peroxide value, particularly under conditions of photo- and thermal oxidation (Table 8.3).

8.7.5.2 Infrared (IR) Spectroscopy

Lipid oxidation can be followed with Fourier transform infrared (FTIR) spectroscopy within the range of 4000 to 700 cm^{-1} (Figure 8.7). Attempts to replace the classic PV

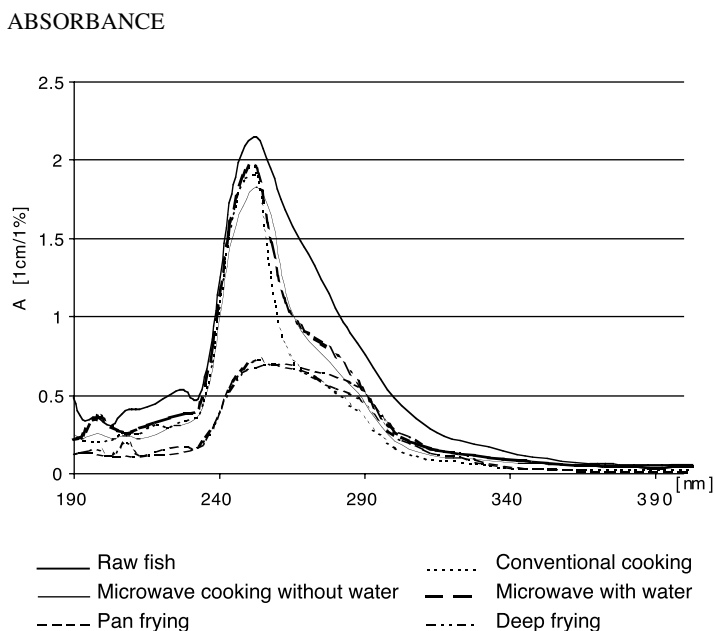


FIGURE 8.6 UV–VIS lipid spectra of raw and heat-treated fish muscle tissue. (From Kołakowska, A., Domiszewski, Z., Bienkiewicz, G., and Szczypiński, M. 2001. Effects of thermal treatment of Baltic herring and sprat on n-3 PUFAs and lipid oxidation, presented at *Lipidforum*, 21st Nordic Lipid Symposium, Bergen, June 5–8.)

TABLE 8.3
Correlation between Indicators of Oxidation in Bream Lipids

Conditions of Lipids Oxidation		PV	AsV	232 nm	268 nm	Ex 365nm	Ex 393nm	Ex 327nm	Em 463nm	Em 415nm	ΔF
UV irradiation/120 min (analyzed after: 0, 30, 60, 120 min)	PV	1									
	AsV	0.96	1								
	232 nm	0.93	0.86	1							
	268 nm	0.93	0.86	0.99	1						
	Ex 365nm	0.88	0.88	0.78	0.84	1					
	Ex 393nm	0.90	0.85	0.98	1.00	0.85	1				
	Ex 327nm	0.71	0.63	0.86	0.90	0.76	0.93	1			
	Em 463nm	-0.82	-0.73	-0.97	-0.94	-0.60	-0.92	-0.82	1		
	Em 415nm	-0.77	-0.67	-0.95	-0.92	-0.58	-0.92	-0.86	0.99	1	
	ΔF	0.95	0.87	0.99	0.98	0.78	0.96	0.82	-0.96	-0.93	1
Temp. 60°C/60 min (analyzed after: 15, 30, 60 min)	PV	1									
	AsV	0.47	1								
	232 nm	0.95	0.72	1							
	268 nm	0.87	0.62	0.93	1						
	Ex 365nm	1.00	0.29	0.97	0.99	1					
	Ex 393nm	0.85	0.67	0.93	1.00	1.00	1				
	Ex 327nm	0.91	0.65	0.96	0.99	1.00	0.99	1			
	Em 463nm	-0.76	-0.70	-0.88	-0.98	-1.00	-0.99	-0.97	1		
	Em 415nm	-0.78	-0.59	-0.86	-0.99	-0.96	-0.99	-0.96	0.99	1	
	ΔF	0.87	0.75	0.97	0.98	0.97	0.99	0.99	-0.97	-0.95	1

Temp. room, light/21 days (analyzed after: 0, 1, 2, 4, 5, 7, 11, 14, 21 days)	PV	1									
	AsV	0.54	1								
	232 nm	0.19	0.60	1							
	268 nm	0.22	0.43	0.87	1						
	Ex 365nm	0.03	0.06	0.64	0.88	1					
	Ex 393nm	0.64	0.60	0.74	0.78	0.63	1				
	Ex 327nm	-0.67	-0.80	-0.51	-0.30	0.03	-0.70	1			
	Em 463nm	0.20	-0.24	-0.47	-0.55	-0.45	-0.20	0.14	1		
	Em 415nm	0.02	-0.30	-0.74	-0.91	-0.92	-0.65	0.15	0.68	1	
Temp. room, dark/21 days (analyzed after: 0, 1, 2, 4, 5, 7, 11, 14, 21 days)	δF	0.83	0.83	0.51	0.44	0.14	0.83	-0.92	-0.05	-0.25	1
	PV	1									
	AsV	0.87	1								
	232 nm	-0.01	0.36	1							
	268 nm	0.23	0.45	0.90	1						
	Ex 365nm	-0.29	-0.18	0.72	0.76	1					
	Ex 393nm	0.59	0.76	0.76	0.87	0.42	1				
	Ex 327nm	-0.72	-0.89	-0.39	-0.36	0.23	-0.73	1			
	Em 463nm	-0.23	-0.32	-0.04	-0.17	0.01	-0.04	-0.09	1		
	Em 415nm	-0.47	-0.61	-0.12	-0.27	0.13	-0.29	0.25	0.92	1	
	δF	0.90	0.90	0.31	0.46	-0.11	0.82	-0.87	-0.02	-0.31	1

Note: δF = fluorescence ratio.

Em = fluorescence at maximum excitation.

Ex = fluorescence at maximal emission.

From Kołakowska, A. and Alchimowicz, M., unpublished data.

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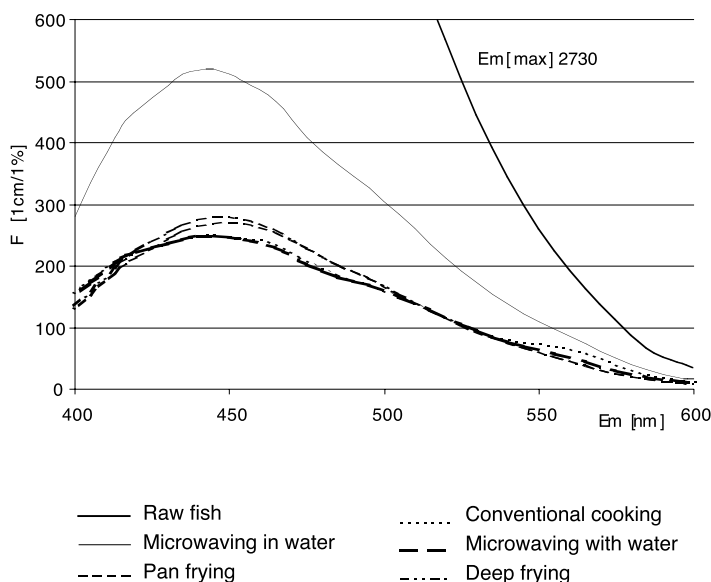


FIGURE 8.7 FT-IR (HATR) lipid spectra of raw and heat-treated fish muscle tissue. (From Kołakowska, A., Domiszewski, Z., Bienkiewicz, G., and Szczypiński, M. 2001. Effects of thermal treatment of Baltic herring and sprat on n-3 PUFAs and lipid oxidation, presented at *Lipidforum*, 21st Nordic Lipid Symposium, Bergen, June 5–8.)

and AsV techniques with the FTIR method have been made for almost 30 years because hydroperoxides and carbonyls show characteristic O – H stretching absorption bands. Van de Voort et al. (1994) described a relationship between the spectral range of 3750 to 3150 cm^{-1} and PV in a series of vegetable oils. The Fourier transform near infrared (FT-NIR) spectral region from 1350 to 1480 nm (13,500 to 14,800 cm^{-1}) was used for predicting PV in palm oil (Moh et al., 1999) and a mid-IR method was proposed. The method is based on reaction of triphenylphosphine with hydroperoxides to form triphenylphosphine oxide, which has a unique and intense band at 542 cm^{-1} (Li et al., 2000).

Dubois et al. (1996) described the application of FTIR, instead of determining AsV, to thermally stressed oils. A strong ($R^2 = 0.99$) linear relationship between spectra, between 2800 and 2600 cm^{-1} (saturated aldehydes) and between 1750 and 1600 cm^{-1} (unsaturated aldehydes) and AsV results, was observed (Che Man and Setiowaty, 1999). FTIR can be used to evaluate oil changes during frying, including *trans* detection (Goburdhun et al., 2001). The most important advantage of IR spectroscopy methods is their rapidity and facility. When the horizontal attenuated total reflectance (ATR) measurement technique is used, a drop of lipid extract can be spread directly onto the surface of the ATR crystal. Recently, the American Oil Chemist's Society (AOCS) adopted this method for the determination of isolated *trans* isomers in fats and oils (Official Methods, 1998). Supercritical fluid extraction coupled directly to IR spectroscopy (Liescheski, 1996) can be applied as well.

It would be most desirable to study lipid oxidation in intact food products without the extraction or sample preparation steps currently necessary (e.g., pelletization, freeze-drying). Perhaps improvements in techniques such as the Fourier transform infrared photoacoustic spectroscopy (Yang and Irudayaraj, 2000) will open such possibilities.

8.7.5.3 Luminescence Spectroscopy

Chemiluminescence is based on light emission from an excited oxygen species; for example, singlet oxygen and triplet carbonyls. Measurement of the ultra-weak chemiluminescence that accompanies autoxidation can be a potentially useful method, but its sensitivity is low. However, a strong light emission is achieved by adding sodium hypochlorite to an oxidized lipid. Consequently, hypochlorite activation can aid in detecting small amounts of lipid hydroperoxides (Burkow et al., 1992). However, evaluation of lipid oxidation and lipid–protein interactions involves fluorescence, a kind of luminescence. When an atom (or a molecule), excited — usually with UV radiation — reverts to its ground state, it simultaneously emits radiation of a longer wavelength (E_m) than that of the excitation wavelength (E_x). Fluorescence can be characterized by analyzing spectra of absorption (E_x), emission (E_m), at a defined E_x and/or E_m , fluorescence intensity

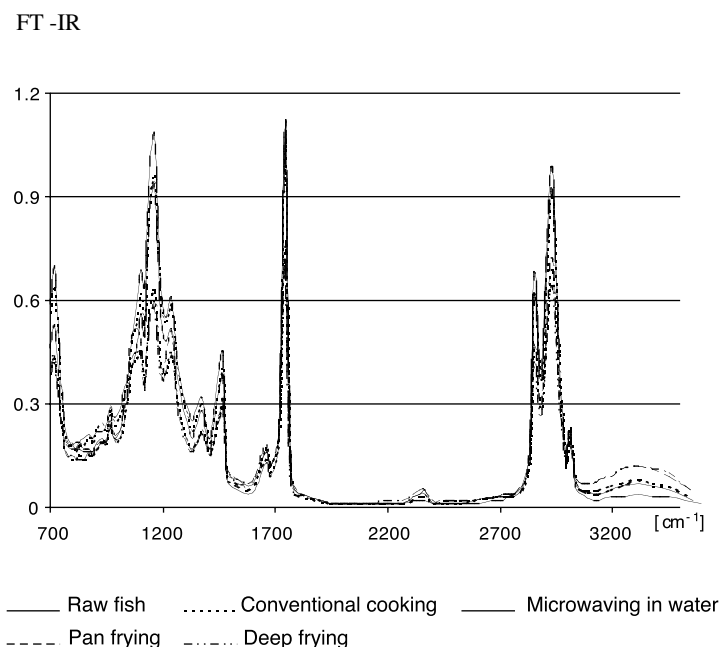


FIGURE 8.8 Fluorescence emission (E_x 365 nm) of lipid in raw and heat-treated fish muscle. (From Kołakowska, A., Domiszewski, Z., Bienkiewicz, G., and Szczygielski, M. 2001. Effects of thermal treatment of Baltic herring and sprat on n-3 PUFAs and lipid oxidation, presented at *Lipidforum*, 21st Nordic Lipid Symposium, Bergen, June 5–8.)

at a defined excitation wavelength (Ex), fluorescence absolute quantum efficiency, or time elapsed between excitation and emission (Deshpande, 2001). The advantages of fluorescence-based analysis include its higher sensitivity relative to that of UV/VIS spectrophotometry and its better selectivity, because some compounds are only fluorescent. Fluorophores are planar compounds containing conjugated bonds. There is, however, no agreement as to which lipid oxidation products are fluorescent. In addition, fluorescence can be quenched by various agents. This quenching phenomenon can be prevented to some extent by the use of certain experimental procedures (Hidalgo et al., 1999; Robinson, 2000). In addition, the quenching itself can be monitored.

Most frequently, fluorescence is measured at a defined wavelength, with a certain emission wavelength. For example, Em 463 nm/Ex 393 nm produced a close correlation with PV for vegetable and fish oils, mainly during catalyzed oxidation and during thermal treatment of fish muscle tissue (Kořakowska et al., 2001) (Figure 8.8). Aubourg and Gallardo (1997) evaluated fish lipid oxidation by applying two Ex/Em maxima ratios (i.e., 393/463 nm and 372/415nm). Direct measurement of fluorescence to determine the amount of lipid oxidation products can be preceded by converting them to fluorophores via, for example, interaction with propylamine (Aubourg, 2000). Solid-sample fluorescence spectroscopy appears promising; it can be used to evaluate the degree of lipid oxidation in food products, such as meat, fish, and milk powder, directly from the intact product, without lipid extraction (Wold, 2001).

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9 Principles of Lipid Analysis

David S. Nichols

CONTENTS

- 9.1 Introduction
- 9.2 Extraction of Lipids from Foods
 - 9.2.1 Introduction
 - 9.2.2 Solvent Extraction
 - 9.2.3 Supercritical Fluid Extraction
 - 9.2.3.1 Description of a Supercritical Fluid
 - 9.2.3.2 Supercritical Fluid Extraction: Pros and Cons
- 9.3 Principles of Lipid Class Separation
 - 9.3.1 Introduction
 - 9.3.2 Solvent Fractionation
 - 9.3.3 Chromatographic Separation
 - 9.3.3.1 Introduction
 - 9.3.3.2 Column Chromatography
 - 9.3.3.3 Thin-Layer Chromatography
- 9.4 Direct Analysis of Lipid Classes
 - 9.4.1 Introduction
 - 9.4.2 Thin-Layer Chromatography–Flame Ionization Detection
 - 9.4.3 High-Performance Liquid Chromatography
 - 9.4.4 Mass Spectrometry
 - 9.4.4.1 Introduction
 - 9.4.4.2 Electron Impact Ionization
 - 9.4.4.3 Chemical Ionization
 - 9.4.4.4 Fast Atom Bombardment
 - 9.4.4.5 Thermospray/Electrospray Ionization
- 9.5 Conclusion
- References

9.1 INTRODUCTION

There are several key developments in the history of lipid analysis that have shaped the current knowledge of lipids in food systems. The first of these is the development of

efficient, quantitative solvent extraction systems for the recovery of pure lipid extracts from a variety of food and natural sources. While taken somewhat for granted today, significant research was devoted to this area in the 1940s, 1950s, and 1960s. Without the establishment of this fundamental area, much of what we know today would not have followed. From these discoveries came the techniques required for the chemical separation of lipid types based on the solubility of different lipids in various solvent systems. These techniques continue to underpin the analysis of lipids today.

Second, major advances were achieved with the development of chromatographic techniques for the separation and identification of lipids. What can now be considered the scientific “field” of chromatography had many of its early advances in the development of lipid separation techniques, from paper chromatography and thin-layer chromatography through to gas chromatography and high-performance liquid chromatography.

Finally, in recent years the continuing revolutions in instrument hardware have had a profound influence on some areas of lipid analysis. The development of supercritical fluid extraction of lipids now provides an alternative to wet solvent extractions, which have basically remained unchanged in principle and practice since the 1960s. Advances in mass spectrometry and analytical chromatography now allow the direct analysis and identification of complex lipids from total lipid extracts without the requirement for solvent fractionation.

9.2 EXTRACTION OF LIPIDS FROM FOODS

9.2.1 INTRODUCTION

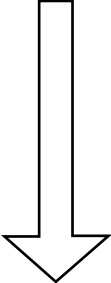
The initial step in the analysis of any lipids from foods is to quantitatively separate the lipid components from other protein, amino acid, carbohydrate, and aqueous ingredients. This should be done in a manner that does not degrade complex lipids or introduce sources of contamination. Two major strategies are discussed for the extraction of lipid components: (1) solvent extraction using various combinations of organic solvents and (2) a more recent innovation known as supercritical fluid extraction.

9.2.2 SOLVENT EXTRACTION

Solvent extraction systems rely on solubility differences between lipids and other food components in a particular system. These solubility differences primarily relate to issues of charge or polarity and the nature of the association between lipids and the other components within the material to be extracted.

However, because lipids represent such a diverse range of compound types, there is also the issue of ensuring that the solvent extraction is capable of obtaining all the lipids present, to yield a total lipid extract or total solvent extract. In essence, this issue is related to the relative polarity of the lipid types and extraction solvents ([Table 9.1](#)). Neutral, or nonpolar, lipids such as hydrocarbons, sterol esters, acylglycerols, and carotenoids can be bound through noncovalent interactions such as Van der Waals forces and through hydrophobic associations between hydrocarbon chains of lipids and hydrophobic protein

TABLE 9.1
The General Gradation of Polarity among Common Lipid Classes and Organic Solvents

Lipid		Solvent
	Least Polar	
Hydrocarbons		Hexane
Wax esters		Cyclohexane
Aldehydes		Diethyl ether
Triacylglycerols		Chloroform
Fatty alcohols		Acetone
Fatty acids		Acetonitrile
Sterols		Ethanol
Diacylglycerols		Methanol
Monoacylglycerols		
Phospholipids		
	Most Polar	

domains within foods. Such lipids can be extracted with relatively nonpolar solvents such as diethyl ether or chloroform. Polar lipids such as phospholipids, glycolipids, and steroids can be bound through hydrogen bonding, and electrostatic and hydrophobic interactions to protein components and are usually membrane associated. The extraction of these lipids requires more polar solvents such as ethanol or methanol to disrupt these molecular interactions. Covalent associations can also exist between fatty acids (FA) (hydroxy or complex branched-chain) that are covalently bound as esters, amides, or glycosides to polysaccharide components. The extraction of bound lipid residues requires initial chemical cleavage through acid or alkaline hydrolysis.

Hence, a mixture of organic solvents, with a range of polarities, is necessary to ensure comprehensive extraction of noncovalently bound lipids. Many methods from the 1940s onward have utilized mixtures of chloroform and methanol for quantitative extraction of lipids. However, the use of more polar organic solvents such as methanol also results in the extraction of unwanted contaminants in the form of sugars, amino acids, and salts. While these contaminants can be removed by purification or “washing” of the solvent extract, there exists a well-established extraction protocol that incorporates a partitioning of the solvent extract against an aqueous phase for the removal of contaminants.

The Bligh and Dyer extraction procedure is an example of a one-step solvent extraction and purification procedure to achieve a quantitative lipid extract. The procedure was originally developed for the extraction of fish tissue, but is equally applicable to the extraction of a wide range of materials with various modifications. The basis of the extraction utilizes a mixture of chloroform, methanol, and water, exploiting the one to two phase relationship of differing proportions of this mixture. It was experimentally determined that the solvent ratio of 2:1:0.8, (v/v/v) of methanol:chloroform:water was not only miscible in a single phase but gave near-quantitative extraction of lipids (approximately 94% from fish tissue — as good

as any other method). Addition of further 1:1 volumes of chloroform:water created a biphasic system, with a (predominantly) chloroform layer containing lipids and a (predominantly) methanol/water layer containing the non-lipid contaminants. Bligh and Dyer noted that around 1% of the total lipid from fish tissues may be lost by partition into the methanol/water phase, and that this fraction contained lipids of particularly high polarity. More recent investigations of the solvent ratios demonstrated that a small increase in the yield of total lipid could be achieved by increasing the proportion of methanol remaining in the chloroform phase and that this was due predominantly to an increased yield of phospholipids.

Such solvent systems are calculated on the basis of the total volume of water in the extraction system, including any moisture content that may be present in the material to be extracted. It is therefore common to either dehydrate the sample prior to extraction or derive an estimate of the moisture content to appropriately adjust the volume of water added.

9.2.3 SUPERCRITICAL FLUID EXTRACTION

9.2.3.1 Description of a Supercritical Fluid

The manipulation of lipids with supercritical fluids is based on the discovery that, in general, supercritical fluids can more easily dissolve compounds than liquid solvents. Until 1869, only three phases of matter were described. At that time, a critical phenomena was discovered in the phase behavior of carbon dioxide. Under the combined extremes of pressure and temperature, a fourth phase was discovered: the supercritical fluid (SF). The generalized phase diagram for a pure substance is shown in [Figure 9.1](#). The phase behavior one is usually accustomed to follows the solid arrow, where at normal pressures a substance will respond to increased temperatures by first crossing the equilibrium point between a solid and a liquid (line B–T) and then the equilibrium line between a liquid and a gas (line T–CP). It is also possible for a substance to pass directly from a solid to the gas phase (sublimation; line A–T) under conditions of combined low pressure and temperature. However, under conditions of combined high pressure and temperature, a critical point is reached (CP) where the properties of the substance become intermediate between those of the liquid and gas phases. The SF possesses a density roughly two orders of magnitude higher than the gas phase and about one third that of the liquid, while the properties of the SF as a solvent are comparable to that of the liquid. Hence, while there is no decrease in solvation properties, an SF offers benefits in density (read viscosity and volume) over a liquid.

9.2.3.2 Supercritical Fluid Extraction: Pros and Cons

Several compounds have been investigated for SF extractions. However, when efficiency, cost, and safety issues are considered, the sole viable option is often concluded to be carbon dioxide. Because the critical point temperature (T_c) and critical point pressure (P_c) values for carbon dioxide are 31°C and 73 atm, respectively, a dedicated extraction instrument with high-pressure pumps and control valves is required for SF extractions. The benefit of SF procedures is the liberation of the extractions from

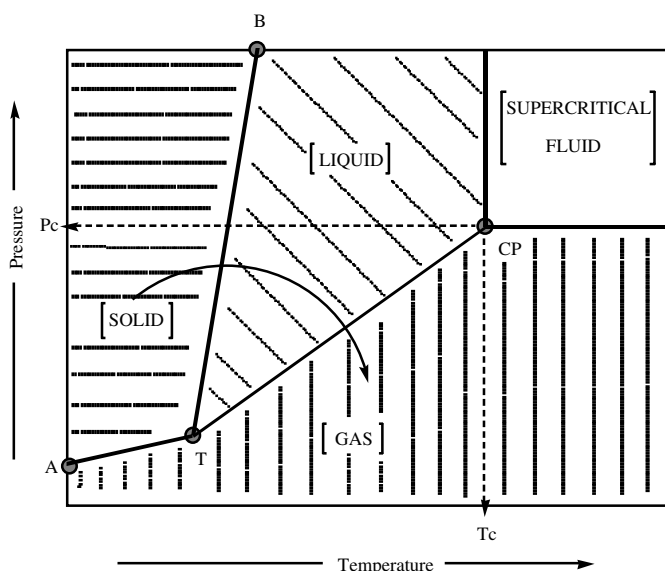


FIGURE 9.1 Idealized phase diagram for a pure compound describing the formation of a supercritical fluid. Abbreviations: T, triple point; CP, critical point; Pc, critical point pressure; Tc, critical point temperature.

(relatively) large volumes of chlorinated organic solvents. Increasing concerns over solvent toxicity and disposal have fueled the development of SF techniques. This has been particularly true in the industrial setting where the problems of organic solvents are magnified and the cost of dedicated high-pressure extraction equipment can be more easily justified.

The low polarity of carbon dioxide appears as a limitation to its use in extraction technologies requiring a total lipid extract, because more polar lipids cannot be extracted. Many of the extraction protocols used for these applications rely on a “modified” solvent system based on the addition of small volumes of a polar organic solvent (methanol or ethanol) to the extraction system. Such application for the extraction of lipids in the food industry is widespread, particularly with plant-based oils. However, the potential problems with the extraction of total lipids by SF has also yielded specific applications in the selective extraction or concentration of lipid components. Examples exist of the separation of FA from triacylglycerols (TAG) and squalene from sterols.

9.3 PRINCIPLES OF LIPID CLASS SEPARATION

9.3.1 INTRODUCTION

Total lipid extracts from natural or food sources are likely to contain a diverse mixture of lipid classes. While one or two lipid classes may dominate on a proportional basis, it can often be the amount or composition of minor lipid classes that are of particular interest. Conversely, the presence of minor lipid classes can interfere

with the determination of the properties of interest from the major classes. In such cases, fractionation of lipid classes is often required to enable individual analyses of the lipid present to be undertaken. The type of fractionation approach can also depend on the characteristics of the lipid extract. For example, those from animal sources are likely to have a high proportion of polar lipids, with the remainder being neutral or nonpolar. Extracts or oils from plant seeds are likely to be the reverse. There are two primary strategies for the separation of lipid classes: (1) solvent fractionation based on the specific chemical properties of certain lipid classes and the resultant differences in solubility; and (2) a suite of chromatographic techniques based on differences in lipid class polarity.

9.3.2 SOLVENT FRACTIONATION

There are two main procedures for the solvent fractionation of total lipid extracts (Figure 9.2). Both are primarily aimed at the isolation of total FA from the lipid extract for analysis. By reaction of lipid classes containing acyl residues, the FA liberated can be separated from the remaining neutral lipids (Figure 9.2, route A), or derivatized directly to a form that can be analyzed together with neutral lipid components (Figure 9.2, route B).

Route A follows the protocol of an initial saponification reaction. Here, a base-catalyzed hydrolysis cleaves the ester linkages of acyl residues in phospholipids, acylglycerols, and wax esters to yield an amalgamation of total FA (as salts), long-chain alcohols (if wax esters were present), and remaining lipid classes that do not contain ester linkages and are therefore unaffected by the reaction process (hydrocarbons, isoprenoids, ether linkages of O-alkyl residues). The high polarity of the FA allows them to be partitioned into an aqueous extract, leaving the isolated fraction of “nonsaponifiable” lipids. Following acidification of the FA fraction, they may be re-extracted back into an organic solvent and further derivatized for analysis, e.g., to FA methyl esters for gas chromatographic analysis.

Route B does not strictly represent a fractionation protocol but is worthy of highlighting in the instances where an alternative “rapid” procedure is preferred for the analysis of total FA. Here, an acid-catalyzed transesterification can be undertaken, converting total ester-linked acyl residues directly to their methyl esters. As a by-product, nonsaponifiable lipids are also retained in this fraction (hydrocarbons, isoprenoids) and analyzed simultaneously by chromatographic techniques. While more rapid, the technique yields a more complex sample for analysis.

Figure 9.3 gives an example of possible results from solvent fractionation procedures. A total FA fraction has been isolated from the blue whale subcutaneous blubber sample and derivatized as methyl esters for gas chromatographic analysis (route A). The sample shows a well-separated profile of FA components and no other lipids. The dorsal blubber sample represents an example in which the neutral lipid fraction has been collected. Here, nonsaponifiable lipids are retained in the analysis fraction, in this example ether-linked 1-O-alkyl-*sn*-glycerols derived from 1-O-alkyl-*sn*-2,3-diacylglycerols originally present in the blubber sample. However, there is also the carry-over of some FA liberated from the ester linkages of complex lipids during the saponification procedure. While such carry-over is

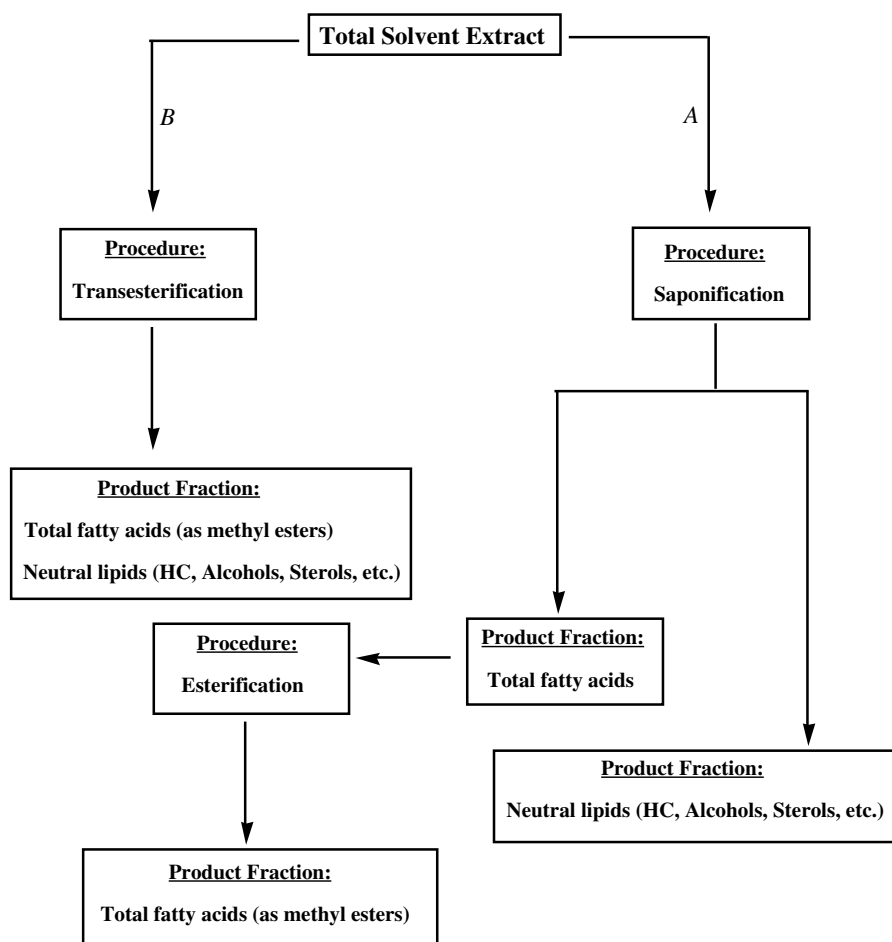


FIGURE 9.2 A flow diagram outlining two strategies for the solvent fractionation of lipid classes based on the hydrolytic reaction of O-acyl ester lipids.

uncommon in the fractionation of most lipid extracts, it may occur due to inefficient manipulation of the solvent fractions or where the proportion of FA liberated during saponification far exceeds that of nonsaponifiable components (in this example, the lipid extract consisted of 92% TAG, 5% FA, and only 1% 1-O-alkyl-*sn*-2,3-diacylglycerol).

9.3.3 CHROMATOGRAPHIC SEPARATION

9.3.3.1 Introduction

The basis of all chromatographic techniques may be considered as the interaction between lipid molecules and a solid matrix of specific character, termed the stationary phase. These interactions are further influenced by the medium in which the

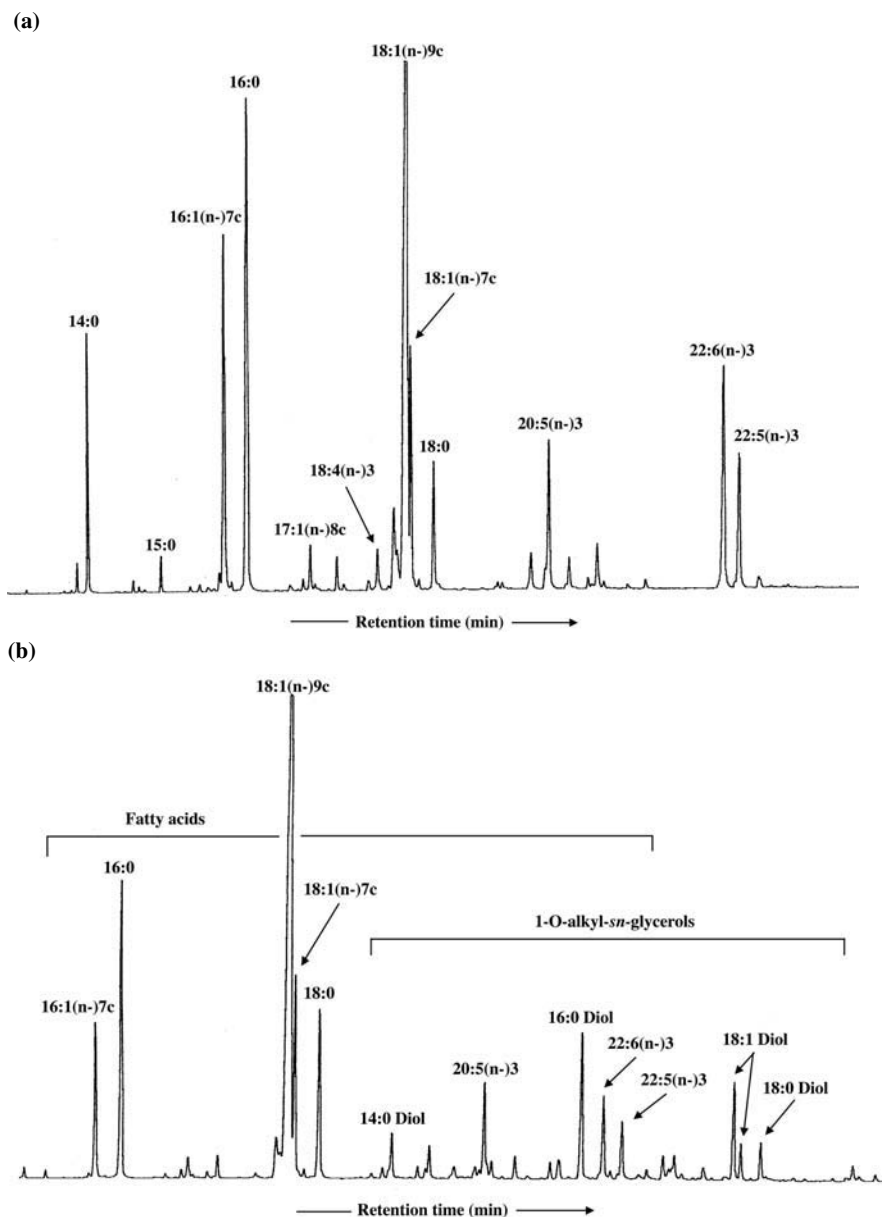


FIGURE 9.3 Partial capillary gas chromatograms of lipid fractions from blue whale (*Balaenoptera musculus*) lipid samples. (a) Total FA (analyzed as methyl esters) derived from the solvent fractionation of lipid classes from a subcutaneous blubber lipid extract by the hydrolytic reaction of O-acyl ester lipids; (b) the neutral lipid fraction, containing nonsaponifiable lipids (analyzed as bis(trimethylsilyl)trifluoroacetamide (OTMS) esters), derived from the solvent fractionation of lipid classes from a dorsal blubber lipid extract. In this case, FA have contaminated the neutral lipid fraction and have been detected as OTMS esters.

lipid sample is contained. This medium can be a liquid (for dissolved lipids) or a gas (for volatilized lipids) and is employed to move the lipid sample over or through the stationary phase. It is therefore known as the mobile phase.

The major characteristics of lipids that are exploited to chromatographically separate different classes are polarity and the degree of ionization. The polarity of a lipid significantly influences properties such as solubility, volatility, and nonspecific binding to other polar materials. The degree of ionization determines the amount of intermolecular interactions, which reduces compound volatility. In addition, such interactions with polar solvents increases compound solubility and can increase the strength of interactions between compounds and polar stationary phases. Hence, the common rationale of all chromatographic systems is to select an appropriate stationary and mobile phase to exploit the differences in polarity and ionization between lipid classes. The degree of lipid class separation can either be “simple,” yielding polar and nonpolar fractions, or “complex,” enabling the separation and isolation of acylglycerols, FA, sterols, glycolipids, and phospholipids.

Chromatographic systems can be classified according to the physical arrangement of the phases employed. In each case, the stationary phase is held in place as a packing of small particles, either in a column or as a layer on a flat surface. The mobile phase then moves through the column or over the surface layer.

9.3.3.2 Column Chromatography

Column chromatography falls into the category of liquid–solid chromatography; the stationary phase consists of a solid matrix through which the liquid mobile phase passes, usually under the influence of gravity only. The process relies on the partitioning or adsorption of lipid classes onto the stationary phase. Lipid classes can then be washed (eluted) from the stationary phase with increasing polarity and strength of the mobile phase. This may involve a step-wise change in the solvents of the mobile phase (isocratic elution) or a gradually changing mixture of solvents (gradient elution). For this technique, the stationary phase is arranged as a tightly packed cylindrical column covered with the initial mobile phase (Figure 9.4). The lipid sample is placed on the surface of the solid phase; as continual addition of the mobile phase progresses, lipid components are separated by the differing rates at which they proceed through the column, based on their interaction with both phases. Common stationary phases used for this technique include silica (silicic acid), alumina, and ion-exchange resins. Well-established procedures using defined mobile phases are available for the separation of all lipid classes, and relatively large amounts of material may be applied to such columns (Kates, 1986).

The separated lipid classes can then be collected as discrete fractions in solvent. This is particularly useful for either the direct detection and quantitation of isolated lipid fractions or for further derivatization and analysis of lipid components. For example, acylglycerols, wax esters, and phospholipid classes can be separated by column chromatography and each fraction reacted as in Section 9.3.2 to analyze the FA present in each lipid class.

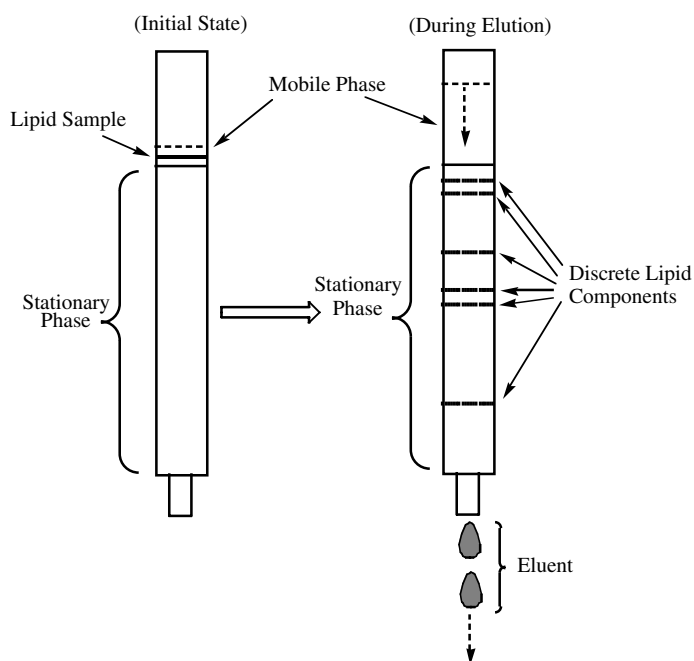


FIGURE 9.4 Schematic representation of the initial and intermediate stages of the column chromatography procedure. See [Section 9.3.3.2](#).

9.3.3.3 Thin-Layer Chromatography

Thin-layer chromatography (TLC) employs the same principles as those of column chromatography, but yields separations more suited to analytical preparations and the identification of lipids through *in situ* reaction of separated lipid components. TLC methods can achieve a very high degree of separation efficiency and are often used for the separation of individual lipid components from within the same lipid class. For example, the separation and identification of individual phospholipid types from a polar lipid fraction, or the separation of FA based on molecular structure such as chain length or the degree of unsaturation.

In TLC, the stationary phase usually consists of silica (or a derivative thereof), together with a binding agent that bonds the phase in a uniform thin layer to a glass or aluminum plate. After application of the sample to one end of the plate, it stands in a reservoir of the mobile phase ([Figure 9.5](#)). This remains the same composition throughout the procedure and progresses across the stationary phase layer under capillary action, the plate being removed from the reservoir before the solvent front reaches the end of the plate (the plate is then said to be “developed”). Lipid components are thereby separated along the TLC plate in the direction of travel of the mobile phase, based on the same principles as discussed previously. However, unlike column chromatography, where separated components are eluted in solvent fractions, components remain deposited on the stationary phase.

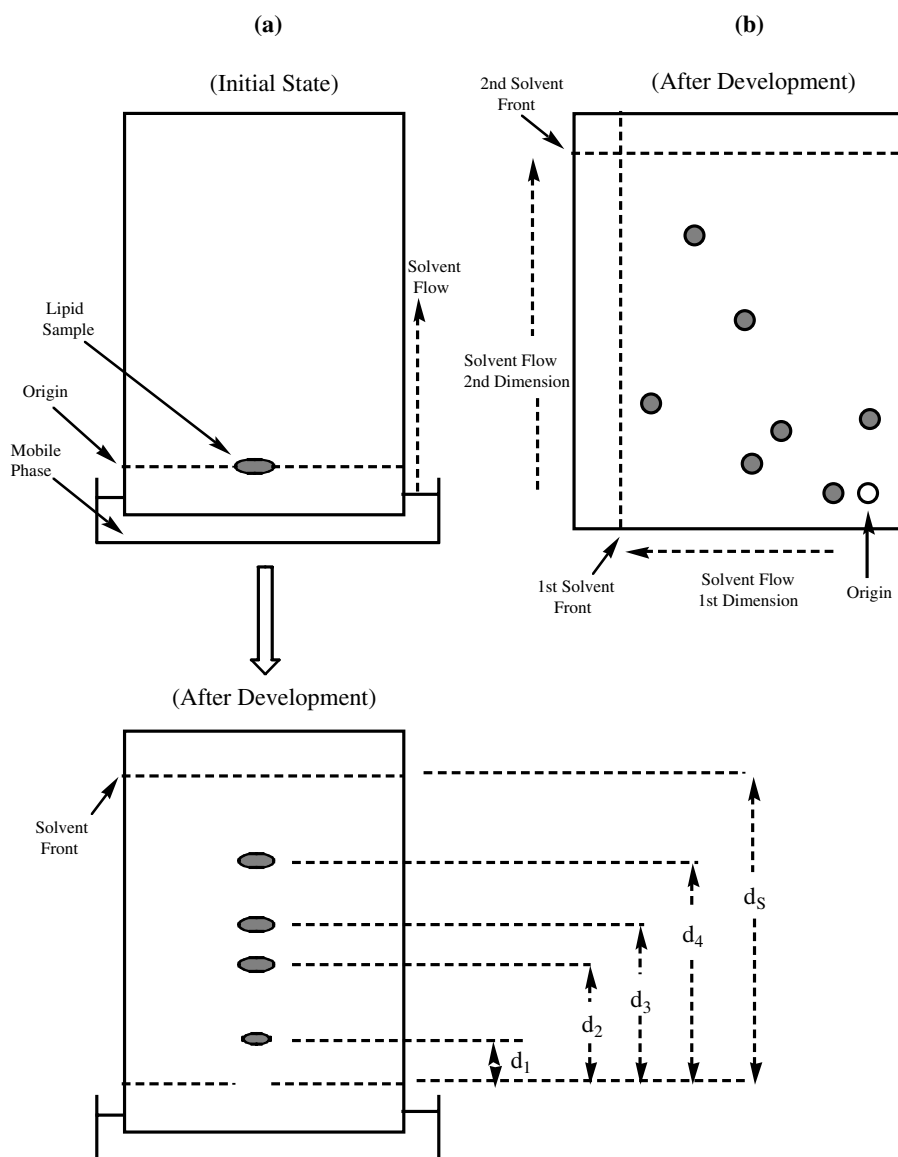


FIGURE 9.5 Schematic representation of the initial and end stages of the thin-layer chromatography (TLC) procedure. (a) One-dimensional TLC. An example of the distances traveled for each separated component is shown. The R_f value for each component can therefore be calculated by (d_x/d_s) , where X is 1, 2, 3, or 4; (b) two-dimensional TLC.

This fact introduces two advantages and one disadvantage of TLC compared with column chromatography. The first advantage is the ability to redevelop the TLC plate in the same or a different mobile phase, thereby improving the separation of components achieved by the initial development. This may be in the same dimension

(referred to as dual or multi-development) or in a second dimension (referred to as two-dimensional) to that of the first development (Figure 9.5). Two-dimensional TLC enables a high degree of separation to be achieved and is particularly useful for very complex mixtures of lipid types, or where the separation of very similar components within a lipid class is required.

The second advantage of bound components following separation is that this enables a further method of component identification based on the carefully measured position of spots on the plate following development. This is described in terms of the distance traveled by each component from the origin relative to the solvent front (Figure 9.5) and is called the R_f value. Characteristic R_f values have been established for lipid components under various conditions. To identify separated components, lipids must be visualized using a stain. Lipid components can be visualized on TLC plates nondestructively through the use of staining compounds such as rhodamine-6G, iodine vapor, or 2',7'-dichlorofluorescence. Many specific stains are also employed to aid in the identification of lipid spots by initiating chemical reactions with specific functionalities (destructive stains). For example, there are stains specific for the phosphate group of phospholipids, for phospholipids containing an amino or choline group, as well as stains specific for glycolipids and sterols.

The disadvantage of TLC compared with column chromatography is one of component recovery for further manipulation. TLC involves the drying of lipids on the stationary phase, thereby risking the oxidation of some components. Many of the stains useful for identification are destructive and therefore decompose the sample by staining. Where nondestructive stains are employed, the spots identified can be scraped from the TLC plate and lipids re-extracted from the stationary phase, although this process is seldom quantitative.

When faced with the separation and identification of a complex lipid mixture, it is often a valuable strategy to employ chromatographic techniques in tandem. Column chromatography is adept at rapidly separating lipid classes. Specific lipid classes can then be analyzed by TLC for the separation and presumptive identification of individual components. Extensive TLC procedures have been described by Stahl (1969).

9.4 DIRECT ANALYSIS OF LIPID CLASSES

9.4.1 INTRODUCTION

The analysis (separation, identification, and quantitation) of lipid classes from total lipid extracts is of prime importance in many food industry applications. While the chemical and chromatographic approaches outlined are elegant, well-established, and robust, they are relatively time-consuming and rely on separate stages of separation, identification, and quantitation.

Direct analysis of lipid classes enables faster and more efficient usage of analytical resources and sample material. Many of the direct analysis techniques are derived from the basic principles of chromatography described above. However, they have evolved specific instrumentation to enable (in most cases) improved separation

and, more important, one or more integrated methods of compound identification and quantitation. The marriage of these three aspects into a closely integrated process is the prime advantage of direct analysis techniques. The disadvantage is that to achieve this marriage, rather expensive and complicated analytical instrumentation is required.

Three example techniques are described in general terms for the direct analysis of lipid classes. Two are derived directly from the chromatographic techniques discussed above, while the third introduces the technique of mass spectrometry for the identification of both simple and complex lipids.

9.4.2 THIN-LAYER CHROMATOGRAPHY–FLAME IONIZATION DETECTION

Thin-layer chromatography–flame ionization detection (TLC-FID) is an analytical technique that combines the separation characteristics of thin-layer chromatography with a rapid and sensitive method of quantitation, flame ionization detection. TLC-FID is particularly useful for the rapid separation and quantitation of total lipid classes.

In this instrumentation, the stationary phase consists of a thin layer of silica or alumina bonded to a quartz rod (0.9 mm × 15 cm). Ten rods can be placed simultaneously in a metal frame and a sample applied to each rod. The rod assembly can then be developed in a solvent system as for a TLC plate. Once developed, the rod assembly is placed within a specialized instrument, the Iatroscan TH-10 (Iatron Laboratories, Tokyo, Japan). The basis of the Iatroscan involves a mobile flame ionization detector (FID). Briefly, an FID consists of two platinum electrodes spaced across a flame of hydrogen burning in air. The FID is positioned in a movable mounting so that it is able to pass along the length of each rod in sequence. Separated components along the rod are therefore combusted by the FID and a current signal is generated. The signal is then displayed as a chromatogram, depicting the rod length and R_f of detected components from which identifications are based (Figure 9.6).

Advantages of the Iatroscan system include the rapidity of the procedure to process multiple samples and a detector system that allows both identification of components (based on known R_f values) and a method for quantitation (described in the following paragraph). TLC-FID systems also offer a further refinement, that of partial scanning of rods by the detector. With this option, certain lipid classes can be separated from a complex mixture by an initial rod development while leaving the remaining lipids at or near the origin. The FID can then be arranged to scan only the upper section of the rod where the initial lipid classes have been separated. Once scanned in this manner, the rods can be redeveloped in the same or different solvent system to further separate the remaining lipid classes together or in an individual series. Such a process in effect yields an “endless” stationary phase, resulting in the possibility of resolving a large number of components.

Disadvantages of the Iatroscan include the limitations of the detector. First, the ionization currents generated by lipids of different classes are not the same. Hence, some lipid classes (e.g., sterols and phospholipids) generate a high ionization current per unit mass while others (e.g., hydrocarbons, TAG, and wax esters) generate a much

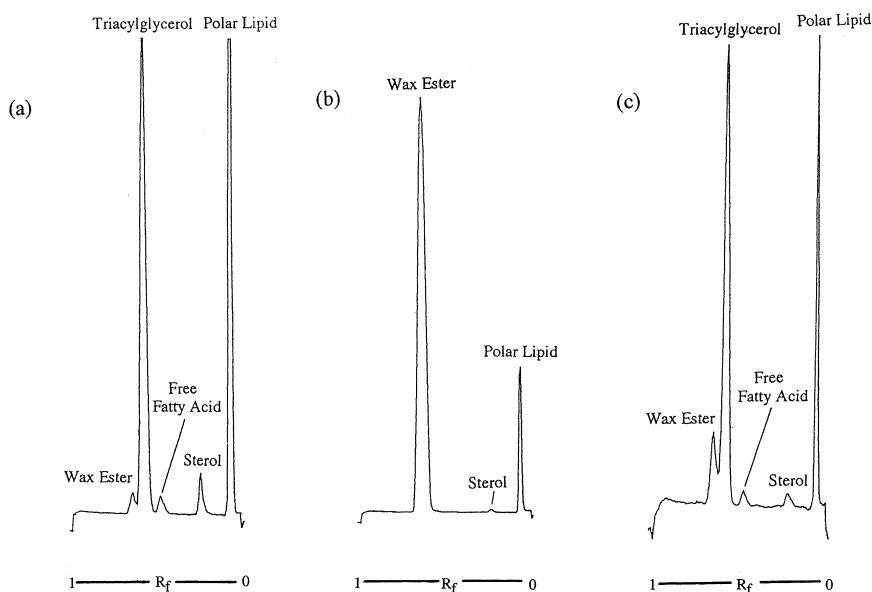


FIGURE 9.6 Thin-layer chromatography–flame ionization detection (TLC-FID) chromatograms of lipid extracts from the deep-sea fish, orange roughy (*Hoplostethus atlanticus*): (a) ovary, (b) muscle, and (c) liver. In this example, lipid classes were separated by a single development in a solvent system (mobile phase) of hexane:diethyl ether:acetic acid (60:17:0.2, v/v/v) with the rod (stationary phase) fully scanned. See [Section 9.4.2](#).

lower detector response per unit mass. This fact necessitates the calibration of the Iatroscan system for each lipid class be quantitated over the concentration range required. Further complications arise from analysis parameters such as hydrogen flow rate and scan speed, which also affect the ionization efficiency. Calibration curves should therefore be produced under the same instrumental conditions to be used for analyses. The material or standards used for lipid class calibrations can also influence quantitation. For example, single-species TAG standards yield higher detector responses than mixed species standards due to the production of sharper peaks. Ideally, the lipid standards used for calibration should be similar in composition to the samples analyzed.

9.4.3 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) represents an advanced derivative of the column chromatography principles already described (see [Section 9.3.3.2](#)). Very small uniform particles of matrix are employed in a very tightly packed column. This guarantees an even flow of mobile phase through the column but requires high pressures to force the solvent through the matrix at a constant and reasonable rate. Coating the matrix particles is the stationary phase, which may comprise a variety of materials bonded to the matrix particles. Polar phases include quaternary ammonium, aminoalkyl, or cyanoalkyl groups. Unlike column chromatography, a range of nonpolar

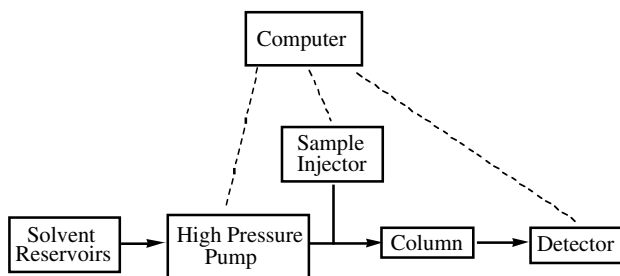


FIGURE 9.7 Schematic arrangement of a basic high-performance liquid chromatography system. See [Section 9.4.3](#).

stationary phases have also been developed, the most common being octadecyl or octyl hydrocarbon groups. Use of these columns is referred to as “reverse-phase” HPLC.

The general components of an HPLC system are described in [Figure 9.7](#). Rather than utilizing a premixed mobile phase, reservoirs of component solvents are provided and are mixed in the appropriate ratios under computer control. This allows the mobile phase composition to be altered during the elution sequence (gradient elution) and represents a significant advantage in improving chromatographic separations. Following passage through the column, the eluant is passed to a detector unit. For many HPLC applications, the most common type is the UV detector, which monitors a fixed wavelength through the flow of eluant, commonly 200 to 210 nm. In such cases, the solvents used must be “UV transparent” and free of UV-absorbing impurities (e.g., “HPLC-grade” solvents). UV detectors can only be used with lipid components that absorb in this region. Some problems can arise with certain lipids. For example, FA do not absorb strongly in this region and must be derivatized with a UV-absorbing group to be quantitated. The requirement for such derivatization negates the major premise of “direct” analysis. Hence, in these situations, alternative techniques or an alternative detection system may be preferred. Other common types include refractive index detectors. Increasingly, mass spectrometric detectors are being utilized in conjunction with HPLC systems due to the advantages offered by the definitive structural detail obtained from the mass fragmentation spectra of lipid components (see [Section 9.4.4](#)).

The general application of reverse-phase HPLC can be employed directly in the separation and quantitation of total lipid classes. However, the high degree of resolution possible from HPLC columns is more frequently employed for the separation and analysis of individual lipid components from complex mixtures or individual lipid classes previously separated by other chromatographic procedures (e.g., column chromatography). Examples include HPLC analysis of acylglycerols (Plattner, 1981; Kuksis, 1994) and phospholipids (Porter and Weenen, 1981).

9.4.4 MASS SPECTROMETRY

9.4.4.1 Introduction

Mass spectrometry as a technique yields a wealth of structural information for the identification of lipids. The degree of information obtained can vary from a simple

molecular weight determination for a purified lipid to the one-step separation, molecular weight determination, and molecular structural determination for each component in a complex lipid mixture. Two factors influence this process: the physical design of the mass spectrometer and the type of ionization used to introduce the lipid sample into the mass spectrometer.

Advances in the production of more compact, more robust, and cheaper mass spectrometers have led to their growing application in routine analysis procedures, for the stand-alone analysis of lipids, and also in combination as “detectors” for other chromatographic systems such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS *viz* HPLC-MS). GC-MS has particular advantages for the analysis of lipids with relatively high volatility (i.e., relatively low molecular weight and polarity) such as hydrocarbons, isoprenoids, and FA (Asselineau and Asselineau, 1984). The separation applications of HPLC for lipid components and lipid classes were discussed above and can be linked online to the mass spectrometer through interfaces such as thermospray or electrospray ionization, usually used to remove the “large” volumes of solvent from entering the mass spectrometer.

While it is beyond the scope of this chapter to address the physical design and fundamentals of mass spectrometers, it is salient to briefly summarize the relevant methods of sample ionization and their differing application to lipid analysis and partner chromatographic systems. The reader is referred to Murphy (1993) for background regarding the various designs and operation of mass spectrometers.

The analysis of lipids by mass spectrometry requires the alteration of the component from its natural state to that of an ion (positive or negative) in the gas phase. A wide variety of techniques are available for the generation of gaseous ions dependent on the volatility of the lipid type. Lipid classes that are relatively volatile (i.e., those amenable to analysis by gas chromatography; hydrocarbons, FA, and isoprenoids) are easily transformed to the gas phase by heating and can then utilize ionization techniques which require a preprepared gaseous form, such as electron impact or chemical ionization. Other lipids that are relatively nonvolatile (e.g., phospholipids) require an ionization technique which incorporates a desorption or desolvation aspect, such as fast atom bombardment or thermospray/electrospray ionization. Each ionization technique yields its own degree of sensitivity, and structural and molecular weight information. The exact information or degree of identification required, along with the lipid properties, may also influence the type of ionization technique employed.

9.4.4.2 Electron Impact Ionization

Electron impact (or bombardment) ionization has been the most widely applied method to date. This technique relies on the analyte being introduced into the ion source of the mass spectrometer (under vacuum) in a preprepared gaseous form. Within the ion source, the neutral analyte molecules come into association with a beam of energetically excited electrons. Transfer of energy from the electrons to the analyte molecule ultimately results in the loss of an electron from the parent molecule (forming a molecular ion, M^+), which then decays by fragmentation into a further series of charged ions (Figure 9.8). The resulting fragmentation ions can

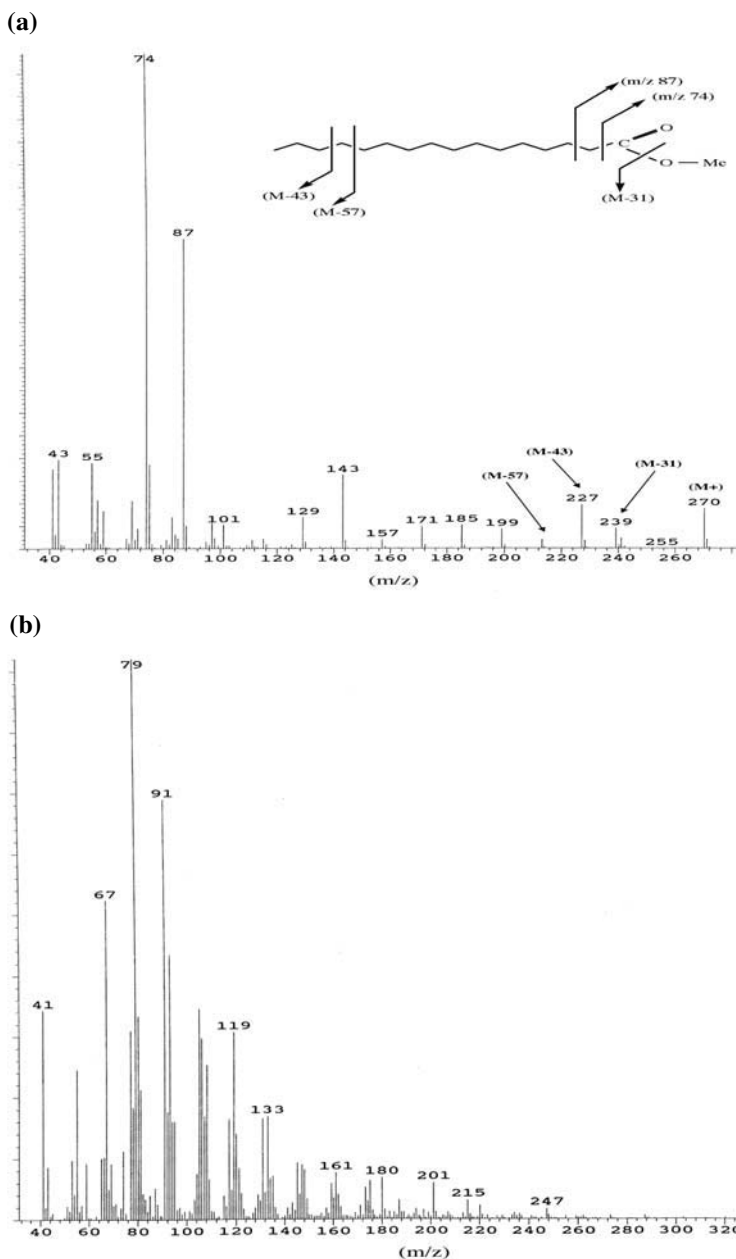


FIGURE 9.8 Examples of electron impact mass spectra for two FA (analyzed as methyl esters). (a) The mass spectrum of methyl hexadecanoate (the derivative of hexadecanoic acid, 16:0, molecular weight 270) demonstrating characteristic fragmentations and resultant ions. (b) The mass spectrum of methyl eicosapentaenoate (the derivative of eicosapentaenoic acid, 20:5(n)-3, molecular weight 316) demonstrating the characteristic ions of polyenoic fatty acids but the lack of higher-molecular-weight ions.

be separated in a magnetic and/or electronic field based on their mass/charge ratio (m/z) and the resultant mass spectrum generated. The primary information gained from this technique is usually the molecular weight of the analyte (represented by the parent molecular ion) and some structural details related to the formation of specific fragment ions. Established databases for electron impact mass spectra of many lipids are readily available (e.g., McLafferty et al., 1991). Two examples of electron mass spectra of FA are given in Figure 9.8. Specific fragmentations give rise to characteristic ions of FA, which in most cases can be interpreted to give unequivocal identification. However, a particular shortcoming of electron impact ionization in lipid analysis is also highlighted in Figure 9.8. The large amount of energy transferred to highly unsaturated FA from electron impact ionization results in a high degree of fragmentation and complex rearrangements. While a mass spectrum characteristic of polyenoic FA is produced, it is dominated by low-molecular-weight ions (Figure 9.8; ions m/z 67, 79, 91) and lacking in high-molecular-weight ions, including the molecular ion. Hence, in isolation, an electron impact mass spectrum of a polyenoic FA is insufficient for complete identification of the compound.

9.4.4.3 Chemical Ionization

Chemical ionization relies on the transfer of charge between an introduced ion and an analyte molecule in the gas phase. Gases such as methane or ammonia can be charged in a modified electron impact ion source and then used as an ion source for the sample. The resultant ionization of the analyte is “soft,” that is, it transfers little excess energy, which markedly decreases the degree of parent ion fragmentation, leaving a high proportion of analyte molecular ions. Hence, where electron impact ionization often results in many fragment ions with the molecular ion sometimes difficult to detect, chemical ionization usually yields a high proportion of the molecular ion, allowing very accurate mass determination of the analyte but less structural information. Further details of chemical ionization procedures have been provided by Harrison (1983).

An important advance in ionization techniques has been the development of atmospheric pressure chemical ionization (APCI). In this technique, the ion source is kept at atmospheric pressure rather than under high vacuum. Following ionization, the sample enters the high-vacuum region of the mass spectrometer through a small orifice. In mass spectrometers associated with HPLC systems, APCI introduces an important benefit, that of eliminating the necessity to introduce large volumes of eluant into the high-vacuum area of a mass spectrometer. In APCI, the majority of solvent is contained within the ion source region where it is utilized as the primary ion source (Henion and Lee, 1990).

9.4.4.4 Fast Atom Bombardment

A significant proportion of complex lipids contain polar structural features that engage in intermolecular associations, resulting in a low volatility and difficulties in manipulating them into the gas phase, which is necessary for electron impact or chemical

ionization. Some polar groups can be chemically derivatized to increase the compound's volatility; however, this increases the molecular weight and can alter the chemical structure of the analyte. Fast atom bombardment (FAB) represents a specific ionization technique for polar lipids or complex lipids of low volatility.

The FAB technique relies on the generation of a high-velocity beam of heavy, neutral gas atoms (e.g., xenon or argon) that is directed against the lipid sample mixed in a "matrix" material (usually glycerol). The matrix absorbs most of the collisional energy but some is transferred to the sample components so that soft ionization events can occur. The ions of sample components thus formed (termed secondary ions) are emitted from the surface of the matrix (desorbed) and can then be analyzed as molecular ions. When a beam of gaseous ions is used (usually cesium ions) rather than neutral atoms for the desorption of sample secondary ions, the technique is referred to as liquid secondary ion mass spectrometry (LSIMS). However, in practice, there is little difference between the outcomes of the two techniques. FAB-mass spectrometry has gained wide application in the analysis of phospholipids and other polar acylglycerols from isolated fractions or directly from complex lipid extracts (Kerwin, 1999). A simplified example is shown in Figure 9.9, in which the phospholipid molecular species from the total solvent extract of a bacterial culture have been analyzed by negative-ion FAB-

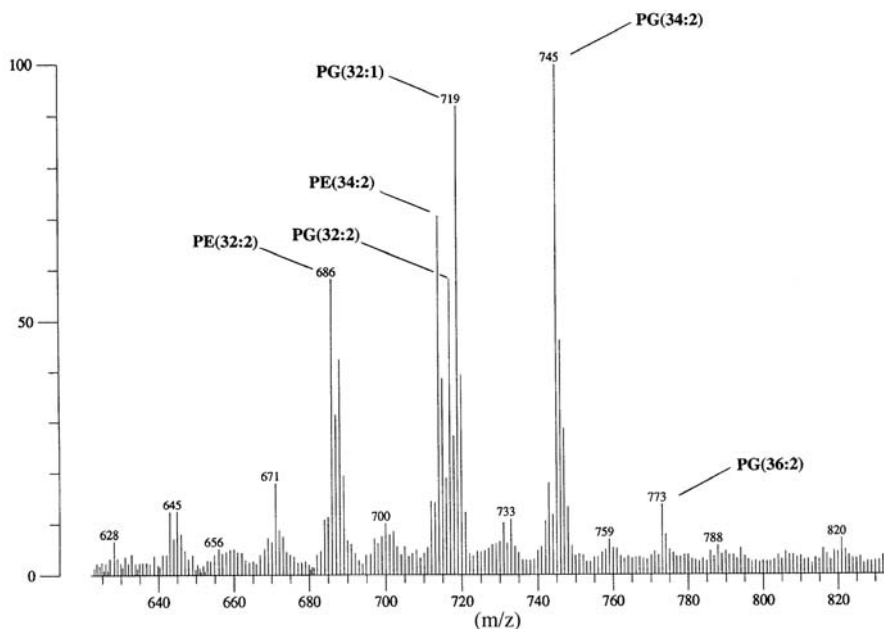


FIGURE 9.9 Example of a partial negative-ion fast atom bombardment mass spectrum of the total solvent extract from a marine bacterium showing phospholipid molecular species. Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

mass spectrometry. Based on the determination of phospholipid molecular ion species, the phospholipid class and combined acyl chain composition of each component can be assigned. Such analyzes, combined with a knowledge of the total FA composition of the sample, can allow specific component speciation to be inferred. For example, with the knowledge that the lipid sample analyzed in [Figure 9.9](#) contains a mixture of saturated and monounsaturated FA from C₁₄ to C₁₈, the phosphatidylethanolamine component PE(32:2) could represent three possible molecular species: PE(16:1/16:1), PE(15:1/17:1), or PE(14:1/18:1). A further refinement to resolve these possibilities would be to utilize FAB-tandem mass spectrometry to identify the acyl residue daughter ions released from the parent molecular species by secondary ionization.

9.4.4.5 Thermospray/Electrospray Ionization

Thermospray and, more recently, electrospray ionization have found wide application as an interface technology between HPLC instruments and mass spectrometers. They represent powerful techniques for the analysis of complex lipids directly from solutions (Henion and Lee, 1990; Murphy, 1993). In most instances, the total HPLC eluant can be sent directly into the heated thermospray ion source. Here, the combination of heat and eluant velocity creates a plume of small-diameter particles suspended in a vapor (nebulization). A strong electric charge forms on the surface of the liquid particles and as the droplets evaporate the increase in charge ionizes analyte molecules that are discharged directly from the droplet into the gas phase. From here, they may enter the mass spectrometer directly.

Electrospray ionization is similar in effect to the thermospray technique and is useful for similar applications. The difference resides in the use of a high electric field to nebulize the sample solution (or sample and eluant), creating droplets with excess electric charge. As the droplet solvent evaporates during traverse of a desolvation chamber, charge transfers to the analyte molecules and these are released as gaseous ions. A further refinement in this technique is the use of electronic lenses to direct ions more efficiently into the mass spectrometer. Because the analyte is not subject to heating, there is also less possibility for thermal decomposition of complex lipid components.

9.5 CONCLUSION

[Figure 9.10](#) illustrates the general approach to analytical lipid analysis described in this chapter, highlighting the major stages and the types of procedures that can be employed. Emphasis has been placed on describing the principles of several widely applicable techniques focused on diverse outcomes. The objective has been to help readers choose an appropriate analysis strategy for their needs and resources.

The approach to the extraction of lipids from samples remains a fundamental area for the majority of analysis techniques. Extraction by organic solvents remains the mainstay of routine procedures although new technologies such as supercritical

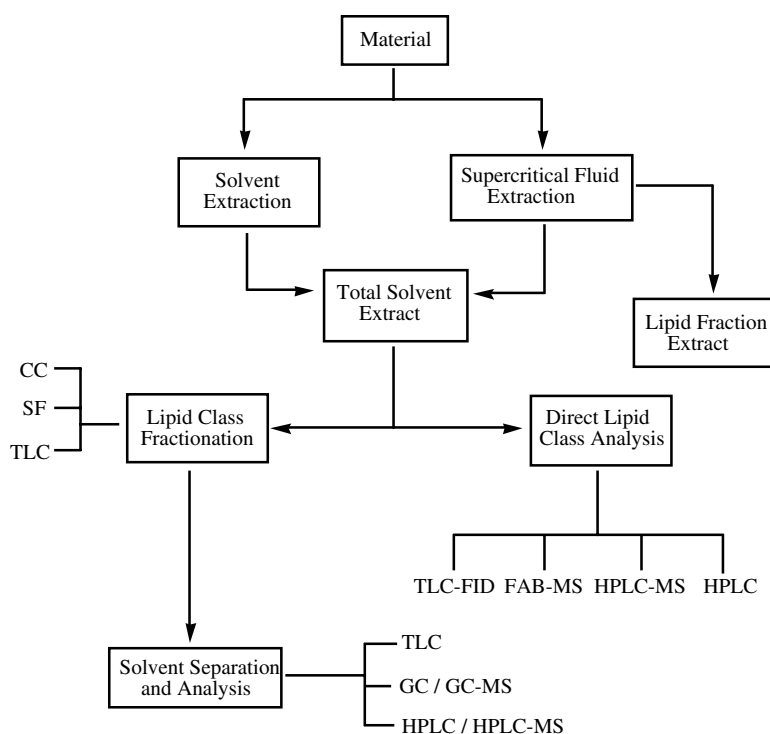


FIGURE 9.10 Flowchart of the general approach to analytical lipid analysis highlighting the major stages and types of procedures that can be employed.

fluid extraction have appealing applications in industrial areas where large volumes of solvent usage is problematic or there are advantageous interactions between the properties of supercritical fluids and certain lipids.

Certain lipid classes (e.g., FA and hydrocarbons) can be fractionated from lipid extracts according to their differing solubilities in solvent systems, particularly following chemical reactions to release ester-linked FA from complex lipids. However, for the separation of complex lipid classes and/or individual components within lipid classes, chromatographic techniques afford robust and reliable methods. Column chromatography is useful for the collection of lipid fractions retained in solvent for further analysis while TLC yields separations more suited to the analytical identification of lipids.

Great advances have been made in the direct analysis of lipid extracts (i.e., those requiring minimal preparative fractionation) by the use of dedicated analytical instrumentation such as TLC-FID and HPLC. Mass spectrometry enables the combination of aspects of lipid separation by molecular weight and structural detail from techniques of ionization-induced fragmentation. Combined with chromatographic procedures, this represents the most powerful but expensive tool available for lipid analysis.

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10 Lipids in Human Nutrition

Roman M. Cichon

CONTENTS

- 10.1 Introduction
- 10.2 Source of Lipids in the Diet
- 10.3 Digestion and Absorption of Lipids
- 10.4 Food Lipids as the Source of Energy
 - 10.4.1 Introduction
 - 10.4.2 Lipids in the Diet and Obesity
 - 10.4.3 Energy Value of Lipids
- 10.5 Essential Fatty Acids
- 10.6 Structural Components
- 10.7 Regulatory Functions
- 10.8 Lipid Requirements and Recommendations
- References

10.1 INTRODUCTION

Lipids form a group of compounds, the chemical nature of which is extremely varied. They have physical, chemical, and physiological properties that make them important both in nutrition and in food technology. Lipids make an important contribution to food characteristics, such as texture and palatability. In addition to fats ingested as food, there are specific lipids synthesized by the human body that are essential to life.

Lipids are insoluble in water and this profoundly affects the particular phenomena associated with their digestion, absorption, transport in the blood, and metabolism at the cellular level. Fat is easily recognized when it accumulates within the body, but the chemical and technical definition of fats and lipids are more difficult. Of nutritional interest are triacylglycerols (TAG), phospholipids (PL), sterols, and some derived lipids — results of the hydrolysis or enzymatic breakdown of simple and compound lipids.

TAG represent the bulk of ingested lipids. The second major group of lipids contained in a normal diet, the PL, contribute only an estimated 2% to the total fat intake, but an additional 12 g PL is secreted in the bile every 24 hours. Other

lipids are either present in such minute quantities that they do not play a role in digestion and absorption, or they are poorly absorbed (e.g., wax and wax-like compounds).

Fatty acids (FA) are the main constituents of food fats and oils as well as of depot fats in man and animals. Dietary fats contain practically no free FA; instead, these are present in the form of TAG.

Diacylglycerols (DAG) are found naturally as minor components in various dietary lipids. Although human adults ingest 1 to 5 g of DAG every day, little attention has been paid to its nutritional characteristics because DAG have been recognized only as intermediates in the process of TAG digestion and absorption.

Food lipids usually contain small amounts of other fat-soluble substances, including flavor components and some vitamins. Animal fats may contain vitamins A and D, and varying amounts of cholesterol, while vegetable fats may contain carotenes, vitamin E, and sitosterol, but not cholesterol.

10.2 SOURCE OF LIPIDS IN THE DIET

In plants, fats are formed from carbohydrates. Thus, when seeds such as sunflower or soybean ripen, their starch content decreases as the fat content increases. The proportion of FA in fat varies from plant to plant, and is also quite variable within a species. Fats from vegetable seeds are among the principal sources of essential FA (EFA), especially of linoleic acid (LA). Vegetable oils obtained from corn, soybean, and sunflower seed contain less than 15% saturated FA and more than 55% polyunsaturated FA (PUFA). However, not all vegetable oils contain such large amounts of PUFA; for example, the fat contained in olive oil and coconut is less than 10% PUFA (Table 10.1).

Animals, including humans, store excess energy almost entirely in deposits of fat, the amount of which is very variable. As in plants, this fat can be made from carbohydrate, but the dietary carbohydrates can be starch, sugar, or cellulose (in ruminants). Animals and humans also lay down fat from their dietary fat. The FA composition in this case reflects that of the diet, except for ruminants whose digestive process normally makes the FA more saturated and/or by isomerization with lower melting point. Animal fats have a high percentage of saturated FA with chain lengths of 14 to 18 carbon atoms. Milk fat contains about 20% 4–14 carbon FA. This short-chain FA (SCFA) and medium-chain FA (MCFA) content is specific for milk fat and is partly responsible for physical properties of butter such as melting point and for its pleasant taste. Beef and pork fats contain about 40% or more of long-chain FA (LCFA) and saturated MCFA. The PUFA content of animal fats, including fish oils, is generally between 4.0 and 30% (Table 10.1). But, contrary to other land animals, the storage fat of hibernating animals (e.g., brown or black bear) is rich in PUFA and contains much of linolenic acid (LNA).

Differences between the animal and vegetable lipids in relation to composition, digestion, absorption, and in the composition and concentration of sterols incorporated in ingested fat affect the metabolism and composition of fat depots.

TABLE 10.1
Recommended Intake of Lipids and Essential Fatty Acids

Recommended Intake as Percentage of Total Energy							Ref.
Level	PUFA					Fats and Oils (%)	
	EFA (%)	n-6 (%)	n-3 (%)	EPA + DHA (%)	n-6/n-3		
Minimum	1÷2						Mohrhauer and Holman, 1963
	3				10 : 1	15÷30 (35)	FAO/WHO, 1978
	3	2.5	0.5		4÷5 : 1		FAO/WHO, 1980
Optimum	4÷10	4÷10			5÷10 : 1	15÷30 (35)	FAO/WHO, 1994
	6÷7	4.8	1.2	0.27	4 : 1		Galli and Simopoulos, 1990
	9	7	1	1	7 : 2	30	

10.3 DIGESTION AND ABSORPTION OF LIPIDS

Food lipids are exposed immediately to the lipolytic activity of a lipase from lingual serous glands (von Ebner) located on the back of the tongue in the region of the circumvallate papillae. A small portion of TAG is hydrolyzed prior to ingestion by exposure to lipases already present in food, for example, in human milk, meat, cheese, vegetables, salad dressings, and soy sauces. However, free FAs are reactive, unpleasant tasting molecules and are tolerated only at very low concentrations.

TAG digestion is not inhibited by the low gastric pH because lipases present in the stomach are active in an acidic environment. The lingual lipase is active in the gastric pH spectrum (pH 2 to 6), and it plays a very important role in TAG digestion of the suckling infant. It forms primarily DAG with a strong preference for hydrolysis of the *sn*-3 position of long-chain TAG (>C14). Similar to most lipases, it has a high activity for hydrolysis of medium-chain TAG (C8-C14) and short-chain TAG (<C8). In addition to lingual lipase, gastric esterase lyses the medium-chain TAG and short-chain TAG, but does not hydrolyze long-chain TAG. The MCFA and SCFA are also partly absorbed by the stomach before gastric chyme reaches the duodenum. Fat entering the intestine is mixed with bile and is further emulsified. The emulsion is then acted upon by lipases secreted by the pancreas. Pancreatic lipase catalyzes the hydrolysis of FA from positions *sn*-1 and *sn*-3 to yield 2-monoacylglycerols (MAG). PL are hydrolyzed by phospholipase A and the major products are PL and FA. Cholesterol esters are hydrolyzed by pancreatic cholesterol ester hydrolase to free cholesterol and FA.

The obligatory steps in TAG absorption are hydrolysis and solubilization of the hydrolytic products. Based on the physicochemical properties of lipids and luminal mucosal membranes, one can predict that (1) TAG and DAG may not permeate the absorptive mucosal membrane and that (2) only 2-MAG and primarily protonated FA can pass through the membrane by diffusion as free monomers in the aqueous phase near the luminal membrane. The efficiency of fat digestion is assured by an excess of pancreatic lipase. Lipase is secreted to the duodenum in about 1000-fold excess. Thus, under optimal conditions, 100 kg TAG could be hydrolyzed in 24 hours instead of the ingested 100 g fat in a normal diet.

The free FA and MAG are absorbed by the enterocytes of the intestinal wall and absorbed lipids are transported in water-soluble form to other tissues. FA with chain lengths shorter than 14 carbon atoms are bound to albumin and preferentially transported directly to the liver via the portal vein. Only a small proportion of MCFA undergoes a conversion to LCFA and esterified to TAG. A very small fraction of LCFA is transported via the portal route. This fraction increases when long-chain TAGs are fed in combination with medium-chain TAGs. The absorbed lipid fractions consist of FA, 2-MAG, some 1-MAG, lyso PL, some PL, fat-soluble vitamins, and small amounts of glycerol and cholesterol. The first step in mucosal transport is re-esterification, and the second step is the synthesis of transport particles: the so-called lipoprotein (chylomicron) and very low-density lipoproteins (VLDL). They enter the bloodstream via the lymph vessels. Lipoprotein lipase located on the interior walls of the capillary blood vessels hydrolyzes the TAG, releasing FA. These enter

the adipose tissue where they are stored, and the muscles and other tissues where they serve as fuel. Consumption of high-fat meals produces more chylomicrons and remnants. The remnants of chylomicron and VLDL are cleared by the liver, usually within a few hours of the ingestion of a fat-containing meal. When fasting, chylomicrons are normally absent in blood plasma.

10.4 FOOD LIPIDS AS THE SOURCE OF ENERGY

10.4.1 INTRODUCTION

The cells of the body, except for red blood cells and those of the central nervous system, can utilize FA directly as a source of energy. Although glucose is normally used by the nervous system, the brain can utilize ketones that are formed from FA during a period of fasting. Regardless of the fat type, lipids — and particularly TAG — are readily digested by healthy individuals. Lipid-deficient diets cannot provide adequate calories and contribute to malnutrition in many parts of the world. Any excess energy, whether derived from dietary carbohydrate, protein, or fat, is stored as TAG within the adipose cells of the body. Adipose cells are found beneath the skin, between muscle fibers, around abdominal organs and their supporting structures called mesentery, and around joints.

Fat in the body is a major storage site for energy from food eaten in excess of need. The storage of fat is a valuable evolutionary mechanism for people who must live a feast-or-famine existence. The ability to store energy in adipose cells as fat is thought to have contributed to the survival of early humans in times of famine. In addition, fats provide most of the energy needed to perform much of the body's work, and especially muscular work. Fat is the major, if not most important, fuel for light- to moderate-intensity exercise.

Because the capacity of the body to change carbohydrate and protein stores is extremely limited, the energy balance is really fat balance, and this could be a key to the metabolic syndrome. Although saturated fats are still the main dietary factors that induce a cascade of dysregulation at multiple metabolic sites, more and more attention is being focused on effect of monoenic FA and the appropriate balance between the n-6 and n-3 classes of PUFA.

Compared with PUFA-enriched diets, diets high in saturated fats could substantially alter the rate of weight gain and fat accumulation and are strongly obesogenic. This may be connected with the resting metabolic rate, the fat oxidation rate, and diet-induced thermogenesis. Animal studies have shown that saturated fats are poorly used for energy and are thus preferentially stored in adipose tissue. Among other dietary fat types with similar carbon chain lengths, monoenic FA are efficiently utilized, as are n-3 PUFA. Both these fat classes, in turn, are somewhat better used for fuel than n-6 PUFA.

Saturated FA are also difficult to shift from adipose depots. They are mobilized less easily as unsaturated FA, and it appears that relative mobilization increases relatively predictably with increasing unsaturation. The most easily mobilized FA were eicosapentaenoic acid (EPA) and arachidonic acid (AA), the precursors of the 3- and 2-series of eicosanoids ([Figure 10.1](#)).

The body may spend less energy assimilating fat than assimilating carbohydrates or protein. The conversion of carbohydrates, proteins, and lipids to fat in the body is approximately at the level of 80, 66, and 96%, respectively. Thus, it can store more calories when fat is eaten than it can store from the same number of calories from carbohydrate and/or protein. In short, it is easier to get fatter on fat calories than on carbohydrate or protein calories. In the over-consumption state, therefore, the following simple relation exists: body depot fat increases as the intake of the dietary lipid increases.

10.4.2 LIPIDS IN THE DIET AND OBESITY

The ingestion of high-calorie food containing lipids has contributed to the enhancement of human health and longevity. However, when such food becomes abundantly available, the problem of obesity becomes apparent. Obesity is generally associated with enhanced lipid consumption. Therefore, to prevent the risk of diseases such as obesity and atherosclerosis, likely to be caused by over-ingestion of dietary lipids, it is necessary to correctly balance the amount and type of lipids, the percent fat energy in the diet, the ratio of animal fat to vegetable oil, and the overall dietary FA composition. To avoid an excess of fats and oils, it is important to balance vegetable oils and fats of land animal origin, and to control the composition of FA that constitute oils and fats. Recently, there has been an attempt to investigate oils and fats from the viewpoint of structural lipids, and change body metabolism by modulating the bonding site of different FA in TAG of food. Particular attention is being paid to the position of SCFA and MCFA in the TAG.

Among various types of fat deposition, visceral fat type obesity is one risk factor for metabolic diseases such as diabetes mellitus, hypercholesterolemia, hyperlipidemia, hypertension, and atherosclerosis. The risk of diseases such as diabetes mellitus and coronary heart disease, as well as all-cause mortality, increases in proportion to the increase in body adipose above optimal, but intra-abdominal distribution of fat in the body is associated more closely with disease risk.

10.4.3 ENERGY VALUE OF LIPIDS

FA are the dietary components that account for the amount of energy liberated during oxidation of lipids in the body. Glycerol is approximately 10% by weight of TAG but accounts for only 5% of the energy. The FA of food impart the high energy value that is of nutritional interest.

FA yield energy by beta-oxidation in the mitochondria of all cells, except those in the brain and kidney. In general, FA are good sources of energy for muscle; for example, 60 to 90% of the energy metabolism of cardiac muscle can be explained by the oxidation of FA. Saturated FA and LCFA undergo the first step of beta-oxidation with different dehydrogenases. Unsaturated FA require two more enzymatic steps than saturated FA to change *cis* double bonds to *trans* and to move them from the alpha to beta position. Even so, oxidation of unsaturated FA, including LA and LNA, is as fast or faster than that of palmitic acid. Very long-chain (>18C) FA are preferentially oxidized by peroxisomes; SCFA and MCFA are also oxidized by

this system. Peroxisomal oxidation is energetically less efficient than mitochondrial oxidation, but yields more heat. This type of oxidation can be induced by diets containing high amounts of fat.

The traditional factor used in calculating the amount of energy of dietary lipid is 9 kcal/g (32.7 kJ/g), compared to 4 kcal/g (16.7 kJ/g) for carbohydrate and protein. These values were introduced by Atwater, and are based on the amount of energy released when these macronutrients are metabolically oxidized, allowing for incomplete intestinal absorption. Other correction factors are inappropriate for the dietary oils and fats commonly consumed; the 9 factor for converting grams of dietary lipid to energy is more suitable and offers consistency.

The amount of energy that might be obtained in the metabolic oxidation of FA depends on the length of the carbon chain and its saturation because energy is obtained by oxidation of carbon and hydrogen. The longer and more saturated the FA, the more carbon and/or hydrogen in the molecule, the more energetic the FA. Thus, stearic acid is more energetic than oleic acid, which is more energetic than palmitic acid.

The heat of combustion for TAG was applied to extracts of various food and, according to Atwater, amounted to 9.45 kcal/g; but for cereals, fruits, and vegetables, the assumed or calculated value was given as 9.30 kcal/g. Atwater used a coefficient of digestibility of 95% for butter, and 90% for fat in cereals and other plant sources. Calculations based on Atwater's data are: $9.45 \times 0.95 \approx 9.00$ kcal/g of fat and $9.30 \times 0.90 = 8.37$ kcal/g. The 8.37 value has been used for several decades to convert grams of fat in cereals, fruits, and vegetables to energy calculated in calories. Thus, the use of Atwater's factor (9 kcal/g) makes no difference in the calculation of total energy intake when both animal and vegetable (mostly oil and margarine) lipids are consumed. In other cases, an overestimation or underestimation of the energy value of food is possible (compare the food of vegans and Western societies).

10.5 ESSENTIAL FATTY ACIDS

The human body has the remarkable ability to synthesize many compounds. Saturated and monoenic FA are obtained either from the diet or by complete synthesis from acetyl-CoA. In contrast, n-3 and n-6 FA, which represent approximately one third of the intracellular FA, cannot be synthesized by animal or humans but are essential components of dietary lipids.

Early on, a substance was regarded by nutritionists as being essential only if death or serious illness resulted from its absence in the diet of a healthy and well-fed individual. Though the substance is necessary for normal function, the human body is unable to synthesize it for itself in adequate amounts. But as the times change, so does the definition.

The biosynthesis of LA and LNA takes place only in the vegetable kingdom and cannot be formed by animals. They can, however, undergo alternating desaturation and chain elongation in animals, thus giving rise to the two PUFA families (Figure 10.2). Two additional families of FA are known, based on oleic acid and palmitoleic acid, but conversion within these two families becomes significant only

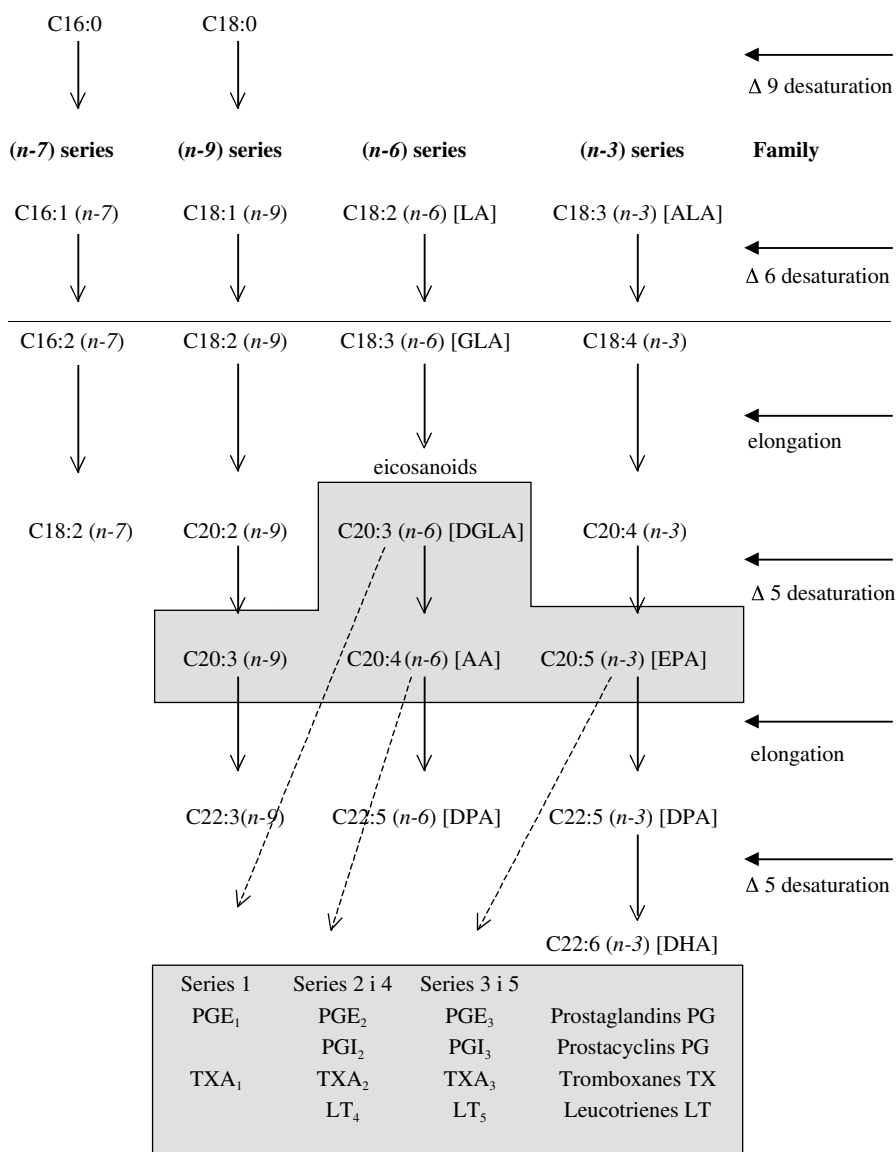


FIGURE 10.2 Unsaturated fatty acid families and their importance in the formation of eicosanoids and long-chain PUFA (LCPUFA).

in animals receiving fat-free or PUFA-free diets. No interconversion between different FA families takes place in the animal kingdom.

Neither LA nor LNA can be produced by animal organisms. Thus, for sufficient prostaglandin biosynthesis via the cyclooxygenase pathway or the leucotriene and hydroxy FA biosynthesis by various lipoxygenases, humans depend on the presence of 18:2, n-6 and 18:3, n-3 FA in the diet. Chain elongation and desaturation of 18:2,

n-6 to 20:3, n-6 and 20:4, n-6 FA occur, and AA is a good substrate for both cyclooxygenase and lipoxygenase. On the other hand, EPA is a poor substrate for cyclooxygenase, but a good one for lipoxygenase.

Important in this respect is that 18:2, n-6; 18:3, n-3; 20:3, n-9 FA; and various *cis-trans* isomers show competitive inhibition with 20:3, n-6; 20:4, n-6; and 20:5, n-3, which are the precursors for the known biologically active prostaglandins and leucotrienes. Administration of an EFA-deficient diet leads to partial replacement of AA by 20:3, n-9, which is a substrate for lipoxygenase but not for cyclooxygenase. Feeding a diet high in PUFA of the n-3 type (e.g., linseed oil, fish oil) results in an enrichment of membrane phospholipids with EPA and docosahexaenoic acid (DHA). This may explain why the FA composition of tissue lipids, including membrane PL, can be modified to some extent by the amount of each class of FA in the diet.

The nutritional significance of PUFA was first recognized by Burr and Burr (1929) and confirmed by Hanson in 1930: administration of LA and/or AA cured the ill-effects of a totally fat-free diet. Induced in rats by fat-free but otherwise nutritionally adequate diets, LNA and AA prevented the symptoms and were termed EFA. A second term, "vitamin F," for these nutrients has been and continues to be used even today by alternative medicine practitioners and the health food press.

For many years, three PUFA — LA, LNA, and AA — were considered to be EFA. However, research performed in the 1960s and 1970s revealed that (1) AA can be synthesized by the body from LA and therefore does not have to be supplied as such in the diet; and (2) LNA has relatively little effect in relieving the skin lesions originally associated with a deficiency of EFA, and did not prevent all the symptoms caused by a lack of fat in the diet. Thus, LA, found widely in foods of both plant and animal origin, was considered the only EFA that could not be synthesized in the body and must be present in the diet.

LNA was not recognized as an EFA until 1978 when Dyerberg suggested that the EFA found in food of marine origin might protect against thrombosis (Dyerberg et al., 1978). However, the conversion of 18:3, n-3 to EPA proceeds efficiently in the rat, but it is much less effective in humans.

Based on the need of LC PUFA n-3 in brain development of humans, Nestel et al. and Simopoulos et al. introduced in 1990 the intake recommendations for EPA and DHA (Simopoulos, 2001). Since then, the requirement for EFA is often extended to include both C18:2, n-6 and C18:3, n-3; and regarding recommendations, EFA is becoming interchangeable with PUFA.

Deficiencies in both n-3 and n-6 EFA are seen clinically and can be differentiated by predominant symptoms. Common symptoms of n-6 FA deficiency include growth retardation, skin lesions, impairment of reproduction, loss of hair, fatty liver, and disorder in the water balance. In contrast, n-3 FA deficiency spares growth and reproduction, but is associated with reduced learning, impaired vision, and polydipsia. Abnormal n-6-to-n-3 ratios have been linked to changes in the vascular membrane lipid composition and the increased incidence of atherosclerosis and inflammatory disorders. In humans, EFA deficiencies have been observed in babies; however, EFA deficiency in adults was unknown until parenteral feeding with a formula diet was administered over a long period of time.

AA is the substrate for most eicosanoid mediators produced by mammalian cells. It is also present in large amounts in phosphatidylinositol, a PL that functions in membrane signal transduction. The need for AA is probably the main reason why LA is essential for health. PUFA, especially of the n-3 type, are preferentially incorporated at the 2-position of most cell membrane PL and are considered to play an important role in both cell membrane integrity and function. Cellular stimulation leads to activation of phospholipase A, followed by mobilization of the FA on the 2-position of cell membrane PL. The types of these FA determine, to a great extent, the types of cyclooxygenase and lipoxygenase products.

DHA, the most abundant n-3 PUFA ordinarily present in tissues, is present in significant amounts in PL of retinal and neuronal membranes. It is required for the proper development and function of the nervous system, and the need for DHA is the main reason why LNA is essential. The cells of the nervous system incorporate DHA because it is more readily available than n-6 DPA, not because DHA possesses special metabolic properties. In this regard, DHA is the main product of LNA metabolism in astrocytes, whereas AA — not n-6 docosapentaenoic acid (DPA) — is the main product of LA conversion. Thus, more DHA than n-6 DPA is available in the brain although the blood supply provides considerably more n-6 PUFA precursors.

The high degree of unsaturation of n-3 and n-6 FA, especially long-chain PUFA such as AA, DPA, EPA and DHA, makes them highly susceptible to oxidation and to generate potential pro-oxidants leading to induce an oxidative stress.

10.6 STRUCTURAL COMPONENTS

Some fat deposits are not accessed during a fast and are classified as structural fat. This body fat (1) holds organs and nerves in place, (2) absorbs shock and protects organs against traumatic injury, and (3) a subcutaneous layer of fat insulates the body against rapid temperature changes or excessive heat loss. Thus, lipids are assisting in internal climate control — and, in a pregnant woman, are protecting her unborn baby. Furthermore, lipids, in particular PL — lecithin, and sphingomyelin — are widely distributed in cell membranes and serve an important role in the structural integrity of the cell membranes. The membranes, consisting of PL, cholesterol, and proteins, serve not only as boundaries between individual cells, but also as a means to compartmentalize several biochemical processes within the cell. The FA composition of the PL contributes significantly to the physical and biochemical properties of the membranes and is closely regulated by the proportion of saturated FA, monoenic FA, and PUFA. PL are water soluble and may aid in the transport of other lipids into and out of cells. Lecithin, as part of bile, emulsifies other lipids, and as a part of lipoproteins, transports TAG and cholesterol. Lecithin is primarily responsible for the hydrophilic properties of the surface coat of chylomicrons and other lipoproteins. Dietary lecithin is an important contributor to blood and brain choline concentrations, which determine the rate of acetylcholine biosynthesis, especially during rapid action of cholinergic neurons.

The brain is rich in sphingolipids, and in sphingomyelin in particular. Sphingomyelins and cerebrosides are important components of the myelin sheath that surrounds nerve axons, and the gray matter of the brain is rich in gangliosides.

The main difference between the various sphingomyelins lies in the nature of the FA, which can have a chain length between 16 and 24 carbon atoms. Lignoceric acid and nervonic acid are abundant. The nature of the fats consumed may influence the ratio of the different FA incorporated in the different sphingolipids of the body. The physiological consequences of this influence, however, remain unknown.

Cholesterol is found in every cell in the human body; its physiological functions are numerous. The fact that cholesterol is observed to accumulate in atheroma, in gallstones, and in renal calculi (all disease conditions) should not overshadow the essential functions of this substance in the body. Among the most important derivatives of cholesterol are the bile acids that originate in the liver and play a major role in the digestion of fats after being secreted into the intestinal tract. The derivative 7-dehydrocholesterol gives vitamin D₃ under the action of ultraviolet radiation from the sun. Other derivatives are the male and female sex hormones and the steroid hormones synthesized by the adrenal glands. The numerous physiological and metabolic functions of these substances are all essential to life.

In addition, cholesterol is essential for the formation of cell membranes and for the maintenance of their integrity. The body cells could not reproduce, grow, or function normally if they were not constantly supplied with cholesterol. The human body therefore depends on specialized mechanisms governing and maintaining cholesterol homeostasis essential for proper body function. Some of these mechanisms involve synthesis of cholesterol in the liver, excretion in the intestine, and feedback reactions. A control mechanism ensures that the synthesis of cholesterol is reduced when the amount of cholesterol intake increases. For some humans, this control mechanism does not work properly and they suffer from hypercholesterolemia.

The solubility of cholesterol esters in water or in body fluids differs significantly, depending on the nature of the FA with which cholesterol is esterified. Their solubility presumably has a bearing on the atherogenicity of cholesterol esters. Thus, LA, LNA, and AA esters would be less atherogenic than esters of monoenoic or saturated FA.

10.7 REGULATORY FUNCTIONS

FA, especially PUFA, as cellular regulators are associated with:

1. Membrane lipid structure
2. Membrane physical properties
3. Intracellular signaling
4. Plasma lipid transport
5. Covalent modification of cellular proteins
6. Eicosanoid formation
7. Gene transcription
8. mRNA stability
9. Cellular differentiation

Particular attention has recently focused on C18:2, 9-*cis*, 11-*trans*, conjugated LNA (CLNA) as a natural anticarcinogenic compound of natural fat of animal (bovine) food.

The FA composition of the lipid bilayer of all cell organelles clearly modulates every cellular function, and affects, for example, the active transporters and ion channels. The LA, and LNA, in the membrane PL exert metabolic control, primarily through their role as precursors of eicosanoids. Interference with the metabolism of n-6 FA plays an important part in the way in which n-3 FA exert their effect on cell function. The interactions take place at different biochemical levels and comprise $\Delta 6$ - and $\Delta 5$ -desaturation, incorporation into PL, eicosanoic formation, and cholesterol esterification. There is evidence that diets high in saturated fat reduce β -adrenergic receptor binding, both centrally and peripherally, presumably through membrane lipid alternations.

In the 1950s, Keys and co-workers pioneered work indicating the very different effects of saturated and unsaturated dietary fats on blood cholesterol levels and, in turn, on coronary heart disease. Based on this work, the lipid hypothesis states that there is a connection between the FA composition of the diet and the cholesterol content of the blood serum, in that saturated FA increase cholesterol, while PUFA decrease it. Later, it was established that the type of fat affected the lipid level more than cholesterol in blood. Keys' research has provided the basis for the recommendation that fats with a relatively low proportion of PUFA be replaced by fats and oils that are rich in PUFA.

Dietary fat depresses gastric secretion, slows gastric emptying and stimulates biliary and pancreatic flow, thereby facilitating the digestive process. Often, the acceptance of food and its palatability depends upon flavor and aroma. Although TAG in the pure state are relatively tasteless, they absorb and retain flavor of food. Furthermore, in combination with other nutrients, lipids provide a texture that enhances palatability and contributes to a feeling of satiety. Finally, dietary lipids serve as carriers for fat-soluble vitamins A, D, E, and K, and as an aid to their absorption in the intestine.

10.8 LIPID REQUIREMENTS AND RECOMMENDATIONS

It appears that the body is capable of adapting to a wide range of fat intakes. Around the world, fat intake varies dramatically. In many countries, fat contributes, and has done so for many generations, 10 to 20% of the energy intake; while in more industrialized countries, dietary fat intake mostly ranges from 30 to 40% of the total energy intake.

There is no required intake of fats and other lipids, with exception of EFA. Thus, there is no established recommended daily intake for lipids. Current dietary guidelines follow recommendations of the FAO/WHO expert consultations on fat and oils in human nutrition published in 1994 as the FAO Food and Nutrition paper No. 57 (FAO/WHO, 1994). General conclusions and recommendations of the consultations regarding lipids intake are as follows:

1. Recommendations on minimum intakes for adults:
 - a. For most adults, dietary fat should supply at least 15% of their total energy intake.
 - b. Women of reproductive age should consume at least 20% of their energy from fat.
 - c. Concerted efforts should be made to ensure adequate consumption of dietary fat among populations where less than 15% of the dietary energy supply is from fat.
2. Recommendations on upper limits of dietary intakes:
 - a. Active individuals who are in energy balance may consume up to 35% of their total energy intake from dietary fat if their intake of EFA and other nutrients is adequate and the level of saturated FA does not exceed 10% of the total energy they consume.
 - b. Sedentary individuals should consume no more than 30% of their energy from fat, particularly if it is high in saturated FA, which are derived primarily from animal sources.
3. Recommendations regarding infant and young child feeding:
 - a. Infants should be fed breast-milk if at all possible.
 - b. The FA composition of infant formulas should correspond to the amount and proportion of FA contained in breast-milk.
 - c. During weaning and at least until 2 years of age, a child's diet should contain 30 to 40% of energy from fat and provide similar levels of EFA as found in breast-milk.
4. Recommendations on intakes of saturated and unsaturated FA:
 - a. Intakes of saturated FA should provide no more than 10% of the total energy.
 - b. Desirable intakes of LA should provide between 4 and 10% of the energy. Intakes at the upper end of this range are recommended when intakes of saturated FA and cholesterol are relatively high.
 - c. Reasonable restriction of dietary cholesterol (less than 300 mg/day) is advised.
5. Recommendations concerning isomeric FA:
 - a. Consumers should substitute liquid oils and soft fats (that is, those that are soft at room temperature) for hard fats to reduce both saturated FA and *trans* isomers of unsaturated FA.
6. Recommendations on antioxidants:
 - a. Tocopherol levels in edible oils need to be adequate to stabilize the unsaturated FA present. Therefore, foods high in PUFA should contain at least 0.6 mg tocopherol equivalents per gram of PUFA. Higher levels may be necessary for fats that are rich in FA containing more than two double bonds.
7. Recommendations concerning EFA intake:
 - a. The ratio of LA to α -LNA in the diet should be between 5:1 and 10:1.
 - b. Individuals having a ratio in excess of 10:1 should be encouraged to consume more n-3-rich foods such as green leafy vegetables and legumes as well as fish and other seafood.

- c. Particular attention should be paid to promoting adequate maternal intakes of EFA throughout pregnancy and lactation in order to meet the requirements of fetal and infant development.

Voit (in 1880), Atwater (in 1895), and Rubner (1903) have recommended fat intake levels of 17, 32, and 16% of total energy, respectively. In the intervening 100 years, and particularly in the past four decades, the fat intake recommendations have slowly evolved from 32% (Atwater) to 16% (Voit and Rubner). On the other hand, the recommendation for EFA intake increased 4 to 5 times, from 1–2% to 4–10% of total energy intake. This change reflects revolution rather than evolution in lipid nutrition.

The requirement for EFA in the daily diet has been established on the basis of the amount of eicosatrienoic acid in blood serum, and a ratio of this FA to AA greater than 0.4 has been taken as a measure of LA deficiency. It was assumed in the 1950s that the minimum requirement for humans was 1% of the calories supplied by food, and the recommendation for daily intake of EFA ranged from 3 to 10 g. In general, 1 to 2% of the energy intake was suggested as a minimum, while recommendations for LA intake of 6% or even of 12% of the calories were regarded as being excessive.

According to more recent opinions of nutritionists and physicians, the ideal diet should consist of one third saturated FA, one third monoenoic FA, and one third PUFA. If this goal were achieved, the ratio of PUFA to saturated FA (P/S) in dietary lipids should be at least 1, and the PUFA content should be at 10% of the energy intake. In diets where PUFA represent 10% of total energy intake, P/S is approximately 1.6 and such a diet is tasteless and unpalatable. Changing the P/S of the diet to 1 or more requires drastic changes in food habits. There is no population known to consume a diet with such a high PUFA level. A recommendation of such high levels of PUFA would lead to a daily intake of 25 to 30 g PUFA, and this can have adverse effects on the digestion and metabolism of lipids. In addition, this may induce early aging of cells; as well, there is the potential toxicity associated with the oxidation of PUFA. Under normal conditions, the cells are protected by natural antioxidants such as vitamin E. Increased dietary intake of PUFA also increases the need for tocopherols. Daily intake at 25 to 30 g PUFA requires at least 15 to 18 mg Eq tocopherol. However, if one takes the value of 0.9 mg Eq vit. E/1 g PUFA, which is recommended for protection of polyenoic FA, then the need for vitamin E would be 50% higher.

In conclusion, a diet containing optimum amounts of energy and essential constituents, wherein the type of dietary fat has no significant influence, is the real basis for good nutrition practices.

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11 Plant Lipids and Oils

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CONTENTS

- 11.1 Introduction
- 11.2 Sources of Plant Lipids
 - 11.2.1 Structural Lipids
 - 11.2.2 Storage Lipids in Seeds
- 11.3 Effect of Plant Species on Lipid Composition and Quality
- 11.4 Effect of Plant Species on the Fatty Acid Composition
 - 11.4.1 Fatty Acid Composition of Traditional Edible Plant Oils
 - 11.4.2 Oils Containing Unusual or Specific Fatty Acids
 - 11.4.3 Oils with Modified Fatty Acid Composition
- 11.5 Changes During Ripening and Postharvest Storage
 - 11.5.1 Changes during Ripening of Plant Seeds
 - 11.5.2 Post-Harvest Changes of Plant Lipids
- 11.6 Effect of Processing on the Functional Properties and Nutritional Value of Plant Lipids
 - 11.6.1 Changes during the Processing of Oilseeds or Oil-Bearing Fruits
 - 11.6.2 Effect of Blanching and Germination
 - 11.6.3 Changes of Lipids during Culinary Food Preparation
- 11.7 Role and Changes of Plant Lipids in Processed Foods
- References

11.1 INTRODUCTION

Plant lipids belong to two categories: (1) structural lipids necessary for the metabolism of cells and all plant tissues; and (2) storage lipids, which serve as a source of energy for germinating plant seeds. Only the latter are used in the food industry, and also for nonedible uses. However, structural lipids may be important with regard to changes in the properties of food during storage and processing because such changes affect the sensory and functional properties of food.

Lipids of algae are not included in this chapter because their lipid composition is more similar to that of marine animals.

11.2 SOURCES OF PLANT LIPIDS

11.2.1 STRUCTURAL LIPIDS

Small amounts of lipids, usually 0.5 to 2.0% (dry weight) are present in the cells of all plant tissues, where they have an essential role in plant metabolism, mainly as constituents of intracellular membranes. Fatty acids (FA) are mostly bound there in polar lipids, such as phospholipids, glycolipids, and mucolipids ([Chapter 5](#)), and only their minor part in neutral lipids, such as triacylglycerols (TAG), waxes, or sterol esters ([Chapter 3](#)). The chemical structures and FA compositions are rather similar in all plant families. Plants of some species contain an unusual acid in the seed oil. In such cases, the acid is either absent or present only in traces in other parts of the plant.

Structural lipids are often bound to proteins in the form of relatively polar lipoproteins with varying ratios of lipidic and protein moieties to become water soluble. Therefore, they are not extracted with nonpolar organic solvents, unless the lipid–protein bonds have been destroyed, either by steam heating or by the addition of lower alcohols. Some lipids are also bound to starch ([Chapter 17](#)).

The composition of lipids on the surface of leaves, stems, and fruits is quite different from that of lipids that form intracellular membranes. Their role is the protection of sensitive plant tissues against the loss of water and other biologically important volatiles. Waxes (i.e., esters of FA with monofunctional alcohols) are the most important components of these lipids. Some plant waxes are of commercial importance, such as carnauba or candellila wax. They are solid at room temperature and in temperate climates, with the exception of liquid jojoba wax, and are plastic or even liquid in tropical climates. They contain bound saturated long-chain FA and alcohols. Waxes on the surface of apples and other fruits from temperate zones are solids or semisolid pastes, consisting of terpenes, ceryl cerotate, ceryl palmitate, and other esters. In the wax from lettuce leaves, higher alcohols prevail, with only small amounts of free FA (Bakker et al., 1998). Other components, such as alkanes, ketones, esters, secondary alcohols, were detected in other vegetables (e.g., in kale or rutabaga).

11.2.2 STORAGE LIPIDS IN SEEDS

Seeds must always contain energy reserves, which are consumed during germination. The energy source can be either starch (or another macromolecular carbohydrate) or lipids (chiefly oils). Both energy sources occur only rarely in the same seed. However, even starchy seeds have high oil content in the germ, for example, in wheat or maize (corn) germs ([Table 11.1](#)). Contrary to structural lipids, seed lipids consist mostly of TAG with a specific steric configuration. Only rarely, they consist of waxes, such as in jojoba seeds.

The lipid content in seeds may be more than 40 to 50% dry weight ([Table 11.1](#)). The seeds are directly used as food (e.g., nuts) or can be processed to yield edible oils (in most cases) or pastes (e.g., peanut butter). During ripening, TAG are synthesized from FA and glycerol so that free FA, monoacylglycerols, and diacylglycerols may be present in unripe seeds in small amounts ([Section 11.5.1](#)).

TABLE 11.1**Lipid Content of Food Materials (% dry weight)**

Lipid Source	Lipid Content	Lipid Source	Lipid Content
Copra (<i>Cocos nucifera</i>)	63–70	Palm fruits (<i>Elaeis guineensis</i>)	30–40
Palm kernels (<i>Elaeis guineensis</i>)	40–52	Olive fruits (<i>Olea europaea</i>)	12–50
Cocoa beans (<i>Theobroma cacao</i>)	54–58	Avocado fruits (<i>Persea americana</i>)	40–80
Shea beans (<i>Butyrospermum parkii</i>)	45–55	Corn germs (<i>Zea mays</i>)	15–20
Cottonseeds (<i>Gossypium hirsutum</i>)	22–24	Wheat germs (<i>Triticum aestivum</i>)	7–12
Hazelnuts (<i>Corylus avellana</i>)	60–68	Rice bran (<i>Oryza sativa</i>)	8–16
Almonds (<i>Prunus amygdalus</i>)	60–65	Wheat flours	0.8–1.6
Sesame seeds (<i>Sesamum indicum</i>)	50–55	Wheat breads	0.9–1.7
Sunflower seeds (<i>Helianthus annuus</i>)	42–63	Chocolate	32–40
Flaxseeds (<i>Linum usitatissimum</i>)	38–44	Beans, bean meal	1.6–1.9
Hempseeds/ <i>Cannabis sativa</i>)	30–35	Peas, pea meal	1.5–1.7
Poppyseeds (<i>Papaver somniferum</i>)	40–51	Mushrooms	3.1–3.4
Walnuts (<i>Juglans regia</i>)	56–59	Fruits (different)	1.0–2.8
Peanuts (<i>Arachis hypogaea</i>)	45–50	Vegetables (different)	1.3–4.0
Soybeans (<i>Soja hispida</i>)	18–22	Potatoes (peeled)	0.7–0.9
Rapeseeds (<i>Brassica napus</i>)	40–48	Sugar	0

From Heimann, W. 1969. *Handbuch der Lebensmittelchemie, Band 4. Fette und Lipide*, Berlin: Springer; and other sources.

The opposite process takes place during the germination ([Section 11.5.2](#)), so that lipids of germinating seeds may also contain mono- and diacylglycerols as TAG degradation products.

In addition to seeds, seed pericarp is sometimes rich in oil, such as in olives, avocado, or palm fruit.

Lipids are distributed in seed cells in tiny droplets (about 1 μm in diameter), emulsified in a protein-containing medium. Small amounts of lipids are bound to proteins and carbohydrates. In the emulsion, lipids are primarily in liquid form, at least at the ambient temperature during the ripening. Under the author's climatic conditions in a temperate climate, the melting point is generally lower than 10°C; and after processing, they form viscous liquids, called oils after olive oil.

In tropical countries, where the ambient temperature is much higher, the melting point of storage lipids may be also higher. After transportation of tropical oils to Europe or North America, the processed lipids often solidify and are called vegetable fats, butters (such as cocoa butter), or even tallow (such as Chinese tallow from *Sapium sebiferum* seeds, containing mostly TAG of palmitic and oleic acids), according to their similarity to the consistency of respective animal products. Low-melting solid tropical fats are often called oils, such as palm oil or coconut oil, especially in English-speaking countries. The degree of unsaturation of seed oils depends not only on the plant species, but also on the climatic conditions and the cultivar.

For edible purposes, TAG have the greatest nutritional importance (see [Chapter 10](#)). Therefore, the processing conditions are selected in such a way as to obtain TAG in high yield and high purity ([Section 11.5.5](#)).

11.3 EFFECT OF PLANT SPECIES ON LIPID COMPOSITION AND QUALITY

Plant lipids consist of neutral lipids, mostly TAG (see [Chapter 3](#)), phospholipids, and glycolipids (see [Chapter 5](#)). From the standpoint of their importance in food, TAG are the most important lipid class. Many different TAG exist in every plant lipid fraction, differing in their acyl groups. Three FA are bound to a molecule of glycerol, but they are rarely identical in plant lipids. Usually, two or three different FA are bound in a molecule of TAG. If the acyls in the 1- and 3-positions are different, the carbon atom at the 2-position becomes asymmetric (see [Chapter 3](#)), such that the number of isomers thus increases. Therefore, it is better to use the *sn* system (see [Chapter 3](#)) in giving the exact structure of a TAG.

The distribution of acyls among different TAG, and even within a single TAG molecule, is not random, because of the stereospecificity of plant lipases. The 1-position is preferably occupied by saturated acyls, while the 2-position mainly by polyunsaturated acyls. The amount of 1-palmitoyl-2,3-dioleoylglycerol in olive oil is approximately 20 to 50 times greater than that of the isomeric 2-palmitoyl-1,3-dioleoylglycerol (Boskou, 1996).

Of course, there is no strict selectivity, which increases the number of isomers present. An example is shown in [Table 11.2](#). In some lipids (for example, in cocoa butter), the number of different major TAG is reduced to just a few, which results in certain specific properties of this fat.

TABLE 11.2
Composition of Triacylglycerols in Edible Oils (%)

ECN	Sunflower Oil	Peanut Oil	Rapeseed Oil
40	(LLL _n)	2.3 (LLL _n)	3.2 (LLL _n)
42	33.4 (LLL , OLL _n , PLL _n)	6.4 (LLL)	9.7 (OLL _n , PLL _n , LLL)
44	37.2 (OLL , PLL, OOL _n)	24.7 (OLL , PLL)	21.7 (OLL , POL _n , PLL)
46	13.7 (POL , SLL, OOL)	30.2 (OOL)	28.4 (OOL , POL, SLL)
48	6.0 (PPL, SOL, OOO)	19.4 (POL , PPL, SLL, OOO)	28.7 (OOO , POO, SOL)
50	6.3 (SOO, PSO)	6.9 (SOO , BLL, PSO)	2.4 (SOO , PPS, PSO)
52	2.8 (SSO, PSS)	4.8 (AOO, BOL, SSO)	2.8 (SSO, PSS)

Note: ECN = equivalent carbon number; fatty acid bound in triacylglycerol; Ln = linolenic; L = linoleic; O = oleic; P = palmitic; S = stearic; B = behenic; A = arachic)

Adapted from Pánek, J. 2000. Changes of triacylglycerol species during lipoxidation, Habilitation dissertation, VSCHT, Prague, Czech Republic.

The reactivity and nutritional value of a particular TAG depend on stereospecific isomerism. If a particular TAG composition is undesirable, it is possible to randomize the product, most often by treatment with alkaline catalysts. In a randomized fat or oil, the distribution of FA among TAG species is quite random. The randomization is particularly important in solid fats because the melting interval and the texture are changed; for example, the melting point of cocoa butter increases from 34.8 to 54.2°C during the randomization.

The specific distribution of FA in a molecule also occurs in polar lipids (see [Chapter 5](#)). However, it does not affect the properties of the respective food product to such a high degree as the distribution in TAG, because polar lipids are only minor components in food.

In a mixture of TAG, such as in edible oils, cooking fats, or margarine, the reactivities of individual TAG species differ significantly because the more saturated species are less reactive. For example, dilinoleoylmonooleoylglycerol is oxidized more easily in a mixture than monolinoleoyldioleoylglycerol (Pánek et al., 1995). Therefore, the TAG composition changes during food processing or storage.

11.4 EFFECT OF PLANT SPECIES ON THE FATTY ACID COMPOSITION

11.4.1 FATTY ACID COMPOSITION OF TRADITIONAL EDIBLE PLANT OILS

The FA composition of plant lipids differs considerably from that of animal lipids as well as marine lipids, even when the most common FA (e.g., palmitic and oleic acids) are present in lipids. While structural plant lipids have similar FA composition in all plant species, storage lipids are somewhat different; their composition is sometimes specific for some plant families or even single plant species.

Seed and fruit lipids of different plant species have been determined according to their texture, and more recently according to their FA composition. (Hilditch and Williams, 1964). They can be classified into groups according to the similar FA compositions of their oils:

Group 1: A group that typically has a high content of lauric and medium-chain FA and low unsaturation ([Table 11.3](#)). Oils from palm seeds belong to this group, such as coconut and palm kernel oil. These oils solidify at temperatures below 25°C and are very stable under storage or during frying.

Group 2: A group of seed oils of some tropical trees, such as cocoa or shea butter, and also palm oil that is produced from the pericarp, not from the seed. These oils are characterized by their high content of palmitic and stearic acids, and are solid at ambient temperature in temperate climates ([Table 11.4](#)).

Group 3: A group of high-oleic acid, low-linoleic acid oils, such as olive oil from the pericarp of olive fruit. Seed oils, such as almond oil, hazelnut oil, or avocado oil, also belong to this group ([Table 11.5](#)).

TABLE 11.3**The Main Fatty Acids of Lipids of Palm Seeds (% total fatty acids)**

Fatty Acid	Coconut Oil	Palm Kernel Oil	Babassu Oil
Octanoic	4.6–9.4	2.1–4.7	2.6–7.7
Decanoic	5.5–7.8	2.6–4.5	1.2–7.6
Lauric	45.1–50.3	43.6–53.2	40.0–55.0
Myristic	16.8–20.6	15.3–17.2	11.0–27.0
Palmitic	7.7–10.2	7.1–10.0	5.2–11.0
Stearic	2.3–3.5	1.3–3.0	1.8–7.4
Oleic	5.4–8.1	11.9–19.3	9.0–20.0
Linoleic	1.0–2.1	1.4–3.3	1.4–6.6

Adapted from AOCS. 1997. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed., Champaign, IL: AOCS Press.

TABLE 11.4**The Main Fatty Acids of Fats Rich in Palmitic and Stearic Acids (% total fatty acids)**

Fatty Acid	Illipe (mowrah) Butter	Cocoa Butter	Shea Butter	Borneo Tallow
Palmitic	23	25–27	4–8	18–21
Stearic	23	33–37	36–41	39–43
Oleic	34	34–35	45–50	34–37
Linoleic	14	3–4	4–8	<1

Adapted from AOCS. 1997. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed., Champaign, IL: AOCS Press.

Group 4: A group with high linoleic acid content, but containing no linolenic acid or only traces. Examples include sunflower, safflower, sesame, peanut, and cottonseed oils (Table 11.5). These oils are very important in the production of edible oils.

Group 5: A group containing linolenic acid as the most unsaturated FA; examples include soybean, rapeseed, hempseed, linseed, and perilla oils (Table 11.5). Soybean oil and rapeseed oil are the two most important edible oils.

Germ oils belong to either group 4 or group 5. If the oil belongs to group 5, the linolenic acid content is very low (Table 11.6).

All unsaturated FA in the previously mentioned groups have a *cis* double-bond configuration; however, some commercially refined edible oils also contain small amounts of *trans*-unsaturated FA. In virgin oils, a *trans*-FA content greater than

TABLE 11.5**The Main Fatty Acids of Liquid Edible Oils (% total fatty acids)**

Oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Almond	4–13	0–10	43–60	20–34	—
Avocado	9–18	<1	56–74	10–17	0–2
Cashew nut	4–17	2–11	61–80	19–22	<1
Coffee bean	35–42	7–11	8–10	36–43	<1
Cottonseed	21–26	2–3	15–22	46–58	<1
Hazelnut	5–7	1–3	72–84	6–22	<1
Hempseed	6–12	1–2	11–16	45–66	15–30
Linseed	7	4	20	17	52
Olive	8–20	1–5	55–83	4–21	<1
Palm oil	40–46	4–7	36–41	9–12	<1
Palm olein	38–43	4–5	40–44	10–13	<1
Palm stearin	48–74	4–6	15–36	3–10	<1
Peanut	8–14	2–4	36–67	14–43	trace
Pecan nut	5–11	1–6	49–69	19–40	0–3
Perilla	6–7	1–2	13–15	14–17	44–64
Poppyseed	7–11	1–4	16–30	62–73	trace
Rapeseed (canola)	3–6	1–3	52–67	16–25	6–14
Safflower	5–8	2–3	8–21	68–83	trace
Sesame	8–10	5–6	36–42	41–48	<1
Soybean	10–13	3–5	18–25	50–57	5–10
Sunflower	5–8	2–7	13–40	40–74	<1
Walnut	7–8	2	17–19	56–60	13–14

Data from AOCS. 1997. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed., Champaign, IL: AOCS Press; Werman, M.J. and Neeman, I. 1987. *JAOCs*, 64, 229–232; and other sources.

TABLE 11.6**The Main Fatty Acids of Cereal Lipids (% total fatty acids)**

Fatty acid	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Corn germ	11–17	2–3	24–42	39–60	<1
Rice bran	16–28	2–4	42–48	16–36	<1
Oat oil	14–23	1–3	29–53	24–48	1–3
Rye germ	9–21	0–2	7–35	48–72	3–8
Wheat germ	12–19	<3	14–23	50–56	3–7

Adapted from AOCS. 1997. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed., Champaign, IL: AOCS Press; Barnes, P.J. 1987. *Recent Advances in Chemistry and Technology of Fats and Oils*, Hamilton, R.J. and Bhati, A., Eds., London: Elsevier Applied Science, 78–108.

0.1% is a sign of improper technology, but concentrations up to 1% are common in oils processed by refining (see [Section 11.6.1](#)). They are formed during crude oil refining, especially deodorization. They may be already present in crude oil because they are formed by enzyme-catalyzed oxidation of polyunsaturated lipids during storage of seeds (see [Chapter 8](#)).

Plant desaturases are not entirely specific; therefore, oleic (9-octadecenoic) acid is accompanied by small amounts of 11-octadecenoic acid. Linoleic (9,12-octadecadienoic) acid is accompanied by small amounts of 9,15-octadecadienoic acid.

11.4.2 OILS CONTAINING UNUSUAL OR SPECIFIC FATTY ACIDS

The lipids of some plant species contain special FA not found in other plant families (see [Table 11.6](#)). They may contain a double bond in a different position (Diedrich and Henschel, 1991a), *trans* double bonds or conjugated double bonds, and acetylenic bonds (Diedrich and Henschel, 1991b), or are substituted with a keto, epoxy, or hydroxy group or even alicyclic or aromatic substituents.

Some are suitable for human nutrition, at least in small amounts (*Umbelliferae* and *Cruciferae* plants); others are consumed only in tropical countries (such as tung oil) and still other oils are used only for nonedible purposes (such as castor oil or oiticica oil). They might even be used for pharmaceutical purposes (e.g., chaulmoogra oil).

11.4.3 OILS WITH MODIFIED FATTY ACID COMPOSITION

Some traditional oils are not suitable for nutrition or are less suitable for specific technological uses (e.g., deep-frying) because of their enhanced resistance against oxidation. Therefore, their FA composition has been modified. Oils can be modified, either by classical breeding methods or, in recent years, by genetic manipulation (see [Chapter 16](#)).

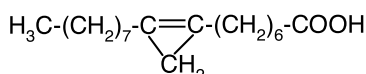
The best example is rapeseed oil (either *Brassica napus* or *Brassica campestris*). The traditional rapeseed oil contained erucic acid as its major FA. Its consumption was found unsuitable from the point of view of health risks. After breeding for many years, rape plant varieties now prevail in Europe and North America that contain less than 1% erucic acid of total FA (called canola oil). The rape plant was found to be very flexible and easily influenced by breeding. One cultivar was developed with reduced linolenic acid content so that it would have better resistance against rancidification. Rapeseed oil with a high stearic acid content is solid at room temperature; thus, it can be used as a substitute for hydrogenated oils in margarine production. Another cultivar has a high lauric acid content and its oil can be used as a substitute for coconut oil in soap making. Another cultivar, having a high arucic acid content, is used for the production of oil for technical purposes. A cultivar was recently announced that contains high percentages of both docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA); this may replace fish oil for pharmaceutical purposes.

Of course, rapeseed oil is not the only modified oil (Krawczyk, 1999). Polyunsaturated FA cause low resistance against oxidation, especially under frying conditions but also on storage. Therefore, high-oleic cultivars are now available in which the polyunsaturated content has been reduced ([Table 11.7](#)). High-oleic and low- or medium-linoleic sunflower oils have been available on the market for

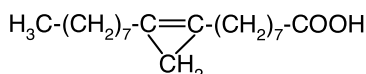
TABLE 11.7**Examples of Unusual Fatty Acids in Plant Lipids**

Source of Lipids	Unusual Fatty Acid
<i>Umbelliferae</i> plants (e.g., parsley)	Petroselinic acid, 6-octadecenoic acid
<i>Brassicaceae</i> plants (e.g., mustard seed)	Erucic acid, 13-docosenoic acid
Elm, <i>Ulmus americana</i> seed	Capric acid, decanoic acid
Pine nut, <i>Pinus pinea</i>	5,11,14-Eicoatrienoic acid
<i>Limnathese douglasii</i>	5,13-Docosadienoic acid
Evening primrose, <i>Oenothera</i> sp.	γ -Linolenic acid, 6,9,12-octadecatrienoic acid
Tung oil, <i>Aleurites fordii</i>	<i>cis,cis,trans</i> -9,11,13-Octadecatrienoic acid
Marigold, <i>Calendula officinalis</i>	<i>trans,trans,cis</i> -8,10,12-Octadecatrienoic acid
<i>Parinarium laurinum</i>	9,11,13,15-Octadecatetraenoic acid
Boleko, <i>Onguekoe gore engler</i>	Isanic acid, octadeca-17-en-9,11-diynoic acid
Sterculia, <i>Sterculia foetida</i>	Malvalic, sterculic acids (Figure 11.1)
<i>Vernonia antihelminctica</i>	Vernolic acid (Figure 11.1)
Chaulmoogric oil	Chaulmoogric acid (Figure 11.1)
Oiticica, <i>Licania rigida</i>	Licanic acid, 4-keto-9,11,13-octadecatrienoic acid
Castor oil, <i>Ricinus communis</i>	Ricinoleic acid, 12-hydroxy-9-octadecenoic acid

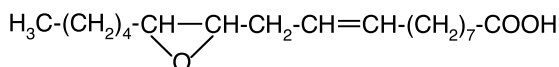
Adapted from AOCS. 1997. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed., Champaign, IL: AOCS Press; Wolf, R.B., Kleiman, R., and England, R.E. 1983. *JAOCs*, 60, 1858–1860; Diedrich, M. and Henschel, K.P. 1991a. *Nahrung*, 35, 85–95; Diedrich, M. and Henschel, K.P. 1991b. *Nahrung*, 35, 193–202; and other sources.



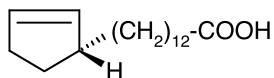
Malvalic acid



Sterculic acid



Vernolic acid



Chaulmoogric acid

FIGURE 11.1 Examples of cyclic acids occurring in plant lipids.

several years (Haumann, 1994), high-oleic sesame oil, safflower oil, and peanut oil have been introduced. Even special cultivars of linseed oil are available that contain trace amounts of linolenic acid and a low percentage of linoleic acid. They

TABLE 11.8**The Main Fatty Acid Groups of Modified Oils (% total fatty acids)**

Oil	Saturated	Monoenoic	Dienoic	Trienoic
Safflower oil:				
traditional	7–10	8–21	68–83	trace
modified	6–8	74–80	13–18	trace
Sunflower oil:				
traditional	7–14	13–41	40–74	trace
modified	6–9	70–87	3–20	trace
Linseed oil:				
traditional	11	20	17	52
modified	4	22	72	2
Peanut oil:				
traditional	18	44	37	trace
modified	13	81	6	trace

Adapted from AOCS. 1997. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed., Champaign, IL: AOCS Press; Párkányiová, L., et al. 2000. *Czech J. Food Sci.*, 18 (Spec.): 125–126.

are suitable for prolonged storage, deep-frying, and microwave heating. The yields and availability of the modified seeds are still not guaranteed, and sufficient experience is lacking in seed and oil processing factories, but it is expected that in the next several years they will be used as much as low-erucic acid rapeseed oil is now.

11.5 CHANGES DURING RIPENING AND POSTHARVEST STORAGE

11.5.1 CHANGES DURING RIPENING OF PLANT SEEDS

The lipid composition in unripened plant seeds is similar to that of structural lipids in other plant tissues and the lipid content is low (up to 3 to 5%, dry weight). During the ripening, lipids are synthesized and the composition of these new lipids corresponds to that of storage lipids. Diacylglycerols are rapidly formed as intermediates during TAG synthesis (e.g., in rapeseed) (Perry and Harwood, 1993). Free FA are incorporated into the diacylglycerol molecule, and their synthesis ceases when TAG synthesis ceases (May and Hume, 1995). At the same time, the content of polar lipids decreases because newly synthesized lipids contain mostly TAG, and thus dilute the polar lipids. On the contrary, the content of phospholipids and galactolipids decreased even during the ripening of tomatoes, in which TAG do not accumulate substantially (Whitaker, 1994).

The increase in lipid content is very fast during the last 2 to 4 weeks prior to full ripeness. The FA composition gradually approaches that of a fully ripe seed. In high-oleic sunflower, the oil first develops similar to that of a traditional

cultivar but oleic acid is preferentially formed during the final stages of ripening. Not only the FA composition, but also their distribution among the TAG molecules is changing; for example, the content of palmitic acid in the 1,3-positions increased but that of oleic acid decreased during ripening of palm fruit (George and Arumughan, 1994). At the same time, the enzymic activities of the respective enzymes are increasing. Tocopherols are formed at the same time so that the stability against oxidation becomes optimal at the stage of full ripeness.

Changes in plant tissues other than the seeds are much smaller prior to harvest ripeness, except for the pericarp in olives and palm fruit, where the changes are similar to those in seeds.

11.5.2 POST-HARVEST CHANGES OF PLANT LIPIDS

After the harvest of seeds, the lipids are relatively stable if the seed is dry. In the case of a rainy season during the harvest, it is necessary to dry seeds within a short time; otherwise, post-harvest changes are very fast and the seed quality deteriorates. Molds growing on the surface of moist seeds contribute to the deterioration of seed lipids. It is preferable to store the harvested seeds intact; they should be free of any broken seeds. Damaged seeds are rapidly spoiled (e.g., poppyseed or peanuts). Nuts are much more stable if they remain unshelled.

Under optimum storage conditions, seeds rich in oil can be stored until the next harvest without significant changes, even in the case of relatively unsaturated lipids such as in poppyseed, hempseed, and walnuts. The optimal moisture content depends on the particular seed, and is approximately 8 to 10% dry weight in most cases. Very dry seeds may be less stable.

Hydrolases have low activity in air-dried seeds (see [Chapter 9](#)), but still the content of free FA in the extract slowly increases if the storage time exceeds several months. Phospholipids are partially converted into lysophospholipids and phosphatidic acids. Hydrolytic changes catalyzed by enzymes are very fast in cereal bran and germ lipids; for example, the free FA content nearly doubled within 2 days in moist rice germ (Dong and Chung, 1999), but it did not change significantly if the material was dried immediately. Therefore, germs and brans should be rapidly processed by steaming. If the free FA content reaches a value greater than approximately 10 mg/kg, bitter substances are formed, and the oil is refined only with difficulty and with great losses. Plant tissues other than seeds have a higher water content and should be rapidly blanched to deactivate enzymes.

Another group of post-harvest processes are enzyme-catalyzed oxidation reactions, such as the degradation by lipoxygenases (see [Chapter 8](#)). Hydroperoxides are slowly formed, even in relatively dry seeds, but are again decomposed by hydroperoxide lyases and other hydroperoxide-cleaving enzymes. Volatile decomposition products cause rancidity, which is particularly intensive in lipids that contain bound linolenic acid. Hay-like off-flavors are produced in green parts of the plants because they are rich in linolenic acid, the oxidation products of which give rise to such off-flavors. If the oxidation reactions have proceeded to a low degree only, they may be perceived as desirable. For example, the typical flavors of cucumbers and virgin olive oil are due to lipid oxidation

products. Even if oxido-reductases are inactivated by heating or if seeds are dry, oxidation may take place via other mechanisms (e.g., autoxidation) (see [Chapter 8](#)).

Contrary to the lipids in animal products, plant lipids always contain natural antioxidants, mostly tocopherols (Hall, 2001; Yanishlieva and Heinonen, 2001). They control the oxidation that occurs during storage, but they are slowly consumed by inhibition reactions with free radicals. Therefore, the resistance of plant lipids to oxidation slowly decreases on storage, especially in the presence of light.

11.6 EFFECT OF PROCESSING ON THE FUNCTIONAL PROPERTIES AND NUTRITIONAL VALUE OF PLANT LIPIDS

11.6.1 CHANGES DURING THE PROCESSING OF OILSEEDS OR OIL-BEARING FRUITS

Oilseeds are crushed, lipid–protein bonds are destroyed by steaming, and the resulting material is expeller-pressed. Cakes obtained in this operation still contain 10 to 25% oil. Therefore, they are extracted with a solvent, primarily hexane. Other solvents are possible, but hydrocarbon solvents are cheaper. The extracted meal still contains 2 to 3% lipids but because it is used as feed, the residual lipids are utilized and are not objectionable. Crude oils obtained by expeller pressing and solvent extraction are usually mixed and refined. The first refining step in the removal of phospholipids (see [Chapter 5](#)) is the process called degumming.

Degummed oils are further refined either by alkali refining or by physical refining. In the alkali refining process, free FA are first removed with an aqueous solution of sodium hydroxide and sodium carbonate; the neutralized oil is washed and dried; and both the chlorophyll and carotenoid pigments are removed by bleaching. The resulting oil still contains some volatile oxidation products, imparting off-flavors to the product. They are removed in the process of deodorization, consisting of the application of superheated steam under reduced pressure at temperatures exceeding 200°C.

In the physical refining process (Čmolík and Pokorný, 2000), phospholipids are efficiently eliminated, and the degummed oil is thoroughly bleached and deodorized. Free FA are distilled off with other volatiles during the deodorization step.

During the refining, the sensory value of the oil is substantially improved but the nutritional value is impaired by partial removal of carotenoids, tocopherols, and phytosterols, and by moderate isomerization of polyunsaturated acyls into their *cis,trans* isomers; linolenic acid is especially sensitive (Čmolík et al., 2000). The amount of *trans* isomers should not exceed 1%. Refined oils are nearly flavorless, tasteless, and colorless, and possess good stability against autoxidation.

Olive fruits are processed in another way (DiGiovacchino, 1996). Because they contain water, they should be processed by pressing very rapidly, without preliminary heating. Such oils are called virgin oils, and are not further refined; this is done to maintain the natural flavor of the substance and to impart olive oil with its specific flavor. It is possible to produce virgin oils from other sources, however, their flavor

is usually not sufficiently acceptable, perhaps with the exception of sunflower, hazelnut, and almond oils.

Palm fruits should also be processed quickly; they are rarely sold as virgin oils and are primarily refined. Because it possesses a nice red or orange color, a part of the carotenoid pigment is sometimes returned back to the refined oil.

Refined oils can be hydrogenated by gaseous hydrogen in the presence of a nickel catalyst. Double bonds of polyunsaturated FA are converted to monounsaturated FA and, to a small extent, to saturated acids. The advantage is that hydrogenated oils are solid; thus, they can be used for the production of margarine, cooking fats, soaps, and many other nonedible products. The disadvantage is that they contain 10 to 40% *trans*-unsaturated FA, which are not desirable for human nutrition in such high amounts.

Solid fats can be produced by an alternative method. High-melting fats, such as palm stearin, animal fats, or fully hydrogenated oils, are mixed with oils and treated with sodium methoxide or an alkali metal. Under these conditions, ester interchange between solid and liquid TAG occurs, and the resulting fat is plastic, similar to hydrogenated oils, but does not contain *trans*-FA.

Low-energy lipids are formed by ester interchange of high-melting (and thus less-digestible) TAG with medium-chain length FA, which are not deposited in the adipose tissue. They have substantially lower energy content than normal fats. The TAG composition can be also modified by interesterification, catalyzed by stereospecific lipases, mostly of microbial origin (Xu, 2000). Cocoa butter substitutes are produced from palm olein by introducing stearoyl residues into the molecules. For more information, see [Chapter 16](#).

11.6.2 EFFECT OF BLANCHING AND GERMINATION

Lipids in fruit and vegetables would be rapidly destroyed by the effect of enzymes and microorganisms; therefore, they are often treated by steam to inactivate the enzymes and kill the microorganisms. The heating should be rapid; otherwise, both hydrolytic and oxidative lipid degradation will take place to a significant degree.

In some technological processes, seeds are germinated as a special step. They are soaked in water to initiate enzymatic processes; and when the germination reaches the appropriate degree, the processes are interrupted by rapid drying. Malting of barley and other cereal grains is a typical example. Lipids are rapidly hydrolyzed during germination, the liberated FA are consumed as an energy source, and therefore the total lipid content decreases (Patterson, 1998). Malted cereals are used for brewing or in the production of breakfast cereals.

11.6.3 CHANGES OF LIPIDS DURING CULINARY FOOD PREPARATION

Changes occur with the addition of water to a food material (e.g., during soaking or dough making). During boiling, lipid degradation is slow because enzymes are soon destroyed, and oxygen dissolved in water is rapidly consumed or removed with steam. If boiling takes a long time, however, hydrolysis might occur and the broth becomes turbid because of dispersed liberated FA and partially esterified glycerol esters.

During extrusion cooking, no perceptible changes occur because the process is very fast and the temperature sufficient for enzyme inactivation is attained in less than 1 min. The content of extractable lipids may be affected by lipid–starch interactions (see Chapter 18). The maximum temperature is usually too low to initiate oxidation reactions, but partial destruction of natural antioxidants does occur.

During baking, lipids are minimally affected, except in the surface layers. Moderate oxidation takes place; and the oxidation products, reacting with proteins, influence the texture. Roasting proceeds at high temperatures, often at more than 200°C, but the process takes only up to 20 min. Nevertheless, oxidation reactions may be pronounced, especially in the case of coffee or peanuts. Microwave heating causes oxidative changes if the time is sufficiently long and the temperature attains high values but, in general, lipid damage is only moderate.

The most severe degradation takes place during deep-frying in oils (see Chapter 16), especially in frying oil and in the outer layers of fried food. Fortunately, plant foods are usually fried in a batter so that inner layers are protected against degradation processes, and several food components inhibit oxidation reactions (Pokorný, 1999).

11.7 ROLE AND CHANGES OF PLANT LIPIDS IN PROCESSED FOODS

Plant lipids increase the nutritional value of food; they contain more essential FA than animal fats. They also contain tocopherols and tocotrienols, which are the most important sources of vitamin E. A disadvantage is that plant lipids (similar to animal fats) considerably increase the content of available energy. Therefore, the consumption of fried foods should be restricted because they are rich in fat.

Lipids affect the functional properties of food; for example, they help to retain carbon dioxide in dough, thus increasing the final volume of bakery products. Polar lipids are useful in improving the cohesiveness of food components; they contribute to the pasting properties of cereal doughs, stabilize the dispersion, and also affect the surface properties. Phospholipids and monoacylglycerols are useful in this respect (see Chapter 4).

The main importance of lipids is their influence on sensory properties. They affect the texture (Chapter 4) and increase the viscosity of the morsel after mixing with saliva; high viscosity is appreciated by most consumers. Their interaction products with amino acids, proteins, and sugars (see Chapter 17) have favorable effects on the color of food surfaces. Coating foods with a layer of oil produces glossy aspect and improves the appearance of the food product.

The most desirable influence of lipids is their effects on the odor and flavor of food products. Plant lipids, being more unsaturated than animal lipids, produce different flavor notes as a result of culinary operations. Flavors originating at roasting or frying temperatures are particularly appreciated.

Fat migration takes place during storage of emulsions, and either lipid or water droplets gradually separate. In chocolate, the ratio of solid to liquid TAG continually

changes due to small variations in the storage temperature. The result is the separation of white solid TAG on the surface, called blooming.

During storage of food products, volatile compounds produced by lipid oxidation cause rancidity, especially if the lipids contain linolenic acid. Dark flours become easily rancid on storage. Crackers and other durable bakery products should be stored in an inert gas atmosphere or be protected by antioxidants. Roasted products, such as peanuts, may change their agreeable flavor if stored in air; therefore, they are stored either in nitrogen or under reduced pressure. Fried products are most sensitive to oxidative rancidification, especially fried products with weak flavor, such as fried bread, French fries, or potato chips. Dry and deep-frozen products are generally rather sensitive to oxidation because of easier access of air into the inner layers of the food product.

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12 Fish Lipids

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CONTENTS

- 12.1 Introduction
- 12.2 Lipid Content and Distribution in Fish Body
- 12.3 Composition of Lipids: The Lipid Classes
- 12.4 Fatty Acid Composition
 - 12.4.1 Fatty Acid Groups
 - 12.4.2 Saturated and Monounsaturated Fatty Acids
 - 12.4.3 Polyunsaturated Fatty Acids
 - 12.4.3.1 Main Types of PUFA in Fish
 - 12.4.3.2 (n-3)/(n-6) Ratio
 - 12.4.3.3 Distribution of PUFA in Lipid Class
- 12.5 Factors Affecting the Lipids in Fish
 - 12.5.1 Biological Role of (n-3) PUFA in Fish
 - 12.5.2 Diet
 - 12.5.3 Seasonal Variations
- 12.6 Susceptibility to Oxidation
- 12.7 Effects of Processing on Fish Lipids
 - 12.7.1 Chilling Fish
 - 12.7.2 Freezing Fish
 - 12.7.3 Cooked Fish
 - 12.7.4 Grilled and Fried Fish
 - 12.7.5 Canned Fish
 - 12.7.6 Smoked Fish
 - 12.7.7 Salted Fish
 - 12.7.8 Marinated Fish
- 12.8 The Role of Fish Lipids in Human Nutrition
- 12.9 Conclusions
- References

12.1 INTRODUCTION

And they had a few small fishes: and he blessed, and commended to set them also before them. So they did eat, and were filled and they took up of the broken meat that was left seven baskets. And they that had eaten were about four thousand; and he sent them away.

(Mark 8: 7–9)

From time immemorial, humans have used fish as food. Fish are a source of highly nutritive protein; vitamins A, D, and particularly B; calcium, phosphorus, rare microelements (iodine, selenium, fluorine); and lipids (fats and oils) that have particularly beneficial health effects. Medical doctors, nutritionists, and scientists have acknowledged these qualities, particularly during the past two decades. However, the correct conclusions could have been drawn much earlier in view of the fact that, 2000 years ago, it was the fish and bread that were multiplied by Jesus who gave them to the listening crowd.

Humans eat fish of more than 1000 fish species; approximately 350 of these species are regarded as commercially valuable. The term “fish products” applies to all marine and freshwater animals, including finfish (i.e., scale fish or bony fish, and sharks and rays) and shellfish (i.e., the crustaceans [e.g., prawns, shrimps, lobsters, Antarctic krill], and mollusks [e.g., squid, oysters, abalone]). The only exceptions are aquatic mammals and frogs (EC Directive 91/493/EEC). Overall, the number of species from aquatic environments consumed by humans is consequently much higher. The marine and freshwater fish consumed by humans are mainly wild-caught. On the other hand, cultured fish contribute about 12% to the world fish catch, but these are mainly freshwater fish. Edible parts of fish include fillets (mainly the muscle tissue called the fish meat, with or without skin), carcasses (e.g., canned), and by-products of some fish species, mainly gonads (roe) and livers (of lean fish).

12.2 LIPID CONTENT AND DISTRIBUTION IN FISH BODY

Lipid content of finfish can vary over a wide range (0.3 to 45%, w/w). In contrast, shellfish, which are mostly crustaceans (prawns, crabs, lobster, crayfish), and mollusks (bivalves, squids) usually only contain 1 to 2% lipids. In Poland, where many high-lipid finfish are consumed, fish can be divided into four basic groups: lean (below 2% lipid), medium fat (2 to 7% lipid), fat (7 to 15% lipid), and high fat (more than 15% lipid) (Table 12.1). This classification differs from the commonly used classification of Lambertsen (1978), who divided fish into the following four classes (Ackman, 1994):

1. Lean (<2% fat): cod, haddock, shellfish
2. Low-fat (2–4% fat): sole, halibut, flounder
3. Medium-fat (4–8% fat): wild salmon
4. High-fat (>8% fat): herring, mackerel, sablefish, farmed salmon

TABLE 12.1
Classes of Fish by Flesh Lipid Content (% w/w)

Group	Examples of Fish Species	Range Lipid Content (% w/w)	Average Lipid Content (% w/w)
Lean	Cod, haddock, whiting, hake, Alaska pollock, blue whiting	0.2–2.0	1.0
Medium fat	Flatfish, sea trout, rainbow trout, tuna, roach, bream	2.0–7.0	5.0
Fat	Herring, sprat, mackerel, horse mackerel, salmon, carp	7.0–15.0	10.5
High fat	Eel, capelin	>15.0	20.0

Complications in arriving at an acceptable classification system stem from the fact that the fat content in the fat fish (e.g., mackerel, herring, sardine, or sprat, and also in some farmed species) tends to vary widely. For example, depending on the season, the lipid content can vary from 4 to more than 30% in mackerel and from 2 to 25% in herring. This is particularly evident in temperate species due to their migratory patterns and highly seasonal feeding and reproductive cycles. Thus, the flesh of these moderate-latitude species generally contains more lipids than the leaner tropical species. For farmed finfish and shellfish, the total lipid content of the diet is important because lipids are a valuable energy source. Therefore, most farmed animals are usually fed high-energy/high-lipid diets to maximize growth rates, and thus their flesh tends to contain more lipid than wild examples of the same species — sometimes twice as much (Dunstan et al., 1996; Nichols et al., 1998). All these factors can result in a particular species being assigned to more than one of the categories shown in [Table 12.1](#), and therefore these categories should only be used as a guide.

The major lipid storage sites in fish vary in different species. However, lipids are primarily located in the subcutaneous tissue, belly flap, muscle tissue, liver, mesenteric tissue, and in the head (Ackman, 1994). The amount of lipid dispersed through the flesh decreases from the head to the tail, where the muscles used for swimming are located. Lipid content in the dark muscle is usually several times greater than that of the white muscles. There is also a considerable amount of lipid located in the myosepta surrounding the muscles (Zhou and Ackman, 1995). The white muscle is responsible for rapid bursts of activity such as sudden fast swimming, while the dark muscle is used for steady, sustained swimming. For this reason, the flesh of fast-moving, migratory species (i.e., many pelagic fish, such as tuna, herring, mackerel, sprat, anchovy, and sardine) contain more dark muscle tissue and more fat than species such as cod, which is generally slower moving (except when darting to catch a prey). The dark muscle contribution to the flesh of clupeids and carangids is about 20% of total muscle, the contribution in mackerel reaching about 26% (Greer-Walker and Pull, 1975). Lean fish usually have mostly white meat, but dark muscle still contributes about 10% in the gadoids.

Fish skin contains more lipid than the muscle tissue. The lipid content in the skin of lean fish (e.g., cod, blue whiting) averages 0.2 to 3.9% w/w. The lipid content

TABLE 12.2**Lipid Content and Composition in Offal of Baltic Sea and Freshwater Fish, in Comparison with Muscle Lipids**

Fish	Date of Catch (month)	Offal					Edible Part (fillet with skin)				
		Lipid (%)		EPA + DHA (%)			Lipid (%)		EPA + DHA (%)		
		w/w	d.m.	Fatty acids	w/w	d.m.	w/w	d.m.	Fatty acids	w/w	d.m.
Herring	Jan	12.30	44.83	22.08	2.71	10.09	7.43	29.72	19.00	1.35	5.40
	Mar	9.15	40.48	16.74	1.53	5.97	9.04	33.48	10.60	0.95	3.47
Sprat	Jan	7.51	28.88	23.35	2.25	8.62	10.89	38.89	17.24	1.88	6.71
	Mar	7.48	30.56	23.20	1.75	7.14	6.10	26.52	19.23	1.44	6.26
Cod (without liver)	Feb	2.77	11.79	16.25	0.45	1.91	0.3	1.53	51.10	0.15	0.76
Rainbow trout	Feb	25.65	79.07	16.15	4.14	17.59	6.66	21.34	17.56	1.16	3.72
Carp	Jan	17.41	60.27	21.09	3.67	12.70	13.83	46.0	15.21	2.10	7.0
Perch	Jan	9.03	32.25	16.93	1.53	5.46	1.45	6.0	24.80	0.36	1.5

Adapted from Kołakowska, A., Kramer M., and Szczygielski M., 2001, *24th World Congr. Exhibition Int. Society for Fat Research (ISF)*, Berlin, September 16–20, 53.

of the skin of fat fish is much higher and can be more than 50% w/w. Among the other organs, only the roe and liver of some fish species play a role as foodstuffs. In cod, the roe can contribute up to 27% body weight (depending on the season), and may contain from 0.3 to 1.5% w/w lipid. The liver contributes between 4 and 9% body weight, and may contain up to 70% w/w lipid. In a number of fish species (excluding the sprat), the lipid content of nonedible offal was higher than in fillets (with skin) (Table 12.2).

12.3 COMPOSITION OF LIPIDS: THE LIPID CLASSES

The lipids of fish flesh are mainly composed of phospholipids, triacylglycerols (TAG), and sterols; minor quantities of metabolic products of these; and small amounts of unusual lipids such as glycolipids and sulfolipids. Triacylglycerols are the storage lipids in almost all commercial fish species. As determined over two annual cycles, the total lipids of fresh Baltic herring muscle (4.2 to 18% w/w) consisted of 51 to 88% TAG, 11.8 to 39% phospholipids, 3.9 to 8.8% cholesterol + 1,2-diacylglycerol, from trace amounts to 7% free fatty acids (FA), from trace amounts to a few percent cholesterol esters and 1,3-diacylglycerol. Baltic sprat had a similar lipid composition (Kołakowska and Kołakowski, 1990; Kołakowska et al., 2000a, 2001d). The TAG content increases with increasing lipid content in muscles.

Because phospholipids are typically the other main lipid class found in fish flesh, the leaner the fish and the higher the proportion that phospholipids contribute to total lipids. For this reason, phospholipids comprise almost 90% of total lipids of lean fish such as cod, with TAG contributing as little as about 1%. Due to anatomical and physiological reasons, the amount of structural lipids (phospholipids) varies between 0.3 and 0.5 per 100 g w/w of fish muscle and does not usually exceed 1% w/w. This is most likely the minimum level of phospholipids essential for the cell and organelle membranes, of which they are a major component. Phospholipids are the major lipid class in most Australian fish, and in mollusks and crustaceans, all of which are typically lean (Table 12.3). In contrast to finfish, which tend to store lipid as TAG, an increase in the lipid content of shellfish is usually due to an accumulation of polar lipids (Nichols et al., 1998). This is similar to the case in Antarctic krill (*Euphausia superba* D.), the phospholipids of which serve as storage lipids along with TAG.

A typical fish polar lipid fraction contains about 60% phosphatidylcholine (PC), 20% phosphatidylethanolamine (PE), and several percent phosphatidylserine and sphingomyelin, while the remainder is composed of other minor phospholipids. In fish, the phospholipid PC:PE ratio is generally 2–3:1. The PC:PE ratio in phospholipids of bream, a freshwater fish (total lipid content of 4 to 6% w/w), was as low as 1.3 to 1.9:1 (Kołakowska et al., 1993, 2000a). A PC:PE ratio of approximately 2:1 was recorded in phospholipids of the light and dark muscle of trout (Ingemansson et al., 1991). Various cod tissues were found to differ in their phospholipid composition. The PC:PE ratios of white and dark muscle of the cod were 3.5:1 and 2.7:1, respectively (Lie and Lambertsen, 1991b). Invertebrate phospholipids contain less PC than those from vertebrates. As shown by a study conducted over two annual

TABLE 12.3**Average Lipid Class Composition of Australian Seafoods**

Lipid (% w/w)	Lipid class composition (%)				
	WE	TG	FFA	ST	PL
Fish (n = 151)					
avg. 1.0	2.2	20.2	5.1	3.8	68.8
sd 1.0	10.6	23.1	7.7	1.7	24.2
Shellfish (n = 15)					
avg. 0.9	0.4	11.6	1.9	9.2	76.9
sd 0.5	1.4	17.2	4.0	5.2	14.7
Crustaceans (n = 23)					
avg. 0.8	0.0	2.7	1.2	10.4	85.7
sd 0.4	0.0	9.4	2.1	3.9	7.7

Note: WE – wax ester; TG – triacylglycerols; FFA – free fatty acids; ST – sterols (mainly cholesterol); PL – polar lipids (phospholipids); avg. – average; sd – standard deviation.

Adapted from Nichols, P. et al. 1998, Nutritional Value of Australian Fish: Oil, Fatty Acid and Cholesterol of Edible Species, FRDC Project 95/122, CSIRO Marine Research, Fisheries Research and Development Corporation, 126.

cycles, the phospholipids of fresh krill contain 30.7 to 71.0% of PC, 10 to 31% of PE, from several to 20% of lyso-compounds, and several percent of other compounds. The PC content is three times that of PE; the former serves also as a storage lipid because, as the krill fasted, the PC contents were observed to decrease (Kořakowska, 1985a, Kořakowski and Kořakowska, 1993). A low PC content is also typical of marine plants.

Fish sterol content is relatively stable and ranges between 40 and 60 mg per 100 g edible fish muscle (Ackman, 1994; Krzynowek et al., 1990); 250 to 650 mg per 100 g roe (Sikorski et al., 1990); and 480 to 1150 mg per 100 g oil in livers of cod, herring, menhaden, and salmon (Kinsella, 1987; Kennish et al., 1992). In finfish, 95% of the total sterol is cholesterol. Shellfish usually contain two to three times more total sterol than finfish (Table 12.3). In a study of Australian seafood, the highest sterol contents (120 to 160 mg/100 g) were recorded in shellfish such as prawns, squid, octopus, and scallops (Nichols et al., 1998). In mollusks, such as the oyster *Crassostrea gigas* and abalone *Haliotis* spp., cholesterol is usually the main sterol, but a complex suite of other sterols are present (e.g., Dunstan et al., 1993; Dunstan et al., 1996).

Wax esters may also be a major component of some species' flesh and/or livers (Nichols et al., 2001a). In some Antarctic aquatic animals, including *Euphausia crystalorophias* and *E. triacanta*, wax esters are the storage lipids (Kořakowska, 1987, 1991b). Wax esters are deemed more stable and are more suitable for long-term storage of energy compared to short-term energy stores such as TAG. In addition, wax esters aid in buoyancy and occur in higher amounts in some deep-water fish. The muscle lipids of castor oil fish, coelocanth, and orange roughy can

contain as much as 90% wax esters (Sargent, 1976; Shulman and Love, 1999). Wax esters and, in particular, species containing high levels of both total oil (>20% flesh w/w) and wax esters (>90% of the oil) are considered to have questionable nutritive value for humans (Nichols et al., 2001a, 2001b). The livers of certain sharks may be rich in glycerol ethers and hydrocarbons such as squalene, which are also thought to aid in buoyancy. A number of nutraceutical and cleaning products have been produced from the wax esters, glycerol ethers, and squalene extracted from fish (Nichols et al., 2001b).

12.4 FATTY ACID COMPOSITION

12.4.1 FATTY ACID GROUPS

Most fish lipids contain FA. The main groups of FA can be saturated (SFA), monounsaturated (MUFA), or polyunsaturated fatty acids (PUFA). The proportion of each FA group in the total lipid content from fish tissues differs and depends primarily on the total lipid content (and hence lipid class composition) and can be affected by numerous biological factors. The total lipids of 25 Antarctic fish had on average 23.5, 37.7, 38.1% SFA, MUFA, and PUFA, respectively; 253 temperate Australasian fish had on average 30.5, 24.7, 39%; and 170 tropical Australasian fish had on average 37.5, 22.4, 37.8%

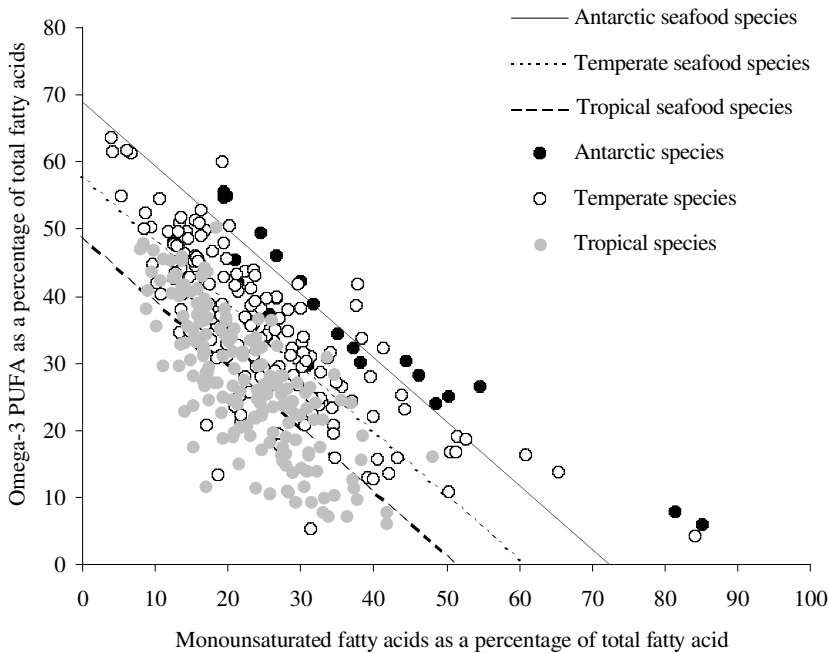


FIGURE 12.1 The relationship between n-3 PUFA and MUFA for species of seafood from the three latitudinal zones. (From Dunstan, G.A. et al. 1999, *Rec. Res. Devel. Lipid Res.*, 3, 63. With permission.)

(Dunstan et al., 1999). Thus, with increasing latitude, the proportion of total MUFA and SFA decreased and PUFA remained fairly constant. The net result was that the lipids were more unsaturated in colder water species. Additional work on lean Australian fish (average lipid content 1% w/w, $n = 151$) were found to contain, on average, 34.2, 21.5, and 44.0% SFA, MUFA, and PUFA, respectively. In medium-fat fish (3.8% w/w, $n = 41$) and Northern Hemisphere fish ($n = 41$), the proportions were 30.7, 23.6, and 45.3%, respectively (Hearn et al., 1987; after Nichols, 1998). For fat fish, such as Baltic herring and Baltic sprat of similar lipid content ($9.7 \pm 2.7\%$ w/w, $n = 59$), the contributions of SFA, MUFA, and PUFA were 31, 42, and 26%, respectively. Lipids of the freshwater bream (average lipid content 4 to 6% w/w, $n = 12$) contained more MUFA and less PUFA than herring and sprat (Kořakowska et al., 1993, 2000a). In all the studies referred to previously, the lowest coefficients of variation were the SFA, while the contributions of MUFA and PUFA were more variable: the two latter groups being inversely correlated both in the lean and fat fish (Nichols et al., 1998; Szczygielski, 2001a). Compared with lean finfish, shellfish and crustaceans with similar lipid contents showed somewhat different proportions of various FA groups. Invertebrate lipids, particularly those of shellfish, generally contained less MUFA and more PUFA than finfish (Nichols et al., 1998). The squid *Notodarus gouldi* (average lipid content 1.43% w/w) contained as much as 65% PUFA (Dunstan et al., 1988), while another squid, *Loligo* sp., with 0.8% lipids, contained as “little” as 45% of PUFA (Nair and Gopakumar, 1978).

12.4.2 SATURATED AND MONOUNSATURATED FATTY ACIDS

Fish lipid SFA are dominated by palmitic acid (16:0) followed by myristic acid (14:0), their combined contributions accounting for about 90% of saturated acids in Baltic herring lipids. There are also a few percent of stearic acid (18:0), which is more abundant in warmer water fish (Ackman, 1994). Also present are 15:0, 17:0, and 20:0, occurring at about 1% or less and generally considered to be of limited nutritive value.

The MUFA are dominated by oleic acid [18:1($n-9$)], followed by the three times less abundant palmitoleic acid [16:1($n-7$)]. Lipids of species such as mackerel and herring contain high amounts of gadoleic [20:1($n-9$)] and cetoleic [22:1($n-11$)] acids. This is considered to be due to a diet of wax ester-rich copepods (and hence long-chain MUFA-rich) (Sargent, 1976). During some periods, 20:1($n-9$) and especially 22:1($n-11$) dominate Baltic herring MUFA (Kořakowska et al., 2001d). The 22:1($n-11$) content can be used as a biomarker of Baltic herring, a fish that has numerous distinct populations migrating within the Baltic Sea. There is a close ($r = 0.96$) inverse relationship between 18:1 and 22:1 over the annual cycle of herring, which could be the result of a shift in the diet, and/or an endogenous synthesis of 18:1($n-9$) by chain-shortening of dietary 22:1($n-9$) (Lie and Lambertsen, 1991b).

12.4.3 POLYUNSATURATED FATTY ACIDS

12.4.3.1 Main Types of PUFA in Fish

The PUFA composition is the most characteristic trait of fish lipids. In aquatic animals, PUFA are usually long-chain (LC) with an ($n-3$) configuration. Quantitatively, they

are mostly eicosapentaenoic acid 20:5(n-3) (EPA) and docosahexaenoic acid 22:6(n-3) (DHA). In most carnivorous fish and invertebrates, DHA is usually more abundant than EPA (up to 2 to 3 times). The proportion of EPA is higher in shellfish (crustaceans, cephalopods, bivalves, gastropods) than in finfish (bony fish, sharks, and rays) (Dunstan et al., 1999).

Small amounts (1 to 3% FA) of docosapentaenoic acid 22:5(n-3) (DPA) may also be present in fish flesh. Exceptionally high amounts of DPA (13 to 14%) were recorded in the abalone in which DPA >> DHA (Dunstan et al., 1996). Mammalian depot fats contain almost equivalent amounts of EPA, DHA, and DPA (Krzynowek et al., 1990; Ackman, 1994). The 18:3(n-3), 18:4(n-3), and 20:4(n-3) acids occur in small (a few percent at most) amounts in fish lipids as well. These acids may be more abundant in freshwater fish. The latter acid was recorded in somewhat higher amounts (about 1%) in rainbow trout, oysters, and blue mussels (Nichols et al., 1998). The flatfish lipids were found to contain the very long-chain 24:6(n-3) FA, contributing as much as 6 to 9% of total FA in the flesh, 3% in the liver, and 6% in the viscera (Ota et al., 1994).

The (n-6) PUFA family is represented in fish by the following acids: 16:2(n-6), 18:2(n-6), 20:3(n-6), 20:4(n-6), 22:2(n-6), 22:4(n-6), and 22:5(n-6). Some (n-6) PUFA, such as 18:2(n-6) and 22:5(n-6), may contribute a few percent; however, most are present in marine fish in only trace amounts. The main exception is arachidonic acid 20:4(n-6) (AA), which can reach high proportions in the flesh of finfish (e.g., 14.9% FA in temperate leatherjacket *Eubalichthys mosaicus*; Dunstan et al., 1988) and shellfish (e.g., 14.3% FA in temperate abalone *H. rubra*; Dunstan et al., 1996), which feed on macroalgae.

Unusual FA found in some fish include furan FA and nonmethylene-interrupted dienoic acids (NMI) (Ota et al., 1994). NMI were also present in abalone at 3 to 6% of FA (Dunstan et al., 1996), oysters at 4.5% (Dunstan et al., 1993), and at approximately 20% in the dusky shark (Nichols et al., 1998). It is the presence of these FA, as well as branched-chain SFA and MUFA, which means that the sum of SFA, MUFA, and PUFA do not equal 100%.

12.4.3.2 (n-3)/(n-6) Ratio

The ratio of (n-3) PUFA to (n-6) PUFA can be used to facilitate identification of high (n-3) PUFA foodstuffs. In general, the (n-3)/(n-6) ratio is higher for marine foodstuffs than terrestrial foodstuffs. This ratio is affected by a number of environmental and biological factors in fish. For example, a strong inverse relationship between (n-3) PUFA and MUFA was revealed in marine species from Antarctic to temperate to tropic waters (Dunstan et al., 1999). The slopes of this relationship were not significantly different for fish from different regions, but the intercept on each axis was reduced in lower-latitude waters due to the increased proportions of SFA and (n-6) PUFA present in warmer-water species. Also, the average (n-6) PUFA contents in seafood from the Antarctic, temperate, and tropical zones were 2.4, 7.8, and 10.5% of total FA, respectively; thus, the (n-3)/(n-6) ratio increased with latitude (Dunstan, et al., 1999).

Freshwater fish contain higher proportions of (n-6) acids and lower (n-3), allowing differentiation between freshwater and marine species based on the ratio of these two

types of PUFA (Ackman, 1994; Sargent, 1995; Nichols et al., 2001b). The average (n-3)/(n-6) ratio in marine Baltic herring lipids was 5.1, but ranged from less than 3 to 12; the average ratio in sprat was 6.1 and ranged from more than 5 to 7. Of freshwater fish, the average ratio was 1.6 in bream; 2.9 in roach; and 4.5 to 5 in rainbow trout, crucian carp, and carp, although some batches of carp had average ratios down to 0.1 (Kořakowska et al., 1993, 2000a). Among three fish species of similarly low (i.e., slightly below 2% w/w) lipid content, the freshwater burbot and marine garfish showed an almost identical ratio of 13.8 to 13.9, while a ratio as low as 9.1 was found in freshwater zander (Kořakowska et al., 2000a). Lipids of the saltwater barramundi showed an (n-3)/(n-6) ratio twice as high as in the same species from a freshwater habitat, with similar differences in lipid content (Nichols et al., 1998). Differences between marine and freshwater fish are clearly more pronounced in offal lipids. Baltic fish (herring, sprat, cod) offal lipids showed the (n-3)/(n-6) ratio to range from 7 to 8, the freshwater fish offal ratio (included in [Table 12.2](#)) being 2.8 ± 0.27 , very similar to that in muscle lipids. Conversely, the offal and muscle lipid ratios differed widely in herring and cod (Kořakowska et al., 2001c).

The ratio (n-3)/(n-6) was much lower in cultured sweet smelt than in wild individuals of this species (Jeong et al., 2000). The (n-3)/(n-6) ratio in muscle lipids of common farmed fish species (rainbow trout, European catfish, pike, grass carp, crucian carp, carp, ide, tench) varied from 0.74 (ide) to 3.45 (pike) (Bieniarz et al., 2000). The flesh of abalone (*Haliotis* spp.) and barramundi (*Lates calcarifer*), when fed formulated feeds, also had reduced (n-3)/(n-6) ratios. However, coincident with the elevated total lipid and (n-6) PUFA contents, the animals had elevated amounts of LC (n-3) PUFA (Dunstan et al., 1996; Nichols et al., 2001b). The (n-3)/(n-6) ratio is lower in farmed fish due to the composition of the feeds they are fed; that is, typically high vegetable oil plant product diets rich in 18:2(n-6). Also contributing to the reduced levels of DHA in the tissue may be a lower demand for (n-3) PUFA resulting from conditions under which they are kept (i.e., lower mobility, reduced need to forage) (Shulman and Love, 1999).

12.4.3.3 Distribution of PUFA in Lipid Class

Long-chain PUFA tend to be preferentially esterified at the 2-position in fish and invertebrate triacyl-*sn*-glycerol. The SFA and/or MUFA tend to be preferentially esterified at the *sn*-1 and/or *sn*-3 positions. The proportions of DHA in positions 1, 2, and 3 were 9, 64, and 26%, respectively, in sardine and depended on the total DHA content in the TAG and on the FA composition. The distribution of DHA, DPA, and EPA was affected by total amounts of 20:1 and 22:1 in triacyl-*sn*-glycerol (Ando, 1992). In Atlantic salmon storage fat and in cod liver oil, 72.6 and 74.4% of DHA, respectively, occurred in the 2-position of TAG (Aursand et al., 1995). As little as 3.2% DHA and 4.6% EPA were esterified to the 2-position of TAG in harp seal oil. EPA was evenly distributed in cod liver oil and muscle lipids of Atlantic salmon, with 37.8 and 39.7% EPA, respectively, being found in the 2-position (Aursand et al., 1995).

In phospholipids, PUFA are also preferentially located at position-2. In tuna phospholipids, the *sn*-2 position contained elevated levels of PUFA (DHA, EPA,

and AA) and 18:1(n-9), whereas elevated levels of SFA (16:0 and 18:0) and 18:1(n-7) were located in the *sn*-1 position (Aubourg et al., 1996). In phospholipids of cod muscle, roe, and brain, DHA is also mostly located in the *sn*-2 position. Interestingly, in retinal membranes of marine fish, the di-22:6(n-3) molecular species accounts for some 70% of the total PE and for 60% of the phosphatidylserine (Sargent, 1995).

12.5 FACTORS AFFECTING THE LIPIDS IN FISH

12.5.1 BIOLOGICAL ROLE OF (N-3) PUFA IN FISH

There are many interacting external (temperature, salinity, food availability) and internal factors, including species, sex, physiological status (gonad maturity, condition, age), that determine and affect the lipid content and composition of aquatic organisms. The (n-3) PUFA are essential for the normal development of embryos, larvae, and the nervous system, and for the proper functioning of the sense organs of marine and freshwater fish.

Differences in composition may occur within a species, depending on the physiological status and sex. Muscle lipids in fat fish (e.g., pelagic fish) are used as an energy source for locomotion, are stored and later transported to gonads for reproduction, and are utilized during spawning migration and actual spawning. Those processes proceed differently in various species, or even subspecies (e.g., Baltic herring and sprat). The processes are quantitatively and qualitatively different in males and females of the same species, explaining the different lipid contents and compositions between sexes (Kořakowska, 1991a). The DHA level in the red muscle of male humpback salmon is a condition indicator during the reproductive period; the DHA content in the phospholipids was higher in leaders and lower in recessive individuals. This acid plays an important reproductive role in females as well. It is transferred from muscles to the liver and gonad products and influences the survival of eggs and larvae. The reproductive capacity of the females can be deduced from the level of DHA in their TAG (Shulman and Love, 1999).

DHA also plays an important role in adaptive processes (temperature, salinity, and oxygen adaptations), as well as in the motoric and social behavior of fish (Shulman and Love, 1999). There is a correlation between the fish DHA content and mobility of salt and freshwater fish (Reinhardt and van Vleet, 1986; Shulman and Love, 1999, and references therein). The highest DHA content in flying fish was found in the brain. The (n-3) PUFA, DHA in particular, also play an important role in adaptation to changed environmental conditions and immunity to infections and parasitic diseases (Shulman and Love, 1999; Sargent et al., 1999).

A comparison of FA composition in lean fish flesh from various areas shows similar amounts of total PUFA but variable proportions of individual (n-3) PUFA in them. The pattern of variation seems clear: freshwater fish contain less (n-3) PUFA than marine fish and the content of (n-3) PUFA increases with increasing latitude (Dunstan et al., 1999), which can be explained by adjustment of the membrane function to environmental constraints. The (n-3)/(n-6) ratios in the same fish species (*barramundi*) from salt and fresh water were 1.66 ± 0.57 and 0.66 ± 0.15 , respectively, and

primarily resulted from a lower DHA content in the freshwater fish, the mean contents amounting to 76 and 40 mg/100 g, respectively (Nichols et al., 1998).

Phospholipids, as structural lipids, are rich in LC PUFA. Various organs of a species show similar FA compositions to each other (Watanabe et al., 1995). The FA profile of phospholipids can be used for identification (taxonomic) purposes (Grahl-Nielsen and Mjaavatten, 1995; Joensen and Grahl-Nielsen, 2001). However, the PUFA compositions of the total phospholipids of various species of fish and shellfish are not strikingly different (Ackman, 1994), but the differences that do occur involve the (n-3) PUFA, particularly DHA. The lipids of lean fish and shellfish have the most PUFA. Cephalopods (e.g., octopus, squid) contained the highest average proportion of DHA when compared to other taxonomic or dietary groups from Antarctic, temperate, and tropical zones (Dunstan et al., 1999).

n-3 PUFA are also important for the growth rates of wild and cultured species. However, in the abalone (*Haliotis laevis*), which normally feeds on low-lipid macroalgae, high levels of dietary lipid (>4% dry weight) not only increased the tissue lipid level but actually reduced growth rates (Dunstan et al., 2000).

12.5.2 DIET

One of the main factors affecting the lipid composition of fish is diet. This is because all natural foodstuffs contain lipids, and some of the dietary lipids are essential nutrients for maintenance of normal growth, disease resistance, fecundity and survival. Because of their importance for these functions, dietary PUFA can be incorporated into structural lipids, stored for future requirements, or used to produce longer chain, more highly unsaturated FA or biologically active molecules eicosanoids (e.g., prostaglandins and leucotrienes). Whether stored as lipid reserves or used for structural purposes, lipid accumulation from the diet will change the lipid composition of fish tissues. Fish diet does not affect the FA composition of phospholipids as much as it does that of the TAG. The TAG reflects the lipid composition of the diet, as shown by numerous nutritional and comparative studies on fish. However, fish can adjust their FA composition according to environmental pressure or physiological demands. This is evident from a comparison of the lipid compositions of flesh and diet. However, the FA compositions of various fish fed identical diets may differ (Kořakowska et al., 2001d).

EPA, some DHA, and shorter-chain C16 and C18 (n-3) PUFA are produced by microalgae, macroalgae, Thraustochytrids, and some bacteria (Lewis et al., 1999). Phytoplankton is consumed by zooplankton, the filter feeding shellfish, and some finfish species; and macroalgae is consumed by grazing shellfish and some finfish. These herbivores are eaten by carnivorous shellfish and finfish, which in turn are eaten by higher-order carnivores. Fish take up the (n-3) PUFA from their food, as essential nutritional components, which they cannot synthesize *de novo*. Along this simplified food chain, the animals can perform limited chain elongation and desaturation of the dietary (n-3) PUFA. For this reason, the herbivores (e.g., abalone, oysters, mussels) and low-order carnivores (e.g., crustaceans) tend to contain more EPA and less DHA than high-order carnivores, which in turn contain less EPA than DHA (e.g., tuna, mackerel, shark, squid, octopus) (Dunstan et al., 1996, 1999). Also,

fish feeding directly on AA-rich macroalgae tend to have elevated proportions of AA relative to other fish, due to the accumulation of this PUFA (Dunstan et al., 1999).

Marine plankton contain more long-chain (n-3) PUFA than freshwater plankton. This is regarded as the major reason for the basic difference in the composition of FA between marine and freshwater fish as discussed in [Section 12.4.3.2](#). In contrast to marine fish, most freshwater fish (e.g., carps, tilapias, eels, and the anadromous salmonids) consume substantial amounts of 18:3(n-3) and 18:2(n-6) derived from terrestrial and aquatic plants and insects, and substantially less DHA with their natural diet. Therefore, most freshwater fish and some marine species have active elongase and desaturase systems, allowing a rapid conversion to DHA and AA from shorter-chain (n-3) and (n-6) FA, respectively (Sargent, 1995). As much as 70% of 18:3(n-3) in rainbow trout diets is converted to DHA, while the predacious pike is not capable of converting 18:3(n-3) into long-chain (n-3) PUFA and must obtain its DHA from the diet (Shulman and Love, 1999; Bieniarz et al., 2000). The very long-chain PUFA 24:6(n-3) present in flatfish is most likely dietary, but it can also be obtained by elongation of DHA (Ota et al., 1994) or during the conversion cycle to DHA. As opposed to freshwater fish, marine species cannot convert C18 to C20 (n-6) PUFA; thus, AA is also an essential FA in marine fish (Sargent et al., 1999).

12.5.3 SEASONAL VARIATIONS

A comparison of different fish species in terms of their lipid compositions can be misleading if seasonal variations in each of the species are ignored. Fish muscle lipid content and composition change from season to season ([Figure 12.2](#)), the changes depending on fish species, age, sex, habitat, region, and year. The complex interaction of seasonal changes in diet, reproductive and migratory status, temperature, and salinity can induce these changes. The mean annual lipid content in Baltic herring flesh changed from one year to the next, but seasonal variability was similar between years and showed a very similar coefficient of variation. Seasonal changes in lipids of the edible parts and in the whole sardine showed a similar pattern in different years (Nunes et al., 1990). Wider between-season differences are observed in fish of the temperate regions, with tropical fish showing much smaller changes. No studies dealing with variability in the Antarctic and/or Arctic fish through a full annual cycle have been reported; the Antarctic krill lipid content varied from 1.0 to 7.8% w/w, coefficient of variation, depending on the year amounting from 44 to 49% (Kořakowska, 1985a and unpublished data).

Seasonal variations in flesh lipids are most pronounced in high-fat pelagic fish. In lean fish, seasonal changes are greater in the liver than in muscles. Shulman and Love (1999) attribute the seasonal changes in fish lipids to variations in water temperature. However, although the lipid content and FA composition in Baltic sprat and herring (members of the same family living in the same location) are on average similar over an annual cycle, the pattern of seasonal changes is not identical in each species. For this reason, Baltic herring and sprat, caught at the same time from the same area, may differ significantly in terms of lipid content, composition, and susceptibility to oxidation (Kořakowska et al., 2001d). The differences primarily stem from different diets. In bream, a medium-fat fish, seasonal variations in lipid

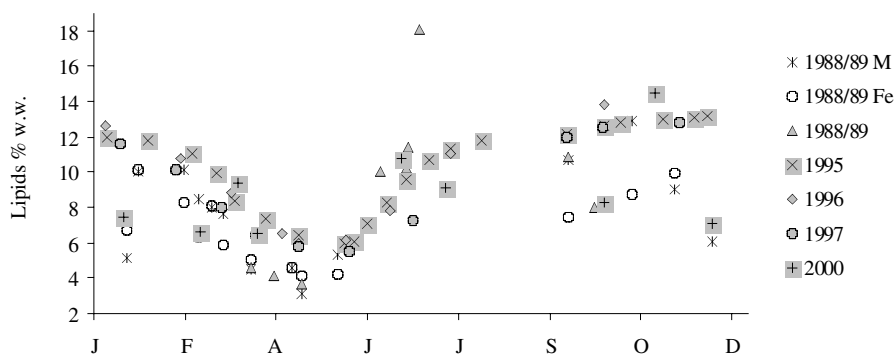


FIGURE 12.2 Seasonal variation in lipid content of Baltic herring, data from several years. Fe - female, M - male; 1988/1989, 1995, 1996, 1997 (Adapted from Kołakowska, A. and Kołakowski, E., *Lipids, in Usefulness of Fish to Processing*, Kołakowski, E., Ed., Report CPBR 10–16/1.5.6.45, Szczecin, 1990); 1995, 1996, 1997 (Adapted from Szczygielski, M., 2001, *Folia Univ. Agric. Stetin*, 218, 129); 2000 (Adapted from Kołakowska, A. et al., 2001d, *Acta Ichth. Piscat.*, in press).

content and FA composition are rather small, but susceptibility to oxidation changes by as much as 80% (Kołakowska et al., 1993). Marine fish show more pronounced seasonal variations in lipids than freshwater fish, except for salmon, which is anadromous and its seasonal changes are of a very specific nature.

Seasonal changes in the lipid classes within a species harvested from a certain area are characteristic and reflect the annual biological cycle of the species and its habitat, although they may be shifted somewhat in time. Seasonal changes in phospholipids of herring (Linko et al., 1985), sardine (Bandarra et al., 1997), and sprat (Hardy and Mackie, 1969) were small in comparison to changes in nonpolar lipids. The pattern of variation differs between males and females. The lowest content of total lipid, TAG, and phospholipids are observed in spawning fish. Males and females usually differ in both the extent and rate of those changes. The spawning Baltic herring males and females lost 43% and as much as 70%, respectively, of their TAG. The lower limit of lipid content was lower in females than in males. Once spawning is finished and the fish resume intensive feeding, the muscle lipid reserves become replenished. Replenishment of phospholipids begins rather early but does not proceed rapidly; the increase is initially in TAG, which increased by a factor of 5 over a month (Kołakowska and Kołakowski, 1990). During spring, dietary lipids and those stored in muscles are mobilized for gonad build-up and as a source of energy necessary during the spawning migration and spawning itself. Seasonal changes in the supply of food, its composition and availability (the presence of competitors for food) can also occur and affect the fish's lipid composition accordingly. Each species has its own survival strategy. The strategy involves seasonal changes in lipids, and also in FA composition, susceptibility to oxidation, and lipolytic activity. Mean cholesterol contents in the meat of mackerel, anchovies, and horse mackerel were found to be 156, 49, 85 mg/100 g w/w, respectively (Pozo et al., 1992), but the coefficient of variation over a year in all three species was about 40%, suggesting significant changes with

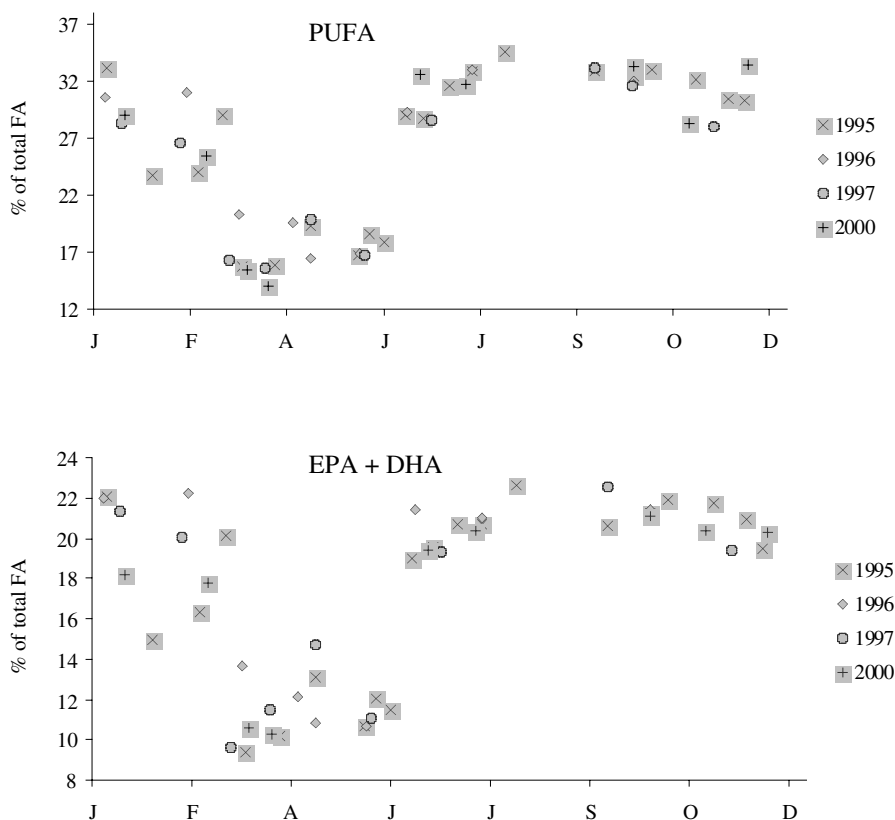


FIGURE 12.3 Changes in PUFA of Baltic herring during annual cycle, data from several years. 1995, 1996, 1997 (Adapted from Szczygielski, M., 2001, *Folia Univ. Agric. Stejn*, in press); 2000 (Adapted from Kołakowska, A., et al., 2001d, *Acta Ichth. Piscat.*, in press).

season. All these changes in the lipid classes are a part of biochemical, physical, and sensory transformations taking place in fish and shellfish throughout the year (Kołakowski et al., 1990; Shulman and Love, 1999).

In fish, there is an inverse correlation between the tissue lipid content and the relative proportions of (n-3) and (n-6) PUFA (Nichols et al., 1998; Dunstan et al., 1999). However, this cannot be regarded as a rule, especially in high-fat fish where the lipid content may vary within wide limits. An increase in lipid content is related to deposition of fat reserves, but a reduction in the muscle lipid content may have various causes (utilization as an energy source during spawning or migration, and/or mobilization of certain fatty lipids in gonads). For this reason, total lipids of the lean, post-spawning Baltic herring contain less (n-3) PUFA (including DHA) than the fattier herring from others seasons (Szczygielski, 2001b; Kołakowska et al., 2001d). The fishing season is most important for the content and composition of lipids. Fishing season-dependent differences in FA composition of fish have been reported for over 60 years (e.g., Lovern, 1938; Gruger et al., 1960; Lambertsen and Braekkan, 1965; Jangaard et al., 1967; Stansby et al., 1990; Ackman, 1994). However, consistent studies on lipid changes over

an entire annual cycle, including the fishing season, are few. In the case of Baltic herring, changes during the year concern all groups of FA, especially MUFA. Spawning fish showed the lowest contents of PUFA and the highest contents of MUFA. MUFA in spawners were dominated by 22:1 (about 27% of all FA), and also by 20:1 which, during the remainder of the year, contributed only a few percent (Szczygielski, 2001a; Kołakowska et al., 2001c). Herring and sprat muscle (n-3) PUFA contents were similar, but herring showed more pronounced seasonal changes than sprat, both in terms of EPA and DHA (DHA seasonal coefficient of variation was 29% and 11% in herring and sprat, respectively). This can be explained by the fact that sprat feed throughout the year, while herring feeding is subject to the strong seasonal availability of food. DHA in total lipids is directly related to food, with the widest variation occurring in intensively feeding herring (coefficient of variation of about 36%). However, Linko et al., (1985) found no relationship between (n-3) PUFA in lipids of herring and of plankton during the period of intensive feeding. Seasonal changes in sardine (Bandarra et al., 1997) were similar to those described for herring, the coefficients of variation being higher for lipid content (72%) and (n-3) PUFA content; the coefficients for DHA and EPA were 117 and 25%, respectively. High seasonal coefficients of variation in FA, including DHA, were recorded in mackerel (Hardy and Keay, 1972), horse mackerel and anchovy (Pozo et al., 1992). Low coefficients were reported for bonito (Saito et al., 1995) and bream (Kołakowska et al., 1993). Consideration of the effects of seasonal variation on fish as a source of LC (n-3) PUFA shows that the most nutritionally valuable Baltic herring are those caught in autumn, following the period of intensive feeding; the least valuable are those caught during spawning (Kołakowska et al., 2001d; Szczygielski, 2001b) (Table 12.4). Unfortunately, it is the spawning populations of Baltic herring and sprat that are the major targets of fisheries in the area.

12.6 SUSCEPTIBILITY TO OXIDATION

Compared with other food lipids, fish lipids are relatively more susceptible to oxidation. This is because of their high degree of unsaturation and low content of antioxidants compared to plant lipids. The α -tocopherol content in fish muscle lipids ranged from 1 to 75 mg/100 g oil (Kinsella, 1987). In herring, the α -tocopherol content ranged from trace amounts to 66 mg/100 g of lipids and varied over the season (Seidler, 1997). The average carotenoid content in fish muscle varied between 0.043 and 0.283 $\mu\text{g/g}$ w/w, but was much higher in the whitefish (*Sebastes* sp.): 0.776 $\mu\text{g/g}$ w/w in muscle, 3.12 $\mu\text{g/g}$ w/w in skin, and 1.45 $\mu\text{g/g}$ in fins (Czeczuga et al., 2000). On the other hand, crustaceans such as Antarctic krill, have exceptionally high carotenoid contents (from about 500 to 2947 $\mu\text{g/g}$) (Kołakowska, 1991c).

The fish muscle prooxidative system, both enzymatic and nonenzymatic, is more active than that in homeotherms; in addition, susceptibility of lipids to UV-catalyzed oxidation is higher (see Chapter 8, Table 8.1). Enzymic NADH-Fe systems were more active than nonenzymic ascorbate-Fe systems in lipid oxidation of cod sarco-plasmic reticulum (Soyer and Hultin, 2000). The most active lipoxxygenases (LOX) are in the gill and skin of fish (Hsieh et al., 1988). Fish gill tissue contains two separate LOX (12- and 15-LOX), each being active toward PUFA (AA, EPA, DHA) but exhibiting different hydroperoxide addition sites. LOX from sardine skin were

TABLE 12.4
n-3 PUFA and Lipid Oxidation in Some Raw and Processed Fish

Product	Lipid (% w/w)	EPA (% FA)	DHA (% FA)	Σ (n-3) PUFA (g/ 100 g product)	PV mg O/100g Lipids	Totox (2PV + AsV)	PV mg O/100 g Product
Herring							
raw	7.0	6.9	13.4	1.42 ^a	7.3	19.8	0.51
cooked	7.1	7	14.6	1.53 ^a	7.2	18.3	0.51
fried	21.6	1.9	4.2	1.31 ^a	7.4	18.4	1.6
Herring							
raw	8.1	7.3	17.8	2.07	3.8	12.3	0.31
salted	8.4	6.5	14.5	1.99	118	267.0	9.98
Herring							
raw	8.2	7.1	13	1.45	9.6	26.0	0.79
marinated	9.4	7	15	1.30	19.4	70.7	1.81
Mackerel							
raw (frozen stored)	33	5	16	6.3	63	134	21
hot smoked	32	7	13	6.4	89	184	28.5
cold smoked	38	7	10	6.4	86	190	32.7
Mackerel							
raw (frozen stored)	28	6.3	11.6	5.0 ^a	10.9	39	3.1
canned (without precooked drip)	24.7	5.9	11.8	4.4 ^a	4.9	34	1.2
canned (with precooked drip)	32.5	5.5	9.8	4.97 ^a	12.8	46	4.2

^a EPA + DHA

Adapted from Kołakowska, A., Makowska, A., Szczygielski, M., Bąk, M., and Pankiewicz, A., 1998, *29th Sci. Conf. Committee for Food Technology and Chemistry of the Polish Academy of Sciences*, Olsztyn, 35; Kołakowska, A., Kurosz, A., Kępczyńska, K., and Szczygielski, M., 1999, *30th Sci. Conf. Committee for Food Technology and Chemistry of the Polish Academy of Sciences*, Kraków, 232; Kołakowska, A., Domiszewski, Z., Bienkiewicz, G., and Szczygielski, M., 2001, presented at *Lipid-forum, 21st Nordic Lipid Symp.*, Bergen.

more active on α -linolenic acid [18:3(n-3)] than linoleic acid [18:2(n-6)] (Mohri et al., 1990). The skin contains active LOX, carotenoids, and the melanin radical trapping mechanism, which significantly affects fish lipid oxidation. In addition, the skin provides a physical boundary from light and oxygen for subcutaneous lipids. In general, skin lipids undergo more rapid oxidation than muscle lipids (Ke and Ackman, 1976).

Compared with other food lipids, fish lipids are very sensitive to photooxidation, with thermooxidation being less pronounced. The lipid oxidation rate of seafood is species dependent, and is harvest and season dependent in a given species (Figure 12.4). The susceptibility of seafood lipids to UV-catalyzed oxidation (hydroperoxide level and slope of accumulation over time of UV exposure) did not correlate with PUFA, DHA, MUFA, or SFA contents. Sprat and herring lipids showed a weak

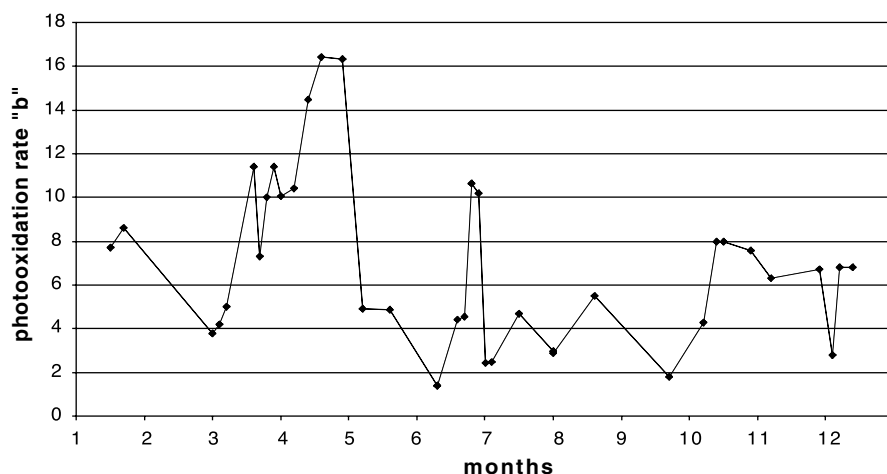


FIGURE 12.4 Seasonal variation in susceptibility to oxidation of Baltic herring lipids. Lipid photooxidation rate ("b") of Baltic herring caught in different months from $y = a + bx$; y-PV mg O/100 g lipids; x-time (min.) exposure of lipids to UV. (From Kołakowska et al., unpublished data and data adapted from Kołakowska, A. et al., 1992, *Quality Assurance in the Fish Industry*, Huss, H.H., Jakobsen, M., and Liston, J., Eds., Elsevier, Amsterdam, 81.)

(but not positive) relationship between hydroperoxide level (after 120 min of UV irradiation) and PUFA and DHA contents. No relationship could be detected between susceptibility of bream lipid to oxidation and FA composition (Kołakowska et al., 1993). A weak relationship did exist between seasonal variability of krill lipid susceptibility to oxidation and carotenoid content (Kołakowska, 1991c). Consequently, oxidation of lipids extracted from various sources and differing in their FA compositions proceeds at different levels. The oxidation process and its rate are not directly dependent on the amount of unsaturated acids: PUFA, MUFA, or DHA. Most likely, the decisive factor is a relationship between activities of pro- and antioxidants contained in the lipids. No losses of (n-3) PUFA were observed to accompany even a substantial oxidation level. As shown by model studies, significant losses of LC (n-3) PUFA were recorded at hydroperoxide levels considerably exceeding those found in commercial fish products (Kołakowska et al., 1998a).

Seasonal changes in the susceptibility of Baltic herring muscle lipid to UV-catalyzed oxidation are larger than changes in lipid content and FA composition (Figure 12.4). This is also true for sprat, bream, and Antarctic krill. Lipid of spawning herring (the lowest lipid phase) was the most susceptible to oxidation (Figure 12.4). Peroxide formation in minced pomfret stored in ice (in a bag) was more pronounced during the season when the fish contained more lipid (Nair and Sankar, 1992). The highest lipolytic activity is recorded during the period of intensive feeding, the lowest activity being typical of the spawning season, particularly in males (Kołakowska and Kołakowski, 1990). Seasonal changes in lipase activity in sardine oil are three times those in ribbon fish (Lopez-Amaya and Marangoni, 2000a). Consequently, nutritive value of fish lipids and changes taking place in lipids during seafood storage and processing are significantly dependent on the fishing season.

12.7 EFFECTS OF PROCESSING ON FISH LIPIDS

12.7.1 CHILLING FISH

Relative to other forms of fish processing, cold storage of fish in ice is not as important to autolytic and microbiological changes of lipids. Lipolysis does not directly affect sensory properties; the sensory effects of oxidation are masked by microbiological spoilage. The initial free FA content (determined immediately after capture) depends on the species and date of harvest as related to the biological cycle (Kořakowska and Kořakowski, 1990). The rate of lipolytic change is faster in whole than in gutted fish and fillets. Lipolysis proceeds more rapidly in muscles of feeding and pre-spawned fish than in spawning fish. In Baltic herring, this was found to be true with respect to lipase and phospholipase (PLA) activities (Kořakowska and Kořakowski, 1990). Fish contain active PLA₂ in their pyloric caeca (Kishimura et al., 2000; Lopez-Amaya and Marangoni, 2000b, and reference therein). Digestive lipolytic enzymes isolated from the intestines of cod (Lie and Lambertsen, 1991a), salmon, and rainbow trout (Halldorsson et al., 2001) prefer to hydrolyze LC (n-3) PUFA. After 10 days of cold storage of whole cod, free FA accounted for 10% of total lipids and after 40 days increased to 55% (Lovern and Olley, 1962). After 10 days of iced storage of Baltic cod, sprat, and herring, free FA accounted 10, 5.5, and 9% of total lipids, respectively (Stodolnik et al., 1971). The presence of free FA resulted from phospholipid and/or TAG decomposition, depending on the species of fish (Olley et al., 1962; Olley and Watson, 1969). Contents of PC and PE in the ice-stored cod flesh decreased slowly during the first 4 days of storage, the decrease becoming more rapid during the subsequent 16 days. During another 30-day storage study, 72.2% of PC and 71.8% of PE were hydrolyzed (Ohshima et al., 1984). Those authors recorded changes in the phospholipid structure and re-esterification of free FA proceeded during cold storage of the cod. After 72 h of cold storage of Antarctic krill, hydrolysis affected 20% of the phospholipids; PC in particular was reduced to half its initial amount (Kořakowska, 1988).

Lipid oxidation in whole ice-stored Baltic herring and bream changed exponentially. The timing of the rapid oxidation phase was batch dependent but did not commence before 3 to 7 days of storage. Rancidity was first noticed not earlier than after 6 days of storage when the analysis of volatile compounds showed aldehyde accumulation. A decrease in some of the volatile compounds (e.g., a 50% reduction in the amount of hexenal-2) was noted between days 8 and 11 of herring storage (Kořakowska et al., 1992a). Changes on storage also occurred in anti- and prooxidants. Antioxidants in ice-stored herring fillets decreased in the following order: α -tocopherol > ascorbic acid > glutathione peroxidase, while a 75% decrease in the activity of enzymatic prooxidants occurred by day 15 (Undeland et al., 1999). LOX in fish were active for up to 48 h of chilled storage. The prooxidative activity due to heme proteins continued for a longer time than that due to LOX (Medina et al., 1999).

No losses of (n-3) PUFA were recorded during the initial 3 to 4 days of cold storage of whole herring and minced and whole bream. After 7 days, the content of

EPA and DHA in bream was reduced by 15 and 30%, respectively (Kořakowska et al., 1996). When compared with storage in ice, the storage of redfish in a modified atmosphere had no effect on malonaldehyde content over 10 days (Pickova et al., 2001).

12.7.2 FREEZING FISH

Lipid deterioration in frozen fish is a very important quality problem. Lipolytic changes were analyzed with respect to their effects on myofibril protein denaturation (Sikorski and Kořakowska, 1990, 1994, 2001). Despite low lipid contents, lipolytic changes were found to be more important in lean fish such as gadoids than in fat fish; the triacylglycerols of the latter probably protect proteins (Stodolnik and Knaśiak, 1981; Haard, 1992). Phospholipid breakdown during frozen storage has been studied extensively in cod (Lovern and Olley, 1962), haddock and lemon sole (Olley et al., 1969). The hydrolysis of phospholipids to form free FA follows a first-order reaction with time. Phospholipid hydrolysis rates reach a peak at approximately -4°C due to the freezing out of water and therefore concentration of substrate and phospholipase and other enzymes (Lovern and Olley, 1962). The asymptote of this reaction becomes lower as the temperature of storage decreases because the substrate must be liquid for the reaction to proceed (Olley et al., 1969). Phospholipases of the Antarctic krill are very active at low temperatures, probably as an adaptation to their habitat where krill enzymes must operate at low temperatures. After 6 months of storage, approximately 70% of phospholipids were hydrolyzed and the free FA content increased by a factor of 6 to 20, accounting for more than 50% of total lipid (Kořakowska, 1986). Such high free FA content found in krill samples from Antarctica had for many years been regarded as a characteristic property of krill rather than an effect of storage.

The extent of lipid oxidation in frozen lean fish is usually high, due to their high PUFA contents and because the membrane lipids are the first to be oxidized (Hultin, 1994). However, if lean fish is stored frozen at an appropriate temperature (e.g., -28°C , but not higher than -18°C), lipid oxidation may not be perceivable until after 1 year of storage, due to the low contribution of lipids to muscle rancidity. This effect produces a flavor described as “cardboardy” and is related to the presence of *cis*-4-heptenal (Hardy et al., 1979). In shellfish, regardless of the absence of direct sensory signals of rancidity, lipid oxidation is evidenced; for example, a 70% decrease in DHA and EPA content after 6 months in prawns at -18°C (see references in Sikorski and Kořakowska, 1990). Lipid oxidation in frozen whole krill and in products obtained through controlled autoproteolysis is clearly inhibited, most probably by carotenoids and products of proteolysis and phospholipid hydrolysis. No (n-3) PUFA losses were found in whole krill stored for 3 months at -18°C , nor in krill precipitate stored for 8 months (Kořakowska et al., 1994).

Lipid oxidation during frozen storage of fat and medium-fat fish is an important quality problem, primarily due to effects on sensory properties such as rancid off-odor (appearing earlier than off-flavor), orange-brown discoloration, and texture changes. Changes in peroxide value (PV) and carbonyl compounds such as anisidine value (AsV), benzidine value, carbonyl value, and thiobarbituric acid (TBA) proceed

in a similar way during frozen storage of herring, mackerel, sprat, horse mackerel, and sardine. After approximately 3 months of storage, oxidation products increase rapidly. After approximately 6 months of storage, a decrease is observed, particularly in aldehydes due to their interaction with nitrogenous substances; the intensity of rancid off-odor is also reduced. Next, the amount of all oxidation products increases rapidly. The activity of the enzymatic and nonenzymatic prooxidative system in muscle increased slightly during the first 3 weeks of frozen storage (-18°C) of herring and sprat, compared with that in unfrozen fish, particularly sprat (Kořakowska and Kucharska, unpublished data). During a 24-week-long storage of herring muscle and roe at -25°C , enzymatic activity (most likely that of LOX) remained at a level about 70% of that in unfrozen fish (Samson and Stodolnik, 2001).

The rate of lipid oxidation-induced changes in frozen fish depends on a number of biological and technological factors: species, lipid content, fishing season, extent of dressing, freshness, and conditions of freezing and storage. During storage at -27°C , the lipid oxidation rates in different batches of mackerel, herring, Baltic herring, and horse mackerel were inversely proportional to the lipid contents of fish muscle (Kořakowska et al., 1981a, 1985b, 1990). However, rancidity was sensorily perceived, first in those batches showing the highest lipid content, although the rancid off-odor profile was not identical (Kořakowska et al., 1981a, 1985b, 1985c). In herring and mackerel of low lipid content, the fastest oxidation rate was recorded in the batches harvested during the spawning season. Baltic herring lipids were most susceptible to oxidation at that time and lipids of the fillets stored at 22°C oxidized faster than those in fish caught in other seasons (Kořakowska et al., 1992a, 1992b). Castell and Maclean (1964) and Castell (1965) have suggested that active feeding after spring spawning of cod resulted in greater concentrations of natural antioxidants in the flesh. They showed that, in fish caught and frozen during these months, the cupric ion catalysis of lipid oxidation in muscle homogenates was markedly reduced. Similar effects were found in July and August for salt fractionation of cod muscle, where the lack of lipid hydrolysis was thought to be related to the natural antioxidant balance (Sheltawy and Olley, 1966).

The rate of lipid oxidation during frozen storage of Baltic herring increased in relation to the extent of dressing: whole fish < fillets with skin < minced (without skin) < carcasses < skinless fillets (Kořakowska, 1979). The oxidation rate of dark muscles was faster than that of light muscles, the reverse being the case for lipolysis (Aubourg et al., 1999; Undeland et al., 1998). The presence of skin protects lipids, particularly the subcutaneous deposits, from oxidation. Skin provides mechanical protection and the presence of melatonin has a protective effect; skin also contains an abundance of prooxidants. The prooxidative enzymatic and nonenzymatic activity in the muscle with skin was 50% higher than in skinless muscle (Kořakowska and Kucharska, unpublished data). Minced meat without skin does not become rancid any faster; the oxidation level (PV, carbonyls) of minced herring kept for 3 months at -20°C was lower than that in skinless fillets and in carcasses (Kořakowska, 1979). Formation of volatile aldehydes and loss of α -tocopherol in minced channel catfish were either similar or slightly lower than in intact fillets throughout frozen storage (Brannan and Erickson, 1996). Those authors contend that enhancement of oxidation, which might have been generated through tissue disruption, is balanced

either by enhancement of ascorbic acid or glutathione's antioxidant activity or by others such as phospholipase. Lipid hydrolysis proceeds at the fastest rate in minces, followed by whole fish, both in herring (Kořakowska, 1981b) and in rayfish (Fernandez-Reiriz et al., 1995).

Careful preparation is required during fat fish surimi production to avoid lipid oxidation. Washing increased the (n-3) PUFA contribution to lipids remaining in the mince (Mestiri et al., 1992). However, after two rinses of minced herring, its (n-3) PUFA content decreased, most likely as a result of oxidation. During a half-year storage, both free and bound lipids of washed minces were more oxidized than those in unwashed minces (Kořakowska et al., 1997).

Changes in lipids are little affected by the temperature of freezing, while the temperature of storage has a very significant effect on any changes (Stodolnik, 1981). The rate of free FA formation decreased with decreasing temperature of storage (-5°C , -18°C , -40°C) and was higher for the phospholipids than for neutral lipids in hake mince (de Koning and Mol, 1990) and minced rayfish (Fernandez-Reiriz et al., 1995). Lipid oxidation is faster at higher storage temperatures of (Kořakowski, 1986; Burt and Hardy, 1992; Hultin, 1994), but the relationship is not linear as a function of water activity (a_w). Autooxidation occurs most rapidly at very low a_w levels, with higher levels producing diminution in rates until $a_w = 0.4$ is obtained. Further increases in a_w tend to increase and then once again decrease oxidation rates (Labuza et al., 1972). As found in minced cod and bream, lipid oxidation and (n-3) PUFA losses can be reduced using directional freezing instead of conventional technology; this is most probably an effect of the weak lipid-protein binding that stabilizes lipids (Kořakowska et al., 1994, 1995). Most likely, such interactions are involved in the inhibition of oxidative processes in frozen minces when appropriate mixing technology is applied prior to freezing (Kořakowska, 1981c).

Fish freshness at the moment of freezing is important in determining the type and extent of changes to lipids once the fish is frozen. Cold storage of krill for several hours, and cold storage of whole herring and herring mince for 1 to 2 days prior to freezing, inhibited lipid oxidation during frozen storage of minced fish (Kořakowska, 1981b, 1988, 1989). Lipid oxidation and hydrolysis proceeded at a faster rate in minces and whole fish frozen immediately after capture and mincing, and also in those stored for a prolonged time (6 to 7 days) prior to freezing. However, this was not the case in *Ariomma indica* stored for 0, 6, and 12 h before freezing (Sankar and Raghunath, 1995). Optimal timing of freezing of herring mince and whole fish with respect to inhibition of oxidation varied with duration of pre-mincing storage, fish species, and fishing season.

In general, there were no losses of (n-3) PUFA recorded in frozen fish; but in some studies, (n-3) PUFA contents were higher after freezing than before freezing (Polvi et al., 1991, Xing et al., 1993, Fernandez-Reiriz et al., 1992, 1995). Losses of n-3 PUFA were negligible in frozen minced bream stored for 9 months at -20°C . These losses were much lower than those induced by a 7-day-long storage of bream in ice (Kořakowska et al., 1995). This was most probably due, in part, to the fact that losses of (n-3) PUFA primarily involve the polar fraction, which is why they are less visible in total lipids of fatty fish (Hultin, 1994). Differences in lipid extractability

from fresh vs. frozen muscle tissue may also play a role. Bienkiewicz (2001) demonstrated that under model conditions, PUFA and DHA in particular are preferentially bound by protein. Thus, as a result of freezing, these PUFA are “released,” to a large extent, from being strongly bound to being weakly bound, which makes extraction possible. This may be the cause of the apparently higher PUFA content in frozen fish. Analysis incorporating direct tissue saponification showed that storage of herring and sprat at -18°C for 5 to 6 months resulted in (n-3) PUFA losses from a few percent up to 27%, depending on fish batch (Kořakowska et al., 2000c). In that study, losses of DHA were usually higher than those of EPA.

12.7.3 COOKED FISH

Changes in fish lipids during heating depend on whether isolated lipids or lipid-containing tissues were being heated. The changes can be affected by the “history” of the fish lipids or tissue. This history includes how it was stored, as well as fish species, fishing season, and a number of other biological variables. Heating temperature is more important than duration of heating (Medina et al., 1998).

Our studies on herring, sprat, and bream (Kořakowska et al., 1998, 2001b, unpublished data; Domiszewski and Kořakowska, 2001) showed that the heating of fish lipids at 60, 100, and 160°C (for 2 h) induced an increase in PV and AsV, the increase being proportional to the heating temperature. The maximum PV and AsV depend on heating temperature; the higher the temperature, the faster the maximum is reached. Once the maximum PV and AsV were achieved, the two values were observed to slowly decrease. Changes in the amount of aldehydes are more pronounced than for hydroperoxides. Conjugated dienes and trienes were observed to form at higher temperatures; they were detected as early as during the initial 15 min of heating at 160°C , while their formation at 100°C was slower but continuous. These changes were accompanied by a temperature-dependent decrease in PUFA. At 100°C , a 20% reduction in DHA was observed after 1 h. A 45% decrease in DHA was recorded as early as after 15 min of heating at 160°C , a loss higher than 70% occurring after one hour. EPA losses under the same conditions were about 20% lower than those of DHA. After 120 min of heating at 60°C , herring and bream lipid LC (n-3) PUFA incurred no significant losses.

Medina et al., (1998) found a significant (16%) reduction in DHA after 60 min of heating salmon lipids at 150°C . Heating (particularly at 160°C) of oxidized lipids from herring (PV = 200 mg O/100g lipid) affected some of the changes occurring during thermal treatment, but did not induce losses of LC (n-3) PUFA. Conjugated bonds form more readily in oxidized lipids: they were evident at a temperature as low as 60°C and increased rapidly and linearly at 160°C (Domiszewski and Kořakowska, unpublished data).

Heat-induced changes in the lipids of minced fish muscle differ from those occurring when isolated lipids are heated. Hydroperoxides present in fresh tissue decompose rapidly, as early as during the initial several minutes of heating. The speed with which they disintegrate increases with temperature. Carbonyls present in fresh tissue are disintegrated less rapidly. Further heating for 2 h results in no detectable increase in oxidation products. Heating of previously frozen (-20°C for

3 months) fish minces resulted in a decreased tissue ability to reduce hydroperoxides and carbonyls; PV and AsV decreased linearly with increasing heating time, the decrease being proportional to heating temperature. The differences were rather small in fresh fish, particularly at temperatures greater than 100°C. Losses in (n-3) PUFA recorded during an hour-long heating of muscle tissue of various batches of fresh and frozen-stored (for about 2 to 3 months) herring and sprat were insignificant, except for herring caught during its spawning season. Minces prepared from spawning herring showed approximately a 15% reduction in their DHA content after 30-min heating at 60 and 100°C, a 30% reduction being recorded after heating at 160°C. The reduction in LC (n-3) PUFA during heating of spawning herring muscle was from TAG rather than phospholipids, and was proportional to temperature (Domisze-wski and Kołakowska, unpublished data; Kołakowska et al., unpublished data). Changes in lipids (oxidation, FA composition) during extended microwaving (90 W) from 5 to 40 min of isolated lipids and tissue were similar to those proceeding during conventional thermal treatment, but were faster. After a 40-min long microwaving of fresh minced herring, the EPA+DHA content decreased by 18% relative to raw mince (Kołakowska et al., 1999a).

Thermal treatment of fish for the temperature and time required to cook the flesh usually resulted in no significant losses in LC (n-3) PUFA. For example, microwave cooking of four species of fish (6 min, medium to high power) did not significantly affect the lipid or FA compositions (Hearn et al., 1987). Conventional heating of seven different fish species (foil wrapped, 200°C, 30 min) did not significantly affect the FA compositions (Sinclair et al., 1992). Steaming (for 5 to 10 min) or microwaving (for 2 to 3 min) did not affect the amount of lipid or (n-3) or (n-6) PUFA in the flesh of blue eye trevalla (*Hyperoglyphe antarctica*) (Nichols et al., 2001b) or gummy shark (*Mustelus antarcticus*) (Nichols et al., unpublished data).

While the total lipid and FA compositions in fish do not usually change significantly during cooking, the type of treatment may substantially affects other lipids (Figure 12.5). During conventional heating (120°C, 5 min) and microwaving (10 min, 90 W) of isolated fish lipids and muscle tissue, the *trans* isomer contents did not exceed 0.5% of all FA, but were somewhat higher during microwaving. Under those conditions, oxysterol contents reached 63 and 43 µg/g lipid for isolate lipids, 47 and 46 µg/g lipid for tissue, conventional and microwaving cooked, respectively. The oxysterols composition differed, depending on the heating conditions (Kołakowska, Wąsowicz, and Szyjewski, unpublished data).

Oxidative stabilities of lipids in fish generally increase when heating at elevated temperatures (Sen and Bhandary, 1978; Flick et al., 1992; Fujita et al., 1994). This is the result of lipoxygenase inactivation and the formation of Maillard interaction products, particularly with phospholipids. Directly after thermal processing of fish, the lipids show lower PV, AsV, and fluorescence. However, a 30-min heating of herring minces at 60, 100, and 160°C failed to inhibit oxidation and changes in PUFA during 4 days of storage (at 5°C), compared with raw minces; the latter showed better antioxidative properties (Kołakowska et al., 2001b). Both conventional heating and microwaving of minced herring prior to freezing only slightly inhibited lipid oxidation during a half-year storage at -20°C. No significant changes in LC (n-3) PUFA of minces thermally treated before freezing and in those frozen raw

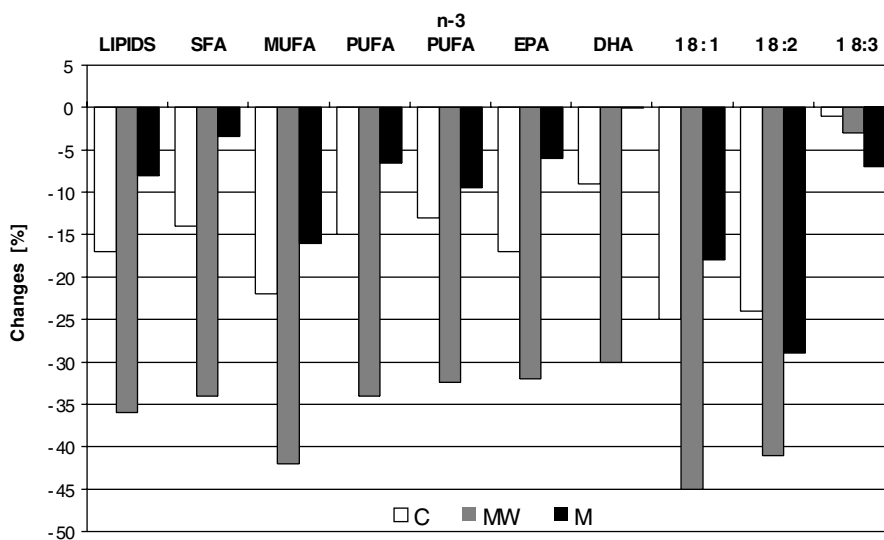


FIGURE 12.5 Changes in lipid content and fatty acids composition in muscle tissue of Baltic herring, caused by cooking: C – conventional cooking in boiling water, MW – microwaving in boiling water, M – microwaving without water. (Adapted from Kołakowska, A., et al., 2001b, *Lipidforum*, 21st Nordic Lipid Symp., Bergen, June 5–8.)

were recorded at that time (Kołakowska et al., 1998d). Our results are in agreement with data published earlier by other authors who reported no significant losses in LC (n-3) PUFA during boiling, cooking, and baking (Gall et al., 1983; Mai and Kinsella, 1979; Piggott and Tucker, 1990) as well as during extrusion cooking of fish (Suzuki et al., 1988).

12.7.4 GRILLED AND FRIED FISH

Grilling and frying of fish induce the largest changes in lipids (Maeda et al., 1985; Toth-Markus and Sass-Kiss, 1993; Nichols et al., 2001b). Due to the absorption of frying fat, frying results in a drastic alteration of fish FA composition (Figure 12.6). Depending on the frying fat used (e.g., sunflower oil, rapeseed oil, hydrogenated frying fat), the lipid content of fried herring doubled or tripled and inversely correlated with the unsaturation of the frying fat used, while lipid oxidation was proportional to the frying fat unsaturation (Kołakowska et al., 2000b). It was the exchange with the frying fat rather than oxidation that caused almost 50% losses of EPA and DHA in fried product dry weight; losses incurred during deep-frying were lower than those caused by pan-frying (Kołakowska et al., 2001b). Similarly, there was an increase in flesh lipid of blue eye trevalla (*Hyperoglyphe antarctica*) by 64% when pan-fried, or three times when deep-fried (Nichols et al., 2001b), but the levels of LC (n-3) PUFA remained unchanged. In contrast, grilling of the same species flesh increased the total lipid and LC (n-3) PUFA levels (on a wet weight basis), presumably due to the decrease in tissue water content (Nichols et al., 2001b).

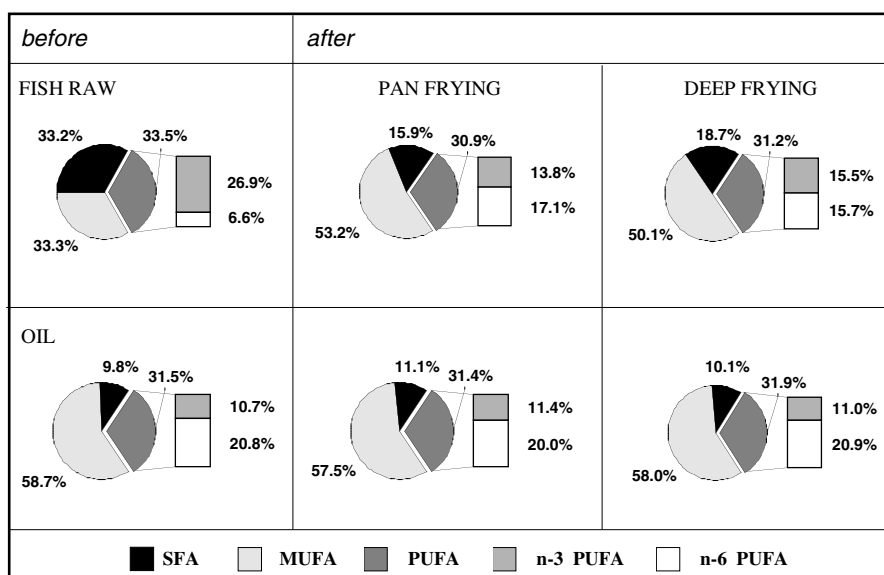


FIGURE 12.6 Fatty acid composition in herring and rapeseed, before and after frying. (Adapted from Kołakowska, A. et al., 2001b, *Lipidforum*, 21st Nordic Lipid Symp., Bergen, June 5–8.)

Fish freshness is important for changes in lipids during frying and during cold storage of fried fish. Fish stored for 1 to 4 days showed progressively increased decomposition of oxidation products (PV, AsV) during frying. This was followed by inhibition of oxidation during storage of fried fish, compared to storage of raw fish and fish fried when fresh. Frying of fish showing signs of deterioration (after 7 days of storage in ice) results in a rather small decomposition of oxidation products and fast deterioration of lipids during storage of fried fish. Although substantial lipid oxidation does occur during storage of fried fish, no direct losses of LC (n-3) PUFA were observed during 10 days of storage of fried herring and sprat at 5°C (the fish were fried when fresh) (Zienkiewicz et al., 2001).

12.7.5 CANNED FISH

Changes in lipids during canning have been extensively followed, particularly by Aubourg et al., (1995, 1996, 1997, 1998). Thermal treatment (cooking and sterilization) causes preferential hydrolysis of DHA from the *sn*-2 position of phospholipids because the amount of DHA at that position is clearly reduced (Medina et al., 1994; Aubourg et al., 1996). PUFA losses during sterilization of minced bigeye tuna and halibut at 124°C were recorded in the free FA fraction only (Koizumi et al., 1986). There is no published evidence of losses of (n-3) PUFA in canned products manufactured from fresh fish, compared with the raw material. Canning of mackerel (manufacture of mackerel in juice), frozen-stored for 4 months, resulted in an increased lipid oxidation level and a reduction in (n-3) PUFA, including 13 and 15%

reductions in EPA and DHA, respectively (Kořakowska et al., 1999b). As a result of the removal of a certain amount of water and stored lipids, the contribution of (n-3) PUFA in precooked fish increases. Lipid composition in canned fish in oil changes after sterilization, the product's covering oil showing the presence of EPA and DHA and the exchange intensifying with time of storage. This leads to a substantial variation in the lipid composition of canned fish, including an "increase" in (n-3) PUFA both in the fish and in the vegetable oil in canned products (Sasaki et al., 1989; Ruiz-Roso et al., 1998).

Lipid oxidation in a canned fish product depends on the storage temperature and on the absorption of metal ions from the can, but is generally slow and takes a number of months (Kořakowska et al., 1983). The fish in the can contains more oxidation products than the covering oil. Addition of α -tocopherol to the latter efficiently inhibited lipid oxidation in fish and in oil for up to a year after canning the herring. After 2 years, the amount of DHA in herring and in the covering oil was higher by 31 and 26%, respectively, in α -tocopherol-enriched products than in those without the additive (Kořakowska and Seidler, unpublished data). An extreme example of canned mackerel stored for 17 years could not be consumed due to sensory changes, but it still contained about half of the initial EPA content and about 33 to 25% of the initial DHA content. Compared with fish juice, covering oil protects fish lipids during prolonged storage (Kořakowska et al., 1985c, 1999b, 2000b). Leaving the thermal drip from fresh fish or from fish frozen and stored for 1 to 2 or 3 months (herring, mackerel, and horse mackerel) formed during precooking in the can, inhibits lipid oxidation during canned product storage. In contrast, drip from fish frozen and stored for longer periods accelerates oxidation.

Although hydroperoxides and carbonyls degrade during precooking and sterilization, there is a correlation between the rancidity of frozen and canned fish (horse mackerel, mackerel, herring), the correlation being stronger for canned fish in juice than for canned fish in oil (Kořakowska et al., 1985c; Czerniejewska-Surma, 1985; Morales et al., 1991). Rancidity was assessed by the sum (converted to w/w) of PV + TBA + Intensity of "off-odor" (in raw and cooked fish) and "off-flavor." The strongest relationship with raw material quality was observed a month after canning, with the relationship weaker thereafter.

Canned fish available on the market are regarded as a good source of LC (n-3) PUFA (Ota et al., 1990; Kořakowska et al., 2000b). Their LC (n-3) PUFA contents depend primarily on the type of raw material, and specifically on its lipid content. However, canned fish may contain an abundance of oxidation products. Some samples of the covering oil of canned sardines available on the Italian and Swiss markets were found to contain *trans*-FA in amounts exceeding the EU-proposed tolerance level (Cavallaro et al., 1996).

12.7.6 SMOKED FISH

Factors involved in smoking — primarily drying, salting, heating, and impregnation with smoke constituents — may have some prooxidative as well as antioxidative effects on lipids in fish muscle (Sikorski and Kořakowska, 2001). Hot smoking of fresh fish, or fish stored frozen for a short time, resulted in increases in hydro-

peroxides, carbonyls, FFA, conjugated FA, and fluorescence in fish lipids, but no reduction in (n-3) PUFA was observed in total lipids of smoked sardine (Beltran and Moral, 1991), mackerel (Kořakowska et al., 1998b), salmon (Cronin et al., 1991), and many other popular fish (Cronin and O'Sullivan, 1990; Wang et al., 1990). Beltran and Moral (1991) demonstrated that the losses of (n-3) PUFA in smoked sardine were from phospholipids and amounted to about 10% of DHA and EPA. When lipids in frozen mackerel were already considerably oxidized (PV = 79 mg O/100 g lipids), smoking resulted in disintegration of hydroperoxides, AsV decreased, but the amount of conjugated dienes increased, while (n-3) PUFA decreased by almost 20% (Kořakowska et al., 1998b) (Table 12.4).

Storage of smoked fish is accompanied by increased oxidation of lipids (Sikorski and Kořakowska, 2001). The rate of oxidation was higher when the mackerel to be smoked was already slightly rancid. After 3 days of dark storage at 4°C, PV was observed to increase by 40%, AsV increased by a factor of 8, but no reduction in LC (n-3) PUFA was recorded (Kořakowska et al., 1998b, 1998c) (Table 12.4). Smoked chub stored at 4°C for as long as 4 weeks showed only a few percent reduction in LC (n-3) PUFA (Wang et al., 1990). After 8 days cold smoked mackerel stored at 4°C, PV increased by 60% and only a few percent reduction in DHA was observed (Kořakowska et al., 1998c).

12.7.7 SALTED FISH

Salting is a traditional technology of fish processing, applied using a variety of fermentation techniques. Salting of fresh herring is accompanied by lipolysis, substantial oxidation of lipids, and losses in (n-3) PUFA (see Table 12.4). The amount of DHA in salted Baltic herring decreased by a few to 20% relative to the raw material, and the salted fish phospholipids retained only trace amounts of PUFA. A 20-day-long storage of those salted herring neither induced further losses of (n-3) PUFA nor did PV or AsV increase, but the number of conjugated bonds doubled and fluorescence increased several fold. Salting of Baltic and open ocean herring after frozen storage (PV = 20 to more than 50 mg O/100 g lipids) resulted primarily in an increase in carbonyls, with no loss of LC (n-3) PUFA (Kořakowska et al., 1998c). Due to loss of water on salting, the (n-3) PUFA content per salted product unit weight is usually not less than in the raw material (Ota et al., 1990; Hyvonen and Koivistoinen, 1994). During prolonged curing of anchovy, PUFA decreased from 10.5 to 7.5%. The (n-3)/(n-6) ratio decreased from 17 to 11 over a 180-day period (Ayensa et al., 1993). PV, TBA, free FA, diacylglyceride, and cholesterol oxide concentrations increased and PUFA decreased during processing and storage of Ghanaian fermented fish products (momoni), the changes being more extensive in crude than in refined-salt-treated fish (Yankah et al., 1996).

Compared with other fish products from the market, the salted products we analyzed showed the highest oxidation level, especially if the additional drying stage is included. Air-drying, in addition to the catalytic effects of salt and its possible contamination with transition metal ions, exposes the product to photooxidation (Bligh et al., 1988; Davis et al., 1995; Smith, 1995). Thus, margarines and spreads made of salted fish such as tuna, salmon, and mackerel — except for mackerel

spreads — have lipids usually low in LC (n-3) PUFA (Kořakowska et al., unpublished data).

12.7.8 MARINATED FISH

There is a paucity of data on the effects of marinating on fish lipids but, due to the low pH of the marinating solution, lipid hydrolysis is one of the main processes affecting the product. Oxidation during a 4-day-long ripening of Baltic herring marinades and during the subsequent 10- to 20-day-long storage in the marinating liquid resulted in little change in PV and AsV, but large changes in batches of spawning herring were observed (Kořakowska et al., unpublished data; and [Table 12.4](#)). Increased amounts of conjugated dienes were recorded after 18 days.

Substantial variations were observed in the FA composition of the marinades studied, especially the MUFA 20:1 and 22:1. Thus, it is difficult to assess changes in those FA present at low concentrations, when raw herring (containing these FA at 16 and 27% of the total FA, respectively) is used. Immediately after marinating, the contribution of (n-3) PUFA to the total FA content was higher than in the raw material due to leaching of lipids from the fish. During a 2-week-long storage of marinades, EPA and DHA content decreased. In those marinades showing signs of spoilage, the proportion of (n-3) PUFA in the total lipids, and particularly when converted to the product weight (w/w and dry weight), was even higher than in the marinades assayed directly after manufacture. This is also influenced by the transfer of protein and lipid degradation products to the marinating liquid. Marinades made of frozen herring showed lower lipid oxidation than the raw material, with no increase in the extent of oxidation being observed during a few days of storage (Kořakowska et al., unpublished data).

12.8 THE ROLE OF FISH LIPIDS IN HUMAN NUTRITION

The history of research on the benefits of fish lipids for the human organism began in the 1950s (Bronte-Stewart et al., 1956; others cited by Enselme, 1962). Intense work began two decades later with the studies of Dyerberg and Bang (1979) in Eskimos and continues today. A spate of publications that have appeared since then, and particularly in the past decade, has left the impression that fish lipids can be a cure for almost everything, as well as a key to longevity. The detailed update in this field on research in humans has been presented, that is, at 24th World Congress of the International Society for Fat Research (ISF), 2001. The beneficial effects of fish lipids in the diet have been attributed to the presence of LC (n-3)-PUFA and apply, in particular, to:

1. Prevention of cardiovascular diseases
2. Development and function of the brain and nervous system
3. Precursors to factors controlling the immune system
4. Prevention or treatment of some types of cancer

Preventive and curative properties have been mostly documented for the importance of LC (n-3) PUFA in cardiovascular disease (CVD). Evidence has accumulated that these FA protect against fatal coronary events, reduce the incidences of myocardial infarction (MI) and sudden cardiac death, and improve the prognosis in MI patients as to morbidity and mortality. LC (n-3) PUFA possess antiarrhythmic and antithrombotic activity, reduce blood pressure and risk of brain hemorrhage, and have been used with good results in patients after angioplasty (Kromhout, 1985; Christensen et al., 1999; Richter, 2001; Rubba and Iannuzzi, 2001; Schacky, 2001). The literature is replete with reports on the benefits of these acids in atherosclerosis, but large intervention studies are still required to clarify their importance (Richter, 2001). It remains to be established which (n-3) PUFAs prevent fatal coronary events and what are the mechanisms of their therapeutic properties in CVD. Nevertheless, it can safely be concluded that fish in the diet protects against coronary heart disease (CHD).

Intense research continues on the importance of fish lipids in the development and function of the nervous system. Electrophysiological studies have revealed the effects of LC PUFA on visual evoked potentials and visual attention. A deficit of (n-3) PUFA in the diet is associated with reduced levels of DHA in the retina and brain tissue. Such deficiencies might have far-reaching consequences during prenatal development when rising levels of DHA reflect maturation of the nervous system. Loss of DHA from the nerve cell membrane leads to dysfunction of the central nervous system in the form of irritability, susceptibility to stress, dyslexia, stereotypic behavior, aggressiveness, reduced learning capacity, impaired memory and cognitive functions, and extended reaction times (Carlson and Neuringer, 1999; Cunnane et al., 2000; Hals et al., 2000). Crawford et al., (1999), in their study of the evolution of the human brain, moved even further to suggest an association between brain size and availability of DHA, which is generous in areas close to water and poor in the African Savannah.

Despite many important achievements, this field of research has suffered from conflicting results and uncertain extrapolations from animal studies. Recent reviews of research in humans (Makrides and Gibson, 2001; Hornstra, 2001) confirm the benefits of (n-3) PUFA during prenatal development and advocate a partial replacement of dietary 18:2(n-6) by LC (n-3) PUFA, particularly for women during the third trimester of pregnancy and when breast-feeding. The infant formula should be enriched for preterm infants whose LC PUFA status at birth is lower than in-term neonates (Makrides and Gibson, 2001; Hornstra, 2001). Prenatal LC PUFA supply and supplementation formulas appear to be important for brain development and fetal growth. Term formula should contain at least 0.2% DHA and 0.35% AA in order to maintain the biochemical LC PUFA status comparable with that of breast-fed infants (Hornstra, 2001). Optimum amounts and ratios of (n-6) and (n-3) LC PUFA remain to be established in the case of preterm formula. Taking a broader look at these findings will certainly help to understand why fish is commonly called "brain food." Note that human nutritionists use the (n-6)/(n-3) ratio, whereas fish technologists use the reverse.

The anti-inflammatory and immunomodulatory properties of (n-3) PUFA in fish oil have been observed on numerous occasions. Decreasing the dietary (n-6)/

(n-3) ratio inhibits T cell proliferation, lymphocyte-derived cytokine production, and cell-mediated immune response. Clinical studies have shown that fish oil supplementation has therapeutic effects in several autoimmune diseases, including rheumatoid arthritis, chronic glomerular disease (IgA nephropathy), multiple sclerosis, inflammatory bowel disease, and psoriasis, but controversy remains over some issues (Belluzzi, 2001). LC (n-3) PUFA differ in their potencies toward individual aspects of the lymphocyte response (Calder, 2001). EPA exerts a particularly strong influence, while DHA seems to selectively modulate the immune system by inhibiting autoimmune reactions and increasing the resistance to bacterial enterotoxins (Calder, 1999, 2001).

Reports have appeared in recent years suggesting that LC (n-3) PUFA inhibit the growth of some tumors (Josyula and Schut, 1999; Singh et al., 1998). It has been shown in humans that the risk of breast tumors is increased by (n-6) and decreased by (n-3) PUFA. DHA is particularly potent, also in the absence of vitamin E (Bougnoux et al., 1999).

The mechanism of the beneficial properties of (n-3) PUFA has not been fully elucidated. A key role has been ascribed to the (n-6)/(n-3) ratio and that of the eicosanoids of either series. An excessive dietary supply of linoleic acid (18:2) produces an unfavorable rise in this ratio, which might explain the increased incidence of chronic inflammatory conditions in modern societies (Lands, 1997). Hals et al., (2000) have confirmed a deficiency of PUFA in children with acute brain damage. By supplementing the diet with (n-6) and (n-3) PUFA, an enrichment of phospholipids with LC (n-3) PUFA only was observed. At the same time, the (n-6)/(n-3) ratio fell to values between 2 and 3, typical for fetal nerve tissue and human milk. The ratio could be further decreased to a value as low as 1 without any risk to human health (Okuyama, 2001). Variations in FA composition and in the physiological properties of individual (n-3) PUFA have made it extremely difficult to establish an optimal value for the (n-6)/(n-3) ratio (Schacky, 2001). Moreover, long-chain FA cannot be totally replaced by α -linolenic acid and the desaturation and elongation of this acid is inhibited by linoleic acid. The biological effects of EPA and DHA are different as well, with DHA playing a more important role in cardiovascular disease and in the development and functioning of the central nervous system, while EPA is more important in immunological conditions. Undoubtedly, competition between (n-3) and (n-6) acids for the Δ -6 desaturase calls for a reduced supply of dietary linoleic acid and increased consumption of EPA and DHA in fish oils. The activity of (n-3) PUFA depends on whether the acids are present in the free form or bound to glycerol and on their position in the TAG or phospholipid molecules. Intestinal absorption of fish TAG, esters, and free FA was 60, 20, and 95% of the administered dose, respectively (Belluzzi, 2001).

A single fish meal per week reduces the risk of sudden death by 52% and the risk of cardiac arrest due to vascular occlusive disease by 70%. This represents the lowest recommended intake of fish. A 2-week diet containing three fish meals per week reduced serum levels of triglycerides, cholesterol, and LDL-cholesterol by 30, 15, and 15%, respectively (Kořakowska and Grala, 2001e). An even higher intake of LC (n-3) PUFA is necessary to reduce symptoms of arthritis. The precise

nutritional requirements of humans for (n-3) PUFA vary with age and remain largely unknown. A daily adequate intake (AI) of approximately 0.65 to 0.8 g EPA+DHA has been identified for adults (Simopoulos et al., 1999). Other estimates of total (n-3) PUFA requirements for beneficial effects to appear should be in the range of 0.85 to 1.5 g/day (von Schecky, 2001) and 1 to 2 g per day (Newton, 1996; Haumann, 1998). A daily fish oil capsule containing 850 to 882mg EPA+DHA (as ethyl esters), for 3.5 years significantly reduced fatal and nonfatal MI and stroke (GISSI-Prevenzione Investigators, 1999).

The negative effects of excessive intake of fish lipids include prolonged blood coagulation times, adverse changes in glucose homeostasis observed in non-insulin-dependent diabetes mellitus (NIDDM), greater susceptibility to *in vivo* oxidation, possible increased exposure to lipophilic contaminants (polychlorinated biphenyls [PCBs], dioxins), and accumulation of lipid peroxidation products. However, there was no tendency to bleed, even with a ninefold higher than recommended consumption of fish lipids (Gerster, 1995). When the (n-3) supply is low or moderate, there is no worsening of glycemic control in NIDDM patients. Furthermore, (n-3) PUFA have beneficial effects on the cardiovascular risk factors in NIDDM (Rubba and Iannuzzi, 2001). Increased intake of PUFA of the (n-6) and (n-3) series raises concerns over the susceptibility of LDL to oxidation and generation of atherogenic products. However, no increase in reactive oxygen species was observed with fish-rich diets that contribute to a higher content of (n-3) acids in LDL, probably because (n-3) PUFA displace arachidonic acid from membranes of monocytes and inhibit the production of superoxide radicals and other reactive forms of oxygen (Frankel, 1998). Unquestionably, the value of fish products as a source of LC (n-3) PUFA depends on whether they contribute to the pool of oxidatively modified lipids (see [Table 12.4](#)), although no loss of EPA or DHA was observed even with high rates of lipid peroxidation (Kořakowska et al., 1998a).

As mentioned previously, another health concern regards the fish' exposure and subsequent absorption of environmental contaminants such as mercury and lipophilic compounds such as PCBs and dioxins (Kořakowska and Kořakowski, 2001a). This can be an issue for certain fish species and fish known to be from contaminated sites. Also, the problem may be greater for fish with high levels of lipid in the flesh and fish products in which all of the animal's soft tissue (including lipid-rich hepatopancreas tissue, etc.) is consumed (e.g., sardines, oysters, and mussels). The long-term consumption of fish flesh contaminated with such compounds could prove to be a major human health hazard, and therefore fish should not be collected from contaminated sites.

12.9 CONCLUSIONS

Due to the presence of LC (n-3) PUFA, fish lipids play an important nutritive and therapeutic role in the human diet. It is already known that LC (n-3) PUFA cannot be substituted by α -linolenic acid, and that EPA and DHA serve different functions in the human body. The value of fish as a source of LC (n-3) PUFA primarily depends on the lipid content and the regions from which the fish are harvested,

with the highest contents generally found in fat fish caught in colder regions (e.g., higher latitudes). The lipid composition of the muscle tissue of fattier fish changes throughout their biological cycle. Therefore, fish may differ in lipid content, FA composition, and therefore susceptibility to oxidation and lipolysis, depending on the fishing season. Lean fish and shellfish, both marine and freshwater, are nutritionally valuable because they contain mainly PUFA-rich phospholipids. Farmed fish, when fed appropriate feeds, may also be a very good source of LC (n-3) PUFA. Because of such variability between species, illustrated books of fish and their lipid compositions such as Yearsley et al., (1999) should be more readily available. The current review shows that lipid composition tables can never be more than a generalization, due to the intrinsic variability of lipids in a given species. Nutritionists, scientists, and large-scale producers of commercial products need to be aware of this.

In general, the processing technologies currently used cause no loss in LC (n-3) PUFA therefore, fish products can be a good source of those FA, provided the raw material is lipid-rich. The dynamics and extent of changes induced in muscle tissue lipids by the processing technology depend on initial lipid composition as well as on the pro- and antioxidative status of the tissue. Thermal stress is more severe in a very fresh fish (immediately after capture). However, for fish approaching deterioration, a moderately extended cold storage of a raw material may enhance lipid stability during and after treatment. Consequently, despite the decreased activity of prooxidative enzymes and α -tocopherol decomposition during storage of fish, the tissue (particularly that of minces) shows a predomination of antioxidative potential (proteolysis products, TMAO decomposition, phospholipid hydrolysis). Also, the presence of some oxidation products can create better conditions for weak lipid-protein interactions that stabilize lipids. Frozen storage of fish, before it is subject to thermal treatment (cooking, canning, frying), can increase the probability of adverse changes in lipids during and/or after treatment.

The examination of fish as a source of LC (n-3) PUFA has to be analyzed in conjunction with the extent of lipid oxidation in a product, particularly because there is no simple relationship between lipid oxidation level and (n-3) PUFA losses. Products that are (n-3) PUFA-rich may also contain high levels of oxidation products if adequate care is not taken during storage and processing. However, seafood remains the key source of beneficial LC (n-3) PUFA for the human population and will continue to do so. Efforts to continue to maximize efficient utilization and processing of this unique resource will therefore greatly assist global and regional fisheries, providing more stringent conservation of resources are implemented.

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13 Milk Lipids

Zofia A. Żegarska

CONTENTS

- 13.1 Introduction
- 13.2 Composition of Milk Lipids
 - 13.2.1 Lipid Classes
 - 13.2.2 Fatty Acids
 - 13.2.2.1 Fatty Acid Composition
 - 13.2.2.2 Factors Influencing Fatty Acid Composition
 - 13.2.3 Triacylglycerols
 - 13.2.4 Compound Lipids
 - 13.2.4.1 Phospholipids
 - 13.2.4.2 Glycolipids
 - 13.2.5 Ether Lipids
 - 13.2.6 Unsaponifiable Lipids
- 13.3 Physicochemical Properties of Milk Fat
 - 13.3.1 Milk Fat Globule and Membrane
 - 13.3.2 Physical Characteristics of Milk Fat
 - 13.3.3 Lipolysis of Milk Fat
 - 13.3.4 Oxidation of Milk Fat
- 13.4 Nutritional Significance of Milk Lipids
- References

13.1 INTRODUCTION

The lipids in milk exist as microscopic globules in an oil-in-water (O/W) emulsion. On the interface between globules and milk plasma is a membrane that stabilizes the emulsion. Many factors can affect the milk lipid content, particularly genetics, feed, stage of lactation, and climate. Cow's milk contains approximately 2.8 to 6% total lipids, 3.5 to 4% on average.

Milk lipids, referred to as "milk fat," have been the object of continuing research. Milk fat is an important component of many dairy products, various food products (e.g., bakery products, chocolate), and also has nontraditional applications (e.g., in cosmetics).

This chapter reviews the chemical composition, physicochemical characteristics, and nutritional aspects of cow's milk fat. The chapter focuses on the unique character

of milk fat — the significant variations in composition, physical properties, and the number of biologically active compounds present.

13.2 COMPOSITION OF MILK LIPIDS

13.2.1 LIPID CLASSES

The lipid fraction of milk is dominated by triacylglycerols (TAG), with small amounts of various other lipid classes (Table 13.1). Milk lipids also contain small quantities of glycolipids, fat-soluble vitamins, ether lipids, and flavor components, mainly lactones, aldehydes, and ketones.

TABLE 13.1
Composition of Milk Lipids

Lipid Class	Weight Percentage
Triacylglycerols	97–98
Diacylglycerols	0.3–0.6
Monoacylglycerols	0.02–0.04
Fatty acids	0.1–0.4
Phospholipids	0.2–1.0
Cholesterol	0.2–0.4
Cholesteryl esters	0.02
Hydrocarbons	0.1

Adapted from Jensen, R.G. 1973. *JAOCS*, 50, 186–192; Jensen, R.G. et al. 1991. *J. Dairy Sci.*, 74, 3228–3243; Roderbourg, H. and Kudzal-Savoie, S. 1979. *JAOCS*, 56(4), 485–488.

13.2.2 FATTY ACIDS

13.2.2.1 Fatty Acid Composition

Fatty acid (FA) residues constitute 90 wt.% of milk lipids. They are bound in acylglycerols (tri-, di-, and mono-), phospholipids (PL), glycolipids, cholesteryl esters, and FA may occur in free form.

Among the edible fats, milk fat has the broadest range of FA. Over 400 FA in milk fat have been identified (Table 13.2). However, only a dozen or so of them are present at the concentration above 1% (Table 13.3). The contents of approximately 30 acids are in the range of 0.1 to 1%, with the great majority of FA being present in trace quantities.

Milk fat is characterized by the presence of relatively high concentrations (about 10 wt.%) of short-chain FA (C4:0 to C10:0). Butyric acid (C4:0) is specific for milk fat of ruminants. Higher saturated FA (>C10:0) occur in amounts of approximately 57%, and palmitic, stearic, and myristic acids dominate. The 15:0 and 17:0 acids, characteristic of ruminant fat, also occur in milk fat as branched FA.

TABLE 13.2
Fatty Acid Composition of Cow's Milk Lipids

Type	Number of Acids ^a	Fatty Acid Identity
Saturated		
Normal	27	2–28,even; 3–27,odd
Monobranched	39	11–24; 13–19; three or more positional isomers, 5–10; Me,Et
Multibranched	16	16–28
Monoenoic		
<i>Cis</i>	61	9–25, positional isomers of 12:1–14:1, 16:1–18:1, and 23:1–25:1
<i>Trans</i>	49	14; 16–25; positional isomers of 14:1–16:1, 18:1 and 23:1–25:1
Dienoic	45	14–26, evens only; <i>cis,cis</i> ; <i>cis,trans</i> or <i>trans,cis</i> ; and <i>trans,trans</i> , unconjugated and conjugated and positional isomers
Polyenoic		
Tri-	10	18, 20, 22; geometric positional and conjugated and unconjugated isomers
Tetra-	5	18, 20, 22; positional isomers
Penta-	2	20, 22
Hexa-	1	22
Keto (oxo)		
Saturated	44	6, 8–10, 12, 14, 15–20, 22, 24; positional isomers
Unsaturated	21	14, 16, 18; positional isomers of carbonyl and double bond
Hydroxy-		
2-position	16	14:0, 16:0–26:0, 16:1, 18:1, 21:1, 24:1, 25:1
4- and 5-positions	9	10:0–16:0, 14:1–6, and 12:1–9
Other positions	60	
Cyclic (hexyl)	1	11; terminal cyclohexyl
Total	406	

^aThe fatty acids were identified by March 1994.

From Jensen, R.G. and Newburg, D.S. 1995. Bovine milk lipids, in *Handbook of Milk Composition*, Academic Press, San Diego, 543–575. With permission.

The average monoenoic acid content of milk fat is around 30%. Oleic acid (*cis*-9–18:1) is the predominant monoenoic acid. It constitutes 95.8% of the positional *cis* isomers of octadecenoic acid. Monoenoic FA also comprise *trans* isomers, mainly *trans*-octadecenoic acids. Cow's milk fat contains 1.3 to 7.3% *trans*-18:1 acids, with an average of 3.7%. The relative proportions of *trans*-18:1 positional isomers in milk fat are given in Table 13.4. *Trans*-16:1 isomers occur in amounts of approximately 0.13% (Precht and Molkenin, 2000).

Polyenoic acids in milk fat (ca. 3.0% of total FA) comprise mainly linoleic acid and α -linolenic acid and its positional and geometric isomers. Milk fat contains *trans*-18:2 isomers in average amounts of approximately 0.5%. The

TABLE 13.3
The Primary Fatty Acids (wt.%) of Milk Fat

Fatty Acids	Summer	Winter
4:0	2.8	3.1
6:0	2.2	2.3
8:0	1.2	1.4
10:0	2.5	3.4
12:0	3.0	3.8
14:0	9.9	12.2
14:1 (15:0 br)	2.1	2.6
15:0	1.5	1.7
16:0	24.5	30.0
16:1 (17:0 br)	3.1	3.3
17:0	1.1	1.2
18:0	11.3	8.0
18:1(<i>cis</i> + <i>trans</i>)	28.8	21.9
18:2	1.4	1.2
18:3 (18:2 <i>conj</i> -)	2.2	1.6

Adapted from Żegarska, Z. 1988. *Acta Acad. Agricult. Techn. Olst.* Suppl. D, 22, 1–46, (in Polish, English abstract).

content of conjugated linoleic acid (CLA) isomers ranges from 0.2 to 2.0%, and *cis*-9, *trans*-11–18:2 acid dominates. The α -linolenic acid content of milk fat is in the range of 0.3 to 0.9% (Żegarska et al., 1996).

13.2.2.2 Factors Influencing Fatty Acid Composition

The milk fat composition is the most variable among natural edible fats. It is influenced by feeding and animal factors such as breed, stage of lactation, health, and age of the cow. The feed has the highest influence on the FA composition. Milk fat from pasture feeding has considerably more C₁₈ FA and less C16:0, C14:0, and C12:0 acids than fat from indoor feeding. Short-chain FA (C4:0 to C8:0) are characterized by the smallest seasonal variation (Table 13.4).

In addition to seasonal factors, significant changes in the FA composition of milk fat can be achieved by manipulating the cow's diet. Milk fat composition is influenced more by dietary fat than by any other dietary component. The effects of fat supplementation on FA composition are dependent on the type and amount of dietary fat. In most cases, fat supplements decrease the proportions of 6:0 to 14:0 acids (*de novo* synthesized) and increase the proportion of long-chain (dietary) FA. Diets containing supplemental fat do not affect the proportion of C4:0 in milk fat.

Differences in FA composition of milk fat exist among various breeds of dairy cows. For example, the milk fat of Jerseys have a higher proportion of short- and medium-chain FA and lower proportions of palmitic and oleic acids than milk fat from Holsteins (Beaulieu and Palmquist, 1995). Milk of Polish-Red cows is char-

TABLE 13.4
Proportion of Positional *trans*-18:1 Isomers of Milk Fat

Isomer	$\Delta 4$	$\Delta 5$	$\Delta 6-8$	$\Delta 9$	$\Delta 10$	$\Delta 11$	$\Delta 12$	$\Delta 13/14$	$\Delta 15$	$\Delta 16$
Proportion	1.6	1.5	4.7	6.9	4.7	43.2	6.3	14.2	7.9	9.8

Adapted from Precht D. and Molkentin J. 2000. *Trans* unsaturated fatty acids in bovine milk fat and dairy products, *Eur. J. Lipid Sci. Technol.*, 102, 635–639.

acterized by higher monoenoic (also *trans* isomers) and polyenoic FA content than the milk of Lowland Black-and-White cows (Żegarska et al., 2001).

Milk fat composition is strongly influenced by the stage of lactation. Initially, the proportion of short-chain FA is low and increases until at least 8 to 10 weeks into lactation. FA with 18 carbon atoms exhibit the opposite pattern (Palmquist et al., 1993).

13.2.3 TRIACYLGLYCEROLS

TAG in cow's milk fat consist of a complex mixture of TAG that differ considerably in molecular weight and degree of unsaturation. The milk fat TAG extend over a wide range of acyl carbon numbers, from C₂₄ to C₅₆, including small amounts of odd-numbered TAG. The composition of milk fat is dominated by TAG with 36 to 40 and 46 to 52 carbon atoms. Feeding season, lactation period, and the individual factors of a cow influence the proportion of individual TAG types (Żegarska and Jaworski, 1981). The average content of saturated TAG in summer milk fat is approximately 33%, increasing to approximately 44% in winter fat (Żegarska, 1988).

It is known that the distribution of FA in milk TAG is not random (Table 13.5). Palmitic acid is preferentially esterified in the *sn*-2-position in high-molecular-weight TAG and in the *sn*-1-position in low-molecular-weight TAG. The *trans*-monoenoic acids are incorporated into TAG in the same manner as their *cis* isomers (Parodi, 1982). The stage of lactation and feed conditions have little effect on the positional specificity of FA (Hawke and Taylor, 1983).

Milk fat contains many different TAG species that are very difficult to identify. However, it has been possible to determine the composition of 223 individual even-numbered TAG (Gresti et al., 1992). The major TAG were 4:0,16:0,18:1; 4:0,16:0,16:0; and 4:0,14:0,16:0 in the amounts of 4.2, 3.2, and 3.1mol%, respectively.

TAG are resistant to the heat treatments used in milk processing. Short-time/high-temperature processing causes virtually no changes in the TAG composition.

13.2.4 COMPOUND LIPIDS

13.2.4.1 Phospholipids

PL represent up to 1.0% of the total lipids in milk. The lactation stage has a significant influence on the content of PL. The major types of milk PL and their

TABLE 13.5
Positional Distribution of Fatty Acids in TAG from
Cow's Milk

Fatty Acid Composition (mol% of total)			
	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -3
4:0	—	—	35.4
6:0	—	0.9	12.9
8:0	1.4	0.7	3.6
10:0	1.9	3.0	6.2
12:0	4.9	6.2	0.6
14:0	9.7	17.5	6.4
16:0	34.0	32.3	5.4
16:1	2.8	3.6	1.4
18:0	10.3	9.5	1.2
18:1	30.0	18.9	23.1
18:2	1.7	3.5	2.3

Adapted from Jensen, R.G., Ferris, A.M., and Lammi-Keefe, C.L. 1991.
The composition of milk fat, *J. Dairy Sci.*, 74: 3228–3243.

approximate percentages are: phosphatidylcholine, 35%; phosphatidylethanolamine, 30%; sphingomyelin, 25%; phosphatidylinositol, 5%; and phosphatidylserine, 3% (Deeth, 1997). The PL of milk and dairy products are concentrated either in the milk fat globule membrane (MFGM) or in other membranous material that is probably derived from the MFGM (Christie et al., 1987). The PL contain longer and more unsaturated FA than the TAG. The milk PL fulfill some very important functions. They stabilize O/W emulsions, are the source of constituents of high nutritional importance, and owing to the location and polyenoic FA (PEFA), they are the foci of autoxidation. During fat centrifuging, about 30% of the PL is retained in skim milk and 45% of cream PL is retained in buttermilk after churning. Buttermilk is thus an attractive source of milk PL.

13.2.4.2 Glycolipids

Milk contains glucosylceramide, lactosylceramide, and gangliosides at approximately 20 to 24 mg/L, assuming 3.3 to 4% milk fat content. Over 70% of the neutral glycolipids and 90% of the gangliosides are associated with the MFGM. Buttermilk high in MFGM is a rich source of gangliosides, even richer than the gray matter of the brain (Jensen and Newburg, 1995).

13.2.5 ETHER LIPIDS

Ether lipids, characterized by an ether bond in *sn*-1 position, are present in small amounts in milk fat. Cows' milk neutral lipids contain approximately 0.01% 1-*O*-

alkyldiacylglycerols and PL contain approximately 0.16% 1-*O*-alkylacylglycerophospholipids (Hallgren et al., 1974).

13.2.6 UNSAPONIFIABLE LIPIDS

The unsaponifiable fraction of milk lipids includes sterols, vitamins (mainly A, E, and D), hydrocarbons, and alcohols. Cholesterol is the major sterol, with an average content of approximately 0.3% of total lipids; 90% of this is in the free state. Small amounts of 7-dehydrocholesterol, lanosterol, and dihydrolanosterol are also present.

Hydrocarbons constitute about 0.1 wt.% of the milk fat, that is, approximately 25% of the total unsaponifiable fat. Squalene is the predominant hydrocarbon. It constitutes 25 to 30% of the total hydrocarbons. Three compounds — phytene-1, phytene-2, and neophytadiene — constitute another major fraction. A minor fraction is composed of hydrocarbons containing an uneven (17 to 35) number of carbon atoms (Roderbourg and Kuzdzal-Savoie, 1979). The β -carotene content of milk fat is usually in the range between 2 and 8 $\mu\text{g/g}$, depending on the season. The level of α -tocopherol during pasture feeding (average 38 $\mu\text{g/g}$ of fat) is almost twice that of the content in winter milk. The average vitamin A content is approximately 30 $\mu\text{g/g}$ in summer milk fat and 20 $\mu\text{g/g}$ in winter milk fat. The vitamin D content in milk fat is very low.

High-temperature processing has little effect on the levels of these vitamins compared with the effects of the season.

13.3 PHYSICOCHEMICAL PROPERTIES OF MILK FAT

13.3.1 MILK FAT GLOBULE AND MEMBRANE

In freshly secreted milk, the fat occurs as minute globules in the aqueous phase of the dispersion. The size of the globules normally varies from less than 0.2 μm to 10 μm , with the majority between 2 and 5 μm . Fat globules in milk are protected and separated from each other by a thin layer, usually called a membrane. Its composition and properties are completely different from either milk fat or plasma. The material of the MFGM constitutes only 2 to 6% of the total mass of the fat globules. Similar to other biological membranes, the MFGM consists largely of proteins (including enzymes), lipids, and saccharides in the approximate ratio of 4:3:1 (Deeth, 1997). The relative proportions of TAG and PL in the MFGM are presented in [Table 13.6](#). The other lipids of the MFGM consist of glycolipids, cholesterol and its esters, di- and monoacylglycerols, FA, and hydrocarbons. Of the fat-soluble vitamins, only α -tocopherol is incorporated into the MFGM (Jensen and Nielsen, 1996). The MFGM is asymmetric, with the outer and inner sides differing considerably in composition. Phosphatidylcholine, sphingomyelin, and the glycolipids (cerebrosides and gangliosides) are predominantly located on the outside, while the neutral lipids, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol are concentrated on the inner surface of MFGM (Deeth, 1997).

The technological processes used in the dairy industry influence the composition of MFGM. During heating, interactions occur between MFGM components and denatured serum proteins, predominantly β -lactoglobulin. An increase in protein

TABLE 13.6
Composition of MFGM Lipids

Classes	Percent of Total Lipids
Triacylglycerols	48.4
Phosphatidylethanolamine	12.3
Phosphatidylinositol	3.8
Phosphatidylserine	6.3
Phosphatidylcholine	13.1
Sphingomyelin	3.4
Other	12.7

From Chen, Z.Y. and Nawar, W.W. 1991. Role of milk fat globule membrane in autoxidation of milk fat, *J. Food Sci.*, 56(2), 398–426.
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material and some losses of TAG of the MFGM have been reported (Houlihan et al., 1992). Agitation may cause a coalescence of fat globules and, as a consequence, a release of the membrane material. In the butter-making process, a large portion of the MFGM is retained in buttermilk. Homogenization of milk causes a disruption of the native milk fat globules into smaller particles of 1 μm or less. The natural MFGM is not sufficient to cover the larger surface area of the fat globules. In homogenized milk, the fat globules are covered with adsorbed milk proteins, particularly casein.

13.3.2 PHYSICAL CHARACTERISTICS OF MILK FAT

The physical properties of milk fat are important in determining its use in dairy and other food products. The significant complexity, both in terms of FA composition and TAG structure, and the variability of milk fat composition affect the physical properties of milk fat. Because of this complexity, milk fat exhibits a melting range between -40 and $+40^{\circ}\text{C}$. In the range of temperatures in which milk fat is used, it is a mixture of solid and liquid phases. The relative proportion of each depends on the chemical composition, temperature and temperature history, and the state of dispersion. The seasonal variations in the FA composition of milk fat affect its solid fat content and the rheological properties of butter. The milk fat from indoor feeding at 10°C may have ten percentage units more of solid phase than the fat from pasture feeding (Żegarska, 1988). Staniewski (2000) observed considerable seasonal variations in the rheological properties of high-quality butter made in Poland. Several functional properties of milk fat, such as hardness, structuring, viscosity, spreadability, plasticity, heat transfer, and compatibility with other fats, have been associated with the solid:liquid ratio (Kaylegian, 1995).

The solid phase of milk fat is present in the form of crystals, while the liquid phase can be present as free liquid, liquid entrapped in the crystal matrix, or liquid adsorbed onto a crystal surface (Kaylegian, 1995). Crystallization of milk fat is more complicated than that of most other fats because of its complex composition. The formation of mixed crystals that contain more than one TAG species and the phe-

nomenon of polymorphism are common in milk fat. Crystallization of globular fat differs considerably from the crystallization of bulk fat. The fat globules provide a natural limit to the growth of fat crystals. The rate of crystallization of emulsified fat is very low and, very often, considerable supercooling is required.

13.3.3 LIPOLYSIS OF MILK FAT

Lipolysis is important from the standpoint of the quality of milk and dairy products. The natural lipoprotein lipase in milk is the main agent in the lipolysis of raw milk. In dairy products, hydrolytic rancidity is primarily caused by heat-stable lipases produced by psychrotrophic bacteria or by residual native lipase that survived heat treatment. The milk lipase and most bacterial lipases preferentially attack the FA residues in the terminal positions. Because milk fat contains relatively high proportions of short-chain FA, which are esterified in the *sn*-3-position, lipolysis increases the level of these FA in free form. The short-chain FA are soluble in water and they may be present in dissociated form (salts) or associated form (acid form), depending on the pH of the aqueous phase of the milk product. The characteristic lipolytic flavors occur when the acids are present in the acid form. Some FA have low flavor thresholds, and even very small amounts cause an unpleasant, rancid flavor of milk and dairy products. Only in some types of cheese (e.g., mold cheeses) is moderate lipolysis desirable for specific flavor.

In raw milk, fat is protected against lipolysis by the intact MFGM. However, the milk of some cows shows lipolysis, called spontaneous lipolysis. The lactation period, feed, and individual cow factors influence this phenomenon. It has been reported that the number of cows producing milk susceptible to spontaneous lipolysis ranges from 5 to 35%.

More important for the milk industry is induced lipolysis. This kind of lipolysis is connected with some damage of the MFGM due to physical treatment, such as agitation, cooling, warming, and/or homogenization. Lipolysis in milk can be readily initiated by vigorous agitation. The amount of lipolysis induced depends on the mode of agitation (e.g., air agitation, pumping, stirring, the severity and duration of agitation). Lipolysis can be induced when fresh milk or cream is subjected to temperature fluctuations (e.g., cooling to 5°C or lower, warming to 30°C and re-cooling to below 10°C). This phenomenon has been termed temperature activation (Kon and Saito, 1997). Homogenization of raw milk results in a very strong activation of lipolysis. That is why it is necessary to homogenize milk after the heating process.

13.3.4 OXIDATION OF MILK FAT

Oxidation of milk fat is one of the most important factors limiting the keeping quality of dairy products. Lipid oxidation yields hydroperoxides that decompose to a broad group of carbonyl compounds, which can contribute to off-flavors in dairy products. The oxidative stability of fat depends on its composition. Compared with many edible fats, milk fat is relatively resistant to oxidation because of its low PEFA content, and high proportion of saturated FA, and the presence of natural antioxidants, principally α -tocopherol and β -carotene. Milk fat from the summer contains

more unsaturated FA and appears to be more susceptible to oxidation than that from the winter period. In fact, summer milk fat may be more resistant due to a higher content of α -tocopherol and β -carotene.

Oxidation of milk fat can be induced by transition metals, especially copper, or by light, but can also occur spontaneously in raw milk. The spontaneous oxidized flavor (SOF) of milk has been known for many years, predominantly as a seasonal problem arising in spring before the cows are let out to pasture. It tends to occur in well-managed, high-yielding dairy herds. It has been suggested that the susceptibility of milk to SOF depends on the balance between α -tocopherol, β -carotene and the PEFA in the neutral lipids and PL (Granelli, 1996).

Processing can affect the autooxidation reaction considerably. On heating, the oxidation-promoting properties of the xanthine oxidase and lactoperoxidase are enhanced due to their denaturation. As a consequence, low pasteurization may favor autooxidation although more intense pasteurization largely decreases the autooxidation rate. H_2S formed on heating and Maillard products contribute to the marked increase in oxidative stability. Homogenization of milk greatly reduces the rate of autooxidation (Walstra and Jenness, 1984).

Oxidative lipid deterioration in milk and dairy products occurs mainly during storage. The rate of oxidation depends on the concentration of dissolved oxygen, the storage temperature, and the presence of antioxidants (α -tocopherol, β -carotene) and prooxidants (especially copper).

13.4 NUTRITIONAL SIGNIFICANCE OF MILK LIPIDS

Milk fat has been a natural part of the human diet for several millennia. The important attribute of milk fat as a food ingredient is its delicate, pleasing flavor. The flavor and mouth-feel of milk fat are far superior to those of any other edible fat. A large number of different flavor compounds occur in milk fat at their flavor thresholds. The palatability of dairy products is also influenced by the fat texture. The taste of milk results to a great extent from the emulsion-colloidal structure; that of butter results from its crystalline structure.

Milk fat is easily digested because of the high proportion of short-chain FA. These acids, located in the terminal position in the TAG molecule of milk fat, are hydrolyzed preferentially and transferred directly from the intestine to the liver via the portal vein. The high dispersion of fat in milk, particularly in homogenized milk, facilitates its absorption.

Milk fat is one of the main sources of vitamin A in the human diet. Better absorption of β -carotene from milk fat than from many other foods is also known. Milk fat is also rich in oleic acid, which has a positive effect on the human health.

According to current knowledge, cow's milk fat contains compounds with negative properties. In the recent past, milk fat was often seen as a potential promoter of coronary heart disease because of its saturated FA and cholesterol content. On the other hand, there are several studies indicating that milk and milk products may not adversely affect blood lipids as would be predicted from its fat content and composition (Pfeuffer and Schrezenmeir, 2000). Recently, it has been shown that different

types of dietary saturated FA do not have equivalent effects on plasma cholesterol levels. The hypercholesterolemic effect of saturated FA is largely due to lauric, myristic, and palmitic acids (Kris-Etherton et al., 2000). Milk fat is not high in cholesterol compared with other animal fats. Moreover, cholesterol occurs in milk fat mainly in the free form, which is more resistant to oxidation than its esterified form.

Trans-fatty acids (TFA) of milk fat are formed in the rumen of cattle via the biohydrogenation of dietary PEFA. In general, TFA are thought to have negative physiological effects. However, the milk fat TFA composition is unique because it is predominantly characterized by *trans*-vaccenic acid, which is a closely associated metabolite of *cis*-9, *trans*-11-octadecadienoic acid. Moreover, *trans*-vaccenic acid is reported to be converted *in vivo* to the *cis*-9, *trans*-11-C18:2 by the Δ 9-desaturase (Griinari et al., 2000). Elaidic acid (*trans*-9-C18:1), which is frequently used in clinical studies, occurs in milk fat in small amounts, 0.23% on average (Precht and Molkentin, 1996).

Recently, it has been shown that cow's milk lipids contain a number of positive bioactive substances, including conjugated linoleic acid (CLA), butyric acid, sphingomyelin, and ether compounds. Milk fat is the richest natural source of CLA. Seasonal variation is very marked, with values in the milk fat from summer up 2 or 3 times higher than winter values. The biologically important *cis*-9,*trans*-11 isomer of CLA constitutes nearly 90% of the total CLA. CLA is reported to have anticarcinogenic, antiatherogenic, and immunomodulating properties. It has also been shown to reduce adipose fat and to act as a growth factor (Parodi, 1999; Kritchevsky, 2000). Compared with milk, other dairy products may have a higher CLA content. Processing may cause free radical oxidation of linoleic acid followed by a reduction, leading to an increase in CLA concentration. Storage of dairy products does not affect the CLA concentration, suggesting that CLA is a stable component (Shantha et al., 1995).

Butyric acid (approximately 3% in milk fat) is a potent antineoplastic agent. It inhibits cell growth and induces differentiation in a wide spectrum of cancer cell lines (Parodi, 1999). It has also been demonstrated that human liver cancer cells can be killed by millimolar concentrations of butyric acid or tributyrin (Watkins et al., 1999).

Milk and milk products are rich sources of sphingolipids, in which sphingomyelin dominates. It is now recognized that sphingomyelin plays an important role in transmembrane signal transduction and cell regulation through its biologically active metabolites (ceramide and sphingosine) (Parodi, 1999). Moreover, milk fat sphingolipids and TAG containing C10:0 and C12:0 FA may protect against food-borne gastroenteritis (Sprong et al., 2001). Cow's milk lipids also comprise ether lipids, which have potent antineoplastic activity at very low concentrations (Parodi, 1999).

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14 Egg Lipids

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CONTENTS

- 14.1 Egg Shell and Albumen Lipids
- 14.2 Egg Yolk Lipids
 - 14.2.1 Introduction
 - 14.2.2 Triacylglycerols of Egg Yolk
 - 14.2.3 Mono- and Diacylglycerols of Egg Yolk Lipids
 - 14.2.4 Phospholipids
 - 14.2.5 Fatty Acid Position in the Glycerol Molecule
 - 14.2.6 The Lipid Components of Lipoproteins
 - 14.2.7 Influence of Diet on Lipid Composition
 - 14.2.8 Positions of Fatty Acids in the Glycerol Molecule
- 14.3 Cholesterol
 - 14.3.1 Introduction
 - 14.3.2 Autoxidation of Cholesterol
 - 14.3.3 Technological Dependencies for the Formation of Oxidation Products of Cholesterol

References

14.1 EGG SHELL AND ALBUMEN LIPIDS

The egg contains lipids on a large scale, even in its outer region. The shell contains 0.045% lipids in the cuticula and 1.35% lipids in the shell membrane (Suyama et al., 1977). Neutral and polar lipids occur in proportions of 5:1 and 6:1, respectively. Neutral lipids consist of mono-, di-, and triacylglycerols (TAG), cholesterol and cholesteryl esters, and free fatty acids (FA). The main components in the neutral lipid fraction of the shell and its adjoining layers are cholesterol and cholesteryl esters, in addition to diacylglycerols; the TAG content is low.

The polar lipid fraction contains mainly sphingomyelins, whereas phosphatidylethanolamines and phosphatidylcholines occur along with cerebrosides (ceramidemono and diglucoside) only in very small amounts. Seventeen different FA are involved in the structure of the lipids; in the neutral fraction of the shell, palmitic, stearic, and oleic acids predominate, and within the membrane, the proportion of linoleic acid is higher. The polar lipids in the cuticula and the membrane are very

similar in composition and arachidonic and behenic acids are found in relatively high concentrations.

Albumen contains up to 0.02% lipids, about 15% of which are phospholipids. TAG make up the main part, but a small amount of a similar phospholipid, such as that in the egg yolk, can also be found. More than half the lipids appear to be bound on albumen. The lipid content is thought to increase during storage at 12°C and 30°C, but not at 4°C. At higher temperatures, TAG and cholesteryl esters — but not phospholipids — move from the egg yolk into the albumen (Sato et al., 1973). Pankey and Stadelman (1969) reported an increase in lipid content at room temperature from 0.0037 to 0.0054% damp weight within 16 weeks.

14.2 EGG YOLK LIPIDS

14.2.1 INTRODUCTION

The fat content of egg yolk is approximately 31.8 to 35.5%; that is, about a third of the whole egg yolk. Lipids occur in different particles (granules, LDL-micelles) and are almost completely associated with proteins. An overview is provided by Ternes (2001). About 70% of the dry mass consists of lipids. Differences in the lipid composition are not only genetically determined, but also occur due to the animals' age and diet. The contents of long-chain (C_{20} and C_{22}) polyunsaturated FA (PUFA) of n-6 and n-3 were 20 to 25% higher in the lipids of egg yolk from younger hens (Nielsen, 1998) (Table 14.1).

At 56 to 64%, the main components are TAG, while the phospholipids represent 21 to 31% in the lipids (Table 14.2). Mono- and diacylglycerols can be found in concentrations of 1.5 to 2.2% in the lipid fraction. The content of cholesterol is about 1.6% of dry matter (Littmann-Nienstedt, 1996).

Ushakova et al. (1979) examined the hydrocarbon content of egg yolk, finding 15 to 30 mg alkanes and cycloalkanes per kilogram egg yolk, 1 to 3 mg/kg n-alkanes, 15 to 17 mg/kg squalenes, and about 1 mg monocyclic arenes/kg egg yolk. Table 14.2 shows the FA composition of whole lipids and several fractions.

14.2.2 TRIACYLGLYCEROLS OF EGG YOLK

The TAG composition of egg yolk and the FA composition are influenced by diet. For example, with a basal diet (Gornall et al., 1970), the C_{52} TAG are the main components at 58.4%, whereas C_{50} and C_{54} TAG are present in nearly equal amounts (19.5 and 17.3%, respectively). C_{48} and C_{46} TAG occur as minor components at 4.8 and 2.0%, respectively (see also Tables 14.11, 14.12, and 14.13A,B). In their FA composition, the TAG correspond to the hen's depot fat. A comparison between the FA composition of egg yolk lipids and TAG lipids is shown in Table 14.2.

The TAG show higher proportions of palmitoleic and oleic acids. The FA composition of TAG in the high-density lipoprotein (HDL) fraction in the granules and the low-density lipoprotein (LDL) fraction of the yolk plasma show similar composition (Tables 14.8 and 14.9).

TABLE 14.1
Fatty Acid Composition of Whole Lipids and Several Fractions in Yolk Lipids
 (% weight)

Fatty Acids	Whole Lipids (1)	Triacyl-glycerols (1)	Mono- and diacylglycerols (2)	Phospholipids (3)	Cholesteryl Esters (4)
C _{14:0}	0.4	0.5	1.1–2.5	—	1.8
C _{16:0}	23.4	23.8	10–20	26.6	—
C _{16:1}	3.4	4.0		1.3	—
C _{17:0}	0.4	0.4			
C _{18:0}	10.2	7.6	2.8	14.8	2.8
C _{18:1}	44.5	49.4	44–61	25.7	38.9
C _{18:2}	12.3	11.2	11–24	18.6	32.7
C _{18:3}	0.6	0.6	traces	0.2	5.0
C _{20:0}	traces	—			
C _{20:1}	0.6	0.6		—	
C _{20:2}	0.1	0.1		0.2	
C _{20:3}	0.2	—			
C _{20:4}	2.5	0.4		6.8	
C _{20:?}	traces	3.5			
C _{22:0}	traces	—			
C _{22:1}	2.5	traces			
C _{22:4}	traces	0.4			
C _{22:5}	0.3	0.1		1.3	
C _{22:6}	0.6	traces		2.2	
C _{24:0}	traces				
C _{24:4}	0.1	0.1	—		
C _{26:1}	0.5	0.1	—		

(1) Kaufmann and Mankel, 1967; (2) Holopainen, 1972; (3) Burghelle-Mayeur et al., 1989; (4) Swaczyna and Montag, 1984

TABLE 14.2
Composition of Lipids in Egg
Yolk in %

Lipids	Holopainen (1972)
Hydrocarbons	0.3
Triacylglycerols	56.1
Mono-, diacylglycerols	6.7
Free fatty acids	0.7
Cholesteryl esters	0.1
Cholesterol	6.0
Phospholipids	30.8

14.2.3 MONO- AND DIACYLGLYCEROLS OF EGG YOLK LIPIDS

Mono- and diacylglycerols are present in egg yolk lipids at 1.5 to 2.2% (Evans et al., 1967). Holopainen (1972) cited contents of 6.7% but this seems to be too high. Mono- and diacylglycerols primarily occur in the HDL fraction of the granules (5.8 to 8.5% in the lipids of the HDL fraction). In the lipids of the LDL fraction from plasma, only low concentrations of 0.4 to 0.7% were found (Tsutsui and Obara, 1979); but more than three fourths of the lipids can be found in the LDL fraction, giving a total concentration of 2% in the lipids.

The FA composition takes an intermediate position between the TAG and the polar lipids, the diacylglycerols correspond somewhat more to the TAG, whereas the monoacylglycerols correspond to the polar lipids (Acker and Ternes, 1994).

14.2.4 PHOSPHOLIPIDS

The main component of the phospholipids is the phosphatidylcholine fraction (PC) at 60 to 73%, followed by the phosphatidylethanolamine fraction (PE) at approximately 15 to 26%. The proportion of sphingomyelins is 2.5 to 4.8%. Phosphatidylinositols and other lipids such as glycolipids occur only in trace quantities (Table 14.3).

In careful examinations, Rhodes and Lea (1957) obtained the composition of phospholipids. Here, for the first time, lysophospholipids were shown to be natural components of egg lipids and not artifacts produced during sample preparation, as the authors explicitly point out. The PC fraction contains 0.9% plasmalogens; the cephalin fraction contains 0.2% phosphatidylserines. Table 14.3 gives an overview of the composition of phospholipids in egg yolk. There is high agreement between the results of all individual authors. Only the results of Holopainen (1972) for sphingomyelins appear to be too high.

A comparison of the FA spectrum of TAG from the whole egg yolk with the phospholipid fraction shows that the concentration of stearic acid is reduced, whereas the proportion of more highly unsaturated FA in the phospholipid fraction increases markedly. Oleic acid (20 to 27% in the phospholipid fraction) is present only half as frequently as in the TAG. The concentration of palmitic acid (approximately

TABLE 14.3
Composition of Phospholipids
in Egg Yolk in %

Phospholipids	
Phosphatidyl cholines	66.0
Phosphatidyl ethanolamines	18.4
Lysophosphatidyl cholines	4.1
Lysophosphatidyl ethanolamines	—
Sphingomyelins	4.8
Plasmalogen	—
Inositol	0.6

TABLE 14.4
**Fatty Acid Composition of Phosphatidyl-
ethanolamine (PE) Fraction and Phosphatidyl-
choline (PC) Fraction in Yolk (% weight)**

Fatty Acids	PE Fraction	PC Fraction
C16:0	21.9	37.1
C16:1	2.2	3.0
C18:0	30.7	7.2
C18:1	20.5	28.0
C18:2	7.5	14.3
C18:3	0.4	0.5
C20:4 (n-6)	9.2	3.1
C22:5 (n-6)	1.3	0.5
C22:6	5.3	3.0

Data from Navarro, J.G., et al., *J. Sci. Food Agric.*, 23, 1287, 1972.

26.6%) is almost as high as that of oleic acid, with an average of 25.7%. The content of stearic and linoleic acids in the phospholipid fraction (18.6%) is notably higher than that in the TAG fraction. While the palmitoleic acid in the phospholipid fraction occurs at lower levels (average = 1.3%), docosahexaenoic acid (C_{22:6}) occurs in higher concentrations at an average of 2.2%. (Table 14.2)

In contrast to TAG, the proportion of unsaturated FA is higher in the phosphatides. Numerous feeding tests have shown that only the quantity and distribution of unsaturated FA within the phospholipid fraction depend on the quantity of monoenoic FA in chicken feed.

There are significant differences in the FA compositions of PC and PE (Table 14.4). In the PE fraction, the concentrations of stearic acid (C_{18:0}) and unsaturated C₂₀ and C₂₂ FA are considerably higher. In contrast, palmitic acid, oleic acid, and linoleic acid are present with increased frequency in the PC fraction.

This unequal distribution of FA in PE and PC is also present in the single lipoprotein fractions (HDL and LDL) (see Tables 14.8, 14.9, and 14.15).

PE are responsible for the antioxidative properties of commercial preparations known as “egg lecithin,” which consist of whole egg phosphatides. Due to the NH₂ group, they can participate in Maillard reactions, which occur not only during heating (boiling), baking, and frying, but also during storage of dried egg products, and which lead to brown-stained, mostly fluorescent compounds (Lea, 1957).

PE are very sensitive to oxygen and autoxidize faster than PC, a process accompanied by an intense brown discoloration. Studies to reduce the oxidation of the PUFA with dietary supplementation of rosemary extract and tocopherols showed a higher oxidative stability of n-3 FA (Galobart et al., 2001; Cherian et al., 1996).

The egg lipid sample, which initially seems to have a simple structure, appears more complex when modern chromatographic separation methods are used to determine sphingolipids and glycolipids, which occur in small amounts (Table 14.5). In

TABLE 14.5

Fatty Acid Composition of Sphingomyelins, Lysophosphatidyl Cholines, Gangliosides, Cerebrosides, and Ceramides in Yolk (% weight)

Fatty Acids	Sphingomyelins (1)		Lysophosphatidyl Cholines (1)		Ganglio- sides (2)	Cerebro- sides (3)	Hydroxy Acids	Ceramides (4)
	Fed with Basal Diet	Fed with	Fed with Basal Diet	Fed with				
		Fish Oil 1.64%		Fish Oil 1.64%				
C _{12:0}	—	—	—	—	0.3	—	—	—
C _{14:0}	—	—	—	—	1.4	—	—	0.08
C _{15:0}	—	—	—	—	0.5	—	—	0.05
C _{16:0}	52.0	23.7	20.6	24.4	26.6	9.6	—	3.54
C _{16:1}	—	3.9	—	6.2	2.3	0.4	—	0.43
C _{17:0}	—	—	—	—	5.7	—	—	0.05
C _{18:0}	—	11.1	6.5	23.6	9.2	2.5	3.5	2.01
C _{18:1}	32.1	21.8	29.6	30.6	22.9	1.8	—	2.55
C _{18:2}	15.2	3.1	30.3	4.7	1.8	—	—	0.59
C _{18:3}	—	1.6	3.2	1.2	—	—	—	—
C _{19:0}	—	—	—	—	—	0.3	—	—
C _{20:0}	—	—	—	—	0.2	0.8	0.6	1.18
C _{20:2}	—	34.8	—	—	—	—	—	—
C _{20:3}	—	—	—	1.2	—	—	—	—
C _{20:4}	—	—	9.8	3.9	—	—	—	—
C _{21:0}	—	—	—	—	—	0.4	—	0.77
C _{22:0}	—	—	—	—	28.3	7.2	19.3	22.73
C _{22:4}	—	—	—	1.2	—	—	—	—
C _{23:0}	—	—	—	—	0.4	3.7	12.2	15.05
C _{24:0}	—	—	—	—	0.4	15.7	22.0	35.66
C _{24:1}	—	—	—	—	0.3	—	—	13.46
C _{26:0}	—	—	—	3.1	—	—	—	1.37

(1) Navarro et al., 1972; (2) Keenan and Berridge, 1973; (3) Momma et al., 1972; (4) Fujino and Momma, 1971.

this way, Fujino et al. (1971) shed some light on the jumble of complex lipid groups. They detected sphingomyelins with 11 different FA (mainly lignoceric, stearic, and behenic acids), cerebrosides with 14 different FA — mainly hydroxy FA — as well as glucose and galactose, and ceramides with 16 different FA (mainly lignoceric acid). An essential N-containing segment in this case is sphingosine, in addition to small amounts of dihydrosphingosine. Apart from this, very low concentrations of gangliosides (glycosphingolipids containing sialic acid) have been found (Keenan and Berridge, 1973). Tests reveal that mainly monosialosyl-N-tetraglycosylceramid with 14 different FA C_{16:0}, C_{18:0}, C_{18:1}, and C_{22:0} are available. Glucose, galactose, hexosamine, and sialic acid are present in proportions of 1.0:1.25:1.95:1.19, respectively. In contrast to ceramides as tested by Fujino and Momma (1971), only small amounts of C_{23:0}, C_{24:0}, and C_{24:1} occur in the gangliosides, but no hydroxy FA. The carbohydrates

glucose, galactose, and hexosamine are present, as is sialic acid. Even these small amounts contribute to the emulsifying characteristics of egg yolk. As early as 1957, Carter et al., (1957) noted the occurrence of plasmalogens in egg (as alkali stable ether lipids), and Lea (1957) found 0.9% plasmalogens in the PC fraction. Do and Ramachandran (1980) characterized those connections in more detail, identifying the 1-*O*-alkyl-*sn*-glycero-3-phosphoethanolamine as the most important ether lipid, apart from a very small amount of 1-*O*-alkyl-*sn*-glycero-3-phosphocholine.

Moschidis et al. (1984) found small amounts of phosphonolipids in the egg yolk. The comparison of the FA composition of minor components shows that in sphingomyelins the amount of palmitic acid is twice that in the egg yolk lipids, whereas there is almost 40% less oleic acid. Linoleic acid at 30% is found at concentrations about three times higher in lysophosphatidylcholines than in egg yolk lipids, and twice as much as in the PC fraction. At 28%, docosanoic acid (C_{20:0} = behenic acid) is present in considerable amounts in gangliosides, as opposed to lower concentrations of oleic and linoleic acids in comparison with the FA spectrum of egg yolk lipids. Momma et al. (1972) also found a high proportion of approximately 67% hydroxy FA in the cerebroside. Many of the hydroxy FA have a chiral center. In any case, Momma et al. (1972) determined a relatively high concentration (15.7%) of tetracosanoic acid (C_{24:0} = lignoceric acid), while smaller concentrations of the main FA of the egg yolk lipids were found in the cerebroside. Table 14.5 shows an overview of the FA spectrum of the minor components.

14.2.5 FATTY ACID POSITION IN THE GLYCEROL MOLECULE

In the TAG molecule, a chiral center develops when both primary hydroxy groups of the glycerol are esterified with two different FA, so that — owing to the position of the FA — a distinction can be made between *sn*-1, *sn*-2, and *sn*-3. The 1-position in the TAG of egg yolk is primarily occupied by palmitic acid, the 2-position by oleic and linoleic acids, while there are oleic acid and saturated FA at the 3-position (Christie and Moore, 1970). In the PC fraction, the 2-position is occupied up to 87% by unsaturated FA (Privett et al., 1962; Gornall and Kuksis, 1971) (Table 14.6).

TABLE 14.6
Stereospecific Distribution of Fatty Acids in Glycerols of Yolk

Fatty Acids	Phospholipids		Triacylglycerols		
	sn-1	sn-2	sn-1	sn-2	sn-3
C _{16:0}	71.8	2.0	71.5	5.1	6.5
C _{16:1}	—	—	6.0	2.6	5.7
C _{18:0}	18.1	0.5	4.1	1.4	8.7
C _{18:1}	7.3	44.2	14.7	56.0	74.6
C _{18:2}	0.6	27.8	2.3	33.5	3.5
C _{18:3}	—	6.9	0.8	1.2	0.6
C _{20:4}	—	1.5	—	—	—
C _{22:6}	—	13.8	—	—	—

TABLE 14.7
Composition of HDL Fraction of Granules and LDL
Fraction of Egg Yolk Plasma

Proteins	Lipoproteins in %	
	HDL Fraction	LDL Fraction
	72.2	14.4
Lipids	27.3	85.6
Neutral lipids	ca.14	64.1
Triacylglycerols	91	80
Mono- and diacylglycerols	8.5	0.7
Sterols	9.2 ^a	5.4 ^a
Steryl esters	1.7	1.6
Hydrocarbons	0.6	0.6
Phospholipids	ca.13.3	21.5
Phosphatidylcholines	85.7	78
Phosphatidylethanolamines	9	15.8
Lysophosphatidyl cholines	5.3	6.0

^a The concentration is lower in the paper of Gornall and Kuksis (1971).

Data from Tsutsui, T. and Obara, T., *Nippon Shokuhin Kogyo Gakkaishi*, 26, 81, 1979.

14.2.6 THE LIPID COMPONENTS OF LIPOPROTEINS

Lipid components in the lipoproteins of the egg yolk differ markedly (Table 14.7). Whereas in the HDL fraction of the granules the lipids constitute 22 to 27% of the dry matter, the LDL fraction constitutes 85% of the plasma (dry matter). Phospholipids can be found in higher concentrations (approximately 50%) in the HDL fraction than in the LDL fraction of the plasma (approximately 20%). Cholesterol and cholesteryl esters are present, at approximately 5% in the HDL fraction and 4% in the LDL fraction of plasma (Gornall and Kuksis, 1971).

By ultracentrifugation ($d < 1.01$ and $d < 1.006$), the VLDL fraction (Evans et al., 1977; Burghelle-Mayeur et al., 1989) can be separated from the LDL fraction. The VLDL fraction contains approximately $92.1 \pm 2.7\%$ of the lipoproteins of the plasma and basically corresponds to the LDL fraction reported by other authors (cf. Table 14.9). The VLDL fraction contains 87% lipids, of which 68% are TAG, 26% phospholipids, and 6% cholesterol and cholesteryl esters (Evans et al., 1977). Burghelle-Mayeur et al. (1989) give similar values for cholesterol, but approximately 10% of the total cholesterol occurs as cholesteryl esters.

There is no significant difference between the FA distribution of TAG in the HDL and LDL fractions (Tsutsui and Obara, 1979; cf. Tables 14.8 and 14.9). When comparing the FA spectrum of egg yolk lipids with the FA spectra of TAG in the HDL and LDL fractions, it can be established that oleic acid is present in 10% higher concentration. In the TAG fraction, the PC and PE fractions differ signifi-

TABLE 14.8

Fatty Acid Composition of Triacylglycerols, Phosphatidylcholines, and Phosphatidylethanolamines in the HDL Fraction of Egg Yolk Granules (% weight)

Fatty Acids	Fraction d > 1.3 (1)	HDL	Triacylglycerols		Phosphatidylcholines		Phosphatidyl-ethanolamines	
			(2)	(3)	(2)	(3)	(2)	(3)
C _{14:0}	—	—	0.3–0.5	0.4	0.3	—	0.2	—
C _{14:1}	—	—	—	—	—	—	—	—
C _{16:0}	27.0	30.0	24.0–25.5	24.2	31.0–35.0	31.6	12.6–18.5	21.8
C _{16:1}	3.2	3.7	4.4–5.9	4.5	1.3–2.7	2.2	0.4–1.4	0.8
C _{18:0}	14.5	12.8	5.9–7.3	7.8	13.5–16.4	16.0	28.5–32.3	41.2
C _{18:1}	40.1	47.6	45.0–50.0	51.5	26.0–44.0	29.6	20.0–30.9	26.0
C _{18:2}	15.2	6.0	5.0–10.0	11.6	6.4–20.1	17.9	5.8–17.3	8.6
C _{20:4}	—	—	—	—	3.4–3.9	2.8	12.2	0.4
C _{18:3}	—	—	—	—	—	—	—	—
C _{20:0}	—	—	—	—	—	—	—	0.7

(1) Evans et al., 1977; (2) Pankey and Stadelman, 1969; (3) Tsutsui and Obara, 1979.

TABLE 14.9

Fatty Acid Composition of VLDL and LDL Fraction, Triacylglycerols, Phosphatidylcholines, and Phosphatidylethanolamines in the LDL Fraction of Egg Yolk Plasma (% weight)

Fatty Acids	Fraction VLDL/LDL		Triacylglycerols			Phosphatidylcholines		Phosphatidylethanolamines	
	(1)	(1)	(2)	(3)	(4)	(2)	(3)	(2)	(3)
C _{14:0}	—	—	0.5	0.4	—	0.3	—	—	—
C _{14:1}	—	—	0.4	—	—	—	—	—	—
C _{16:0}	24.6	25.2	23.0–24.7	24.0	22.3	3.2	32.4	5.0–20.1	21.6
C _{16:1}	3.4	3.5	3.8–6.2	4.6	3.8	1.3–2.1	2.2	0.3–1.1	0.9
C _{18:0}	10.3	11.4	5.2	7.7	6.6	13.1–18.8	16.4	25.7–32.0	40.5
C _{18:1}	48.1	46.6	44.4–57.9	51.6	50.4	24.0–42.3	30.3	24.5–30.0	23.9
C _{18:2}	13.1	13.4	5.4–10.0	11.7	14.6	7.4–12.3	15.2	7.9–11.0	9.5
C _{18:3}	—	—	—	—	0.6	—	—	—	—
C _{20:0}	—	—	—	—	—	—	—	—	1.1
C _{20:4}	—	—	—	—	0.2	2.2–2.8	2.9	12.7–15.9	2.4

(1) Evans et al., 1977; (2) Pankey and Stadelman, 1969; (3) Tsutsui and Obara, 1979; (4) Burghelle-Mayeur, 1988.

cantly. Palmitic acid in the PC fraction is present in higher concentrations, whereas TAG concentrations in the PE fraction are correspondingly lower. On the other hand, the stearic acid in the PE fraction is found in higher concentrations. Palmitoleic acid mainly occurs in the TAG fraction and, in lesser quantities in the PC fraction of HDL and LDL lipids. In the PE fraction of both proteins, the palmitoleic acid content is low. Arachidonic acid is to be found almost exclusively in the phospholipid fraction. In 1969, Pankey and Stadelman found an extremely high arachidonic acid content in the PE fraction, but Tsutsui and Obara (1979) could not confirm this.

14.2.7 INFLUENCE OF DIET ON LIPID COMPOSITION

Dietary fat supplements are frequently used to increase the energy content in the hens' ration. Increased dietary fat does not lead to an increase in the fat content in the egg yolk, but the FA and TAG spectra in egg yolk lipids do change in relation to the fat composition of the feed. There is nearly always a connection between diet and the egg yolk FA spectrum, but the amounts of FA do not completely correspond.

The digested fat will be absorbed as FA and monoacylglycerols through the intestinal epithelium and esterified back to TAG. The TAG produced in the hens' bloodstreams are associated with lipoproteins. The lipoprotein and TAG synthesis occurs *de novo* in the liver and is, among other things, necessary for the formation of egg yolk lipid in the ovaries. Due to this synthesis, the FA distributions in the 1-, 2-, and 3-positions in the TAG are similar in the liver and egg yolk (Hirata et al., 1987). Hens, like many other vertebrates, are not able to synthesize PUFA, especially linoleic acid, from the "non-fats" in the feed (such as carbohydrates and proteins). This explains the conspicuously high proportion of saturated and monoenic FA (such as C_{16:0}, C_{18:0}, C_{18:1}) in the egg yolk fat after a low-fat diet (Table 14.10A,B).

When feed contains higher quantities of PUFA, as in fish or algae oil supplements, the content of linoleic acid and other polyunsaturated FA increases significantly. Even the linoleic acid content of the feed is involved in the amount of unsaturated FA in the egg yolk. Supplements of oils containing linoleic acid, such as safflower, flax, soybean, sunflower, and corn (maize) oil, lead to high enrichment of this acid in the egg lipids (Biedermann et al., 1971).

According to Sim et al. (1972), there is a marked increase of oleic acid content in the egg yolk after supplements of sunflower oil in the feed. The increase of oleic acid is higher than in other feed mixtures with rape oil, soybean oil, or beef tallow. It is remarkable that with other diets low in linoleic acid, a reduction in linoleic acid content in the egg yolk does not conform to the decrease in linoleic acid in the feed. The living organism can produce linoleic acid from certain precursors (e.g., *cis*-2-octenoic acid), where the synthesis required for chain augmentation must be repeated five times for the production of a double bond. Although linoleic acid and linolenic acid are precursors for the production of arachidonic acid (C_{20:4}), docosapentaenoic acid (C_{22:5}), eicosapentaenoic acid (C_{20:5}) (EPA), and docosahexaenoic acid (C_{22:6}) (DHA). According to Reiser (1950), hens can produce eicosatrienoic acid (C_{20:3}).

In contrast to other investigators, Chen et al. (1965) were unable to detect an increase of linoleic acid with a dietary supplement of 10% flax oil or cottonseed oil. The discrepancy can be explained by the fact that at 44% the content of linoleic

TABLE 14.10A**Distribution of Fatty Acids in Dietary Fat and Yolk Lipids Depending on Dietary Fat (% weight)**

Fatty Acids	Fish Oil (1)		Soybean Oil		Coconut Oil (2)		Sunflower Oil (2)	
	a	b (3%)	a	b (12%)	a	b (10%)	a	b (10%)
C _{10:0}	—	—	—	—	7.9	traces	—	—
C _{12:0}	—	—	—	—	40.0	1.0	1.8	traces
C _{14:0}	—	—	0.1	0.2	18.5	7.5	1.8	0.3
C _{16:0}	23.0	26.8	11.4	24.0	12.5	25.5	7.5	20.8
C _{16:1}	7.9	4.9	traces	1.6	—	4.6	—	1.8
C _{17:0}	—	—	0.1	0.4	—	—	—	—
C _{18:0}	7.0	18.2	5.0	8.6	3.6	8.1	4.3	9.2
C _{18:1}	15.8	31.7	24.5	38.1	10.9	39.3	26.7	35.9
C _{18:2}	4.9	11.3	50.3	33.1	4.9	9.0	58.6	27.4
C _{18:3}	4.4	0.4	7.5	1.4	0.3	0.2	0.9	0.2
C _{20:4}	4.9	1.3	—	1.2	—	—	—	—
C _{20:5}	9.5	0.4	—	—	—	—	—	—
C _{20:1}	—	—	0.6	0.2	—	—	—	—
C _{21:1}	—	—	—	—	—	—	—	—
C _{22:4}	6.2	0.2	—	—	—	—	—	—
C _{22:5}	7.5	0.5	—	—	—	—	—	—
C _{22:6}	8.0	4.1	—	0.5	—	—	—	—
Others	—	0.2	0.5	0.8	—	2.0	—	4.3

^a Distribution of fatty acids in dietary fat.^b Distribution of fatty acids in yolk lipids (percentage of dietary fat in the diet).

(1) Navarro et al., 1972; (2) Biedermann et al., 1971.

acid in the animals' diet was already high, so that even in the eggs of control animals, the concentration in the yolk was high at 15%. Obviously, there is an upper limit for the interchange of FA with linoleic acid. Kaufmann and Mankel (1967) thought that a selective influence might play a role during the biosynthesis of egg lipids in contrast to the depot fat of laying hens.

While dietary saturated FA have only a slight effect on the FA in the samples, *trans*-FA can have a considerable influence. With a diet containing 5.5 g *trans*-FA daily in the form of hardened fats, after 16 days the *trans*-FA increased up to 10% at the expense of some proportion of oleic acid in the neutral lipids. Less *trans*-FA are transferred into the phospholipid fraction than into the TAG fraction (Kaufmann and Mankel, 1967). From rapeseed oil (which contains erucic acid), the erucic acid is transferred into the egg (Biedermann et al., 1971). In addition to the increase in C_{18:2} acid in egg lipids, the physical characteristics change, thus increasing the tendency toward oxidation.

Naber and Biggert (1989) showed in their feeding tests that, as the fat content in the feed increases, there is a reduction in lipogenesis in the liver; and the fat is stored in the liver and egg yolk with the same FA as in the dietary fat.

TABLE 14.10B**Distribution of Fatty Acids in Triacylglycerols of Dietary Fat and Egg Yolk**

Fatty Acids	Soybean Oil (4)		Coconut Oil (4)		Lard (4)		Tallow (4)		Safflower Oil (5)	
	a	b (10%)	a	b (10%)	a	b (10%)	a	b (10%)	a	b (30%)
C _{8:0}	—	—	10.3	—	—	—	—	—	—	—
C _{10:0}	—	—	7.4	—	—	—	—	—	—	—
C _{12:0}	—	0.3	49.1	2.2	—	tr.	—	0.4	—	—
C _{14:0}	tr.	1.8	17.4	10.0	1.8	0.7	3.7	1.2	—	0.3
C _{14:1}	—	0.2	—	3.5	0.2	tr.	0.1	0.2	—	0.1
C _{16:0}	11.5	22.1	7.7	27.9	25.9	25.2	25.7	27.1	7.0	19.9
C _{16:1}	—	2.6	—	7.7	3.6	4.7	3.1	4.9	0.0	1.4
C _{17:0}	0.1	0.2	—	0.1	0.4	0.2	1.2	0.5	—	—
C _{17:1}	—	0.2	—	—	0.3	0.3	0.6	0.6	—	—
C _{18:0}	3.7	3.7	22.1	3.7	12.1	3.8	18.0	4.2	3.0	5.1
C _{18:1}	22.2	37.4	4.9	37.8	43.4	55.3	40.7	54.1	13.0	23.6
C _{18:2}	53.6	28.8	1.1	6.4	10.5	9.0	3.6	5.6	76.0	48.7
C _{18:3}	8.6	2.1	—	0.4	0.7	0.1	0.5	0.2	1.0	0.7
C _{20:1}	0.3	0.2	—	0.3	1.0	0.3	0.6	0.3	—	—
C _{20:2}	—	0.2	—	tr.	—	0.1	—	tr.	—	—
C _{20:4}	—	0.3	—	0.1	—	0.3	—	tr.	—	—

^a Distribution of fatty acids in feed fat.

^b Distribution of fatty acids in yolk (percentage of dietary fat in the diet).

tr. = Less than 0.1%.

(4) Hirata et al., 1987; (5) Naber and Biggert, 1989.

Metabolic changes in PUFA such as linoleic acid to less highly saturated acids such as oleic and stearic acids affect the amount of linoleic acid. Dietary fats high in oleic acid should reduce the transformation of stearic and linoleic acids to other FA. The reduction of linoleic acid to palmitic and palmitoleic acids can also be assumed.

Huang et al. (1990) show further connections between dietary and egg yolk fat on closer examination of n-3 FA from menhaden oil. It was demonstrated that the amounts of n-3 FA, EPA, and DHA in egg yolk fat are not proportional to those in the feed, although EPA is metabolized in a high proportion. The quantities of docosapentaenoic acid and DHA in the egg yolk react in inverse proportions to the content in menhaden oil. Differences in the absorption rate of both acids in the intestine are assumed to be responsible.

Linoleic acid is thought to be not only a precursor of arachidonic acid, but also of EPA, DHA, docosapentaenoic acid, and linolenic acid.

Navarro et al. (1972) found that an increase of fish oil in the feed leads to an increase in n-3 FA as compared with the content in the feed, whereas the quantity of n-6 FA was reduced. This corresponds to earlier reports that n-3 FA are preferentially transported into the egg in lipid metabolism.

Caston and Leeson (1990) reported that the feeding of flaxseed increases n-3 FA and slightly increases n-6 FA as well. The more flaxseed oil in the feed, the higher the amounts of linoleic and linolenic acids, as well as EPA and DHA, present in the egg yolk lipids.

Hirata et al., (1987) conducted extensive tests comparing the FA composition of egg yolk after feed supplements of vegetable oil and animal fat. The FA sample of the dietary fat supplement is reflected considerably more clearly in TAG than in polar lipids. Table 14.10B reveals that compared to the supplement of animal fats, the proportion of linoleic acid in the TAG of the egg yolk is highest after a 10% soy oil supplementation, and the proportion of oleic acid the lowest; this also applies to a coconut oil supplementation. Furthermore, after coconut oil supplementation, the TAG contain substantially more myristic, myristoleic, and palmitoleic acids. Because myristoleic acid (C_{14:1}) and palmitoleic acid (C_{16:1}) are not present in coconut oil, but do occur in egg yolk lipids, the production of a double bond in myristic and palmitic acids by fat metabolism can be assumed. Although coconut oil consists of 50% lauric acid (C_{12:0}), only small amounts of this acid can be found in the egg yolk. It has been assumed that hens can use only very small amounts of FA with shorter chains for egg yolk lipid production. Shorter FA in the coconut oil do not contribute to egg yolk FA production. The

TABLE 14.10C
Influence of Dietary Conjugated Linoleic Acid (CLA; 2.5%) on the Fatty Acid Composition of Total Lipids, Triacylglycerols, Phosphatidylcholine (PC), and Phosphatidylethanolamine (PE)

Fatty Acids	Total Lipids	Triacylglycerols	PC	PE
C _{16:0}	24.85	26.2	27.17	16.42
C _{16:1} (n-9)	0.63	1.16	0.22	
C _{16:2} (n-9,12)	0.23	0.69		
C _{16:2} (1) ^a	0.12	0.28		
C _{16:2} (2) ^a	0.13	0.26		
C _{16:2} (3) ^a	0.06	0.08		
C _{17:0}	0.29	0.28	0.25	0.45
C _{18:2} (n-9,12)	19.25	22.45	18.14	15.41
C _{18:1} (n-9)	25.21	33.88	26.45	20.86
C _{18:0}	16.48	10.17	17.60	28.59
CLA (<i>cis</i> -9, <i>trans</i> -11)	2.74	3.17	2.19	2.16
CLA (<i>trans</i> -10, <i>cis</i> -12) 2.54	3.50	1.76	1.50	
CLA (<i>cis</i> -8, <i>trans</i> -10)	0.60	0.57	0.36	0.74
CLA (<i>cis</i> -11, <i>trans</i> -13) 0.94	0.80	0.98	1.32	
C _{20:4} (n-5,8,11,13)	3.91	0.18	2.78	11.34

^a Corresponding to three hexadecadienoic isomer peaks identified by gas chromatograph-mass selective analysis.

Data from Du, M., Ahn, D.U., and Sell, J.L., *Poultry Sci.*, 78, 1639, 1999.

lipid spectrum in the egg yolk lipids differs significantly after supplementation with animal and vegetable fats. The addition of beef tallow or lard supplements results in only minor differences. Numerous reports have confirmed the data of Hirata et al. (1987).

Isomers of conjugated linoleic acids (CLA) have anticancer activity, and immune-enhancing-weight reductions, and antiatherogenic properties. CLA are found in food produced from ruminant animals. The development of CLA-enriched eggs showed a reduction in monoenoic FA and non-CLA PUFA after CLA feeding, and an increase in EPA and DHA. The incorporation rates of different CLA isomers into the whole lipids of egg yolk and TAG, PC, and PE-lipid classes were different. *cis*-9,*trans*-11 and *cis*-10,*trans*-12 CLA were deposited more in TAG, but *cis*-11,*trans*-13 CLA was less. There are large differences in the concentrations of *cis*-8,*trans*-10 CLA in PC and PE (Table 14.10C).

14.2.8 POSITIONS OF FATTY ACIDS IN THE GLYCEROL MOLECULE

Hirata et al. (1987) showed modifications of the TAG spectrum, depending on the feeding fat (Table 14.11). Coconut oil with a high proportion of C₃₂-C₄₂ TAG, which develop due to the high concentration of C_{8:0}, C_{10:0}, and C_{12:0} FA, displaces the TAG composition in favor of low-molecular-weight TAG. A large part of these low-

TABLE 14.11
Composition of Triacylglycerols in Yolk after Feeding Different Fats (% weight)

Number of C-atoms in triacylg.	Soybean oil		Coconut oil		Tallow		Lard	
	a	b	a	b	a	b	a	b
C ₂₈ + C ₃₀	—	—	4.3	—	—	—	—	—
C ₃₂	—	—	13.5	—	—	—	—	—
C ₃₄	—	—	17.3	—	—	—	—	—
C ₃₆	—	—	19.8	—	—	—	—	—
C ₃₈	—	—	17.3	—	—	—	—	—
C ₄₀	—	—	10.7	—	—	—	—	—
C ₄₂	—	0.5	7.5	1.5	—	—	—	—
C ₄₄	—	1.0	4.1	4.2	0.4	—	1.0	—
C ₄₆	—	1.9	2.1	9.5	1.6	0.5	1.9	—
C ₄₈	0.6	4.4	1.6	21.7	7.0	3.9	4.8	1.7
C ₅₀	3.9	9.0	0.9	24.2	19.8	17.5	16.6	12.2
C ₅₂	28.8	56.4	0.7	33.9	48.0	63.4	55.5	67.8
C ₅₄	66.7	26.8	0.2	5.0	23.2	14.9	20.2	18.3

^a Distribution of fatty acids in feed fat.

^b Distribution of fatty acids in yolk.

Hirata, A. et al., *Nippon Shokuhin Kogyo Gakkaishi*, 34(5), 320, 1987.

molecular-weight FA does not reach the egg yolk because they are metabolized in the liver. Feeding of soybean oil, with its high proportion of C₅₂ and C₅₄ TAG, leads to increased C₅₂ and C₅₄ TAG in the egg yolk lipids.

At lower concentrations, linoleic acid in the feed is preferentially transported into the PC fraction. At higher concentrations, linoleic acid is also transported in higher amounts into the TAG and PC fractions (Tables 14.12, 14.13A,B, and 14.14).

TABLE 14.12
Fatty Acid Composition in Triacylglycerols, Phosphatidylcholine (PC)
Fraction, and Phosphatidylethanolamine (PE) Fraction in the HDL and LDL
Fraction Depending on Dietary Fat (% weight)

Fatty Acids	Fatty Acid Composition of Dietary Fat		Triacylglycerols			
	Saff. Oil	Olive Oil	(2)/(3)		HDL Saff. Oil	HDL Olive Oil
			LDL Saff. Oil	LDL Olive Oil		
C _{14:0}	—	—	0.5	0.3	0.5	0.3
C _{16:0}	0.7	11.5	22.8	21.6	23.4	20.8
C _{16:1}	—	1.5	3.4	4.2	3.2	3.8
C _{18:0}	0.3	2.5	6.5	3.6	6.7	4.1
C _{18:1}	18.0	75.5	37.9	63.1	39.0	62.9
C _{18:2}	76.0	7.5	28.9	7.2	27.2	8.2
C _{18:3}	1.0	1.0	—	—	—	—
C _{20:0}	—	0.5	—	—	—	—
C _{20:4}	—	—	—	—	—	—

	PC Fraction				PE Fraction			
	(2)/(3)		HDL Saff. Oil	HDL Olive Oil	(2)/(3)		HDL Saff. Oil	HDL Olive Oil
	LDL Saff. Oil	LDL Olive Oil			LDL Saff. Oil	LDL Olive Oil		
C _{14:0}	—	—	—	—	—	—	—	—
C _{16:0}	29.6	30.2	31.4	31.0	18.6	19.0	16.5	16.8
C _{16:1}	—	—	—	—	—	—	—	—
C _{18:0}	15.5	15.0	17.8	13.4	27.3	25.0	32.5	30.3
C _{18:1}	21.2	38.7	21.5	40.6	17.5	28.5	15.3	27.8
C _{18:2}	28.0	12.3	26.8	10.9	21.5	10.6	20.7	8.4
C _{18:3}	—	—	—	—	—	—	—	—
C _{20:0}	—	—	—	—	—	—	—	—
C _{20:4}	5.6	3.8	3.7	4.1	15.2	16.0	15.0	16.8

(2) Naber and Biggert, 1989; (3) Pankey and Stadelman, 1969.

Hirata et al. (1987) analyzed the position of FA in the TAG depending on dietary fats (Table 14.13A,B). It is clear that preference is also given to the *sn*-2-position for linoleic acid. Cossignani et al. (1994; Table 14.14) show the stereospecific distribution of FA in the PC and PE fractions, in reference to the FA on *sn*-1 and *sn*-2 of lysoPC and lysoPE (Table 14.15). Amate et al. (1999) observed a great difference in the FA composition of lysoPC and lysoPE in comparison to PC and PE fractions.

TABLE 14.13A
Position of Fatty Acids in Triacylglycerols of Yolk Depending on Dietary Fat

Fatty Acids	Triacylglycerols of Coconut Oil (dietary fat)			Triacylglycerols of Yolk (diet with coconut oil)		
	Position	Position	Position	Position	Position	Position
	1 (sn-1)	2 (sn-2)	3 (sn-3)	1 (sn-1)	2 (sn-2)	3 (sn-3)
C _{8:0}	8.3	0.7	21.9	—	—	—
C _{10:0}	6.5	3.4	10.3	—	—	—
C _{12:0}	35.1	80.8	31.4	0.7	2.0	3.9
C _{14:0}	20.4	8.0	23.8	5.8	5.9	18.3
C _{14:1}	—	—	—	0.2	2.9	7.4
C _{16:0}	12.3	1.5	9.3	63.2	9.4	10.1
C _{16:1}	—	—	—	5.1	6.6	11.4
C _{18:0}	3.6	0.6	2.1	3.9	3.7	3.5
C _{18:1}	12.6	3.7	0.6	17.7	52.1	43.6
C _{18:2}	1.3	1.3	0.7	2.3	16.2	0.7
C _{18:3}	—	—	—	—	0.6	0.6
C _{20:1}	—	—	—	0.1	0.3	0.5

Fatty Acids	Triacylglycerols of Soybean Oil (dietary fat)			Triacylglycerols of Yolk (diet with soybean oil)		
	Position	Position	Position	Position	Position	Position
	1 (sn-1)	2 (sn-2)	3 (sn-3)	1 (sn-1)	2 (sn-2)	3 (sn-3)
C _{12:0}	—	—	—	0.7	0.1	0.1
C _{14:0}	—	—	—	1.1	1.8	2.5
C _{16:0}	24.8	6.9	2.8	52.5	9.2	4.6
C _{16:1}	—	—	—	0.7	2.0	5.1
C _{18:0}	9.7	1.4	—	7.4	3.9	—
C _{18:1}	31.2	19.0	16.4	24.0	26.3	61.9
C _{18:2}	32.2	64.8	63.8	12.0	53.9	20.3
C _{18:3}	1.4	7.5	16.6	0.8	2.3	3.2
C _{20:1}	0.3	0.2	—	0.3	0.2	0.1

Data from Hirata, A., et al., *Nippon Shokuhin Kogyo Gakkaishi*, 34(5), 320, 1987.

TABLE 14.13B**Position of Fatty Acids in Triacylglycerols of Yolk Depending on Dietary Fat**

Fatty Acids	Triacylglycerols of Yolk (basal diet)			Triacylglycerols of Yolk (diet with 7% fish oil)		
	Position	Position	Position	Position	Position	Position
	1 (sn-1)	2 (sn-2)	3 (sn-3)	1 (sn-1)	2 (sn-2)	3 (sn-3)
C _{14:0}	0.9	0.2	0.3	1.5	0.5	0.8
C _{16:0}	65.9	2.5	6.7	66.8	3.0	2.8
C _{16:1(n-7)}	3.1		0.3	3.0		
C _{16:1(n-5)}	3.5	1.4	5.4	4.3	2.0	5.9
C _{18:0}	5.8	2.0	8.3	6.2	2.5	11.0
C _{18:1(n-9)}	12.9	42.4	67.7	10.5	40.9	63.8
C _{18:1(n-7)}	1.4	1.2	2.9	1.7	1.7	4.3
C _{18:2(n-6)}	4.2	45.8	7.3	2.8	42.8	5.4
C _{18:3(n-3)}	1.4	1.2	0.6	1.4	1.6	0.3
C _{20:4(n-6)}		0.9	0.1		0.5	0.1
C _{20:5(n-3)}					0.4	
C _{22:5(n-3)}	0.3				1.2	0.2
C _{22:6(n-3)}	0.5				1.9	0.2

Data from Cossignani, L. et al., *Ital. J. Food Sci.*, 3, 293, 1994.

TABLE 14.14

Structural Analysis of Phosphatidylcholine (PC) Fraction and Phosphatidylethanolamine (PE) Fraction in Yolk Depending on Dietary Fat (7% fish oil)

Fatty Acids	PC Fraction (basal diet)		PC Fraction (7% fish oil)		PE Fraction (basal diet)		PE Fraction (7% fish oil)	
	Position		Position		Position		Position	
	1 (sn-1)	2 (sn-2)	1 (sn-1)	2 (sn-2)	1 (sn-1)	2 (sn-2)	1 (sn-1)	2 (sn-2)
C _{14:0}	0.4	0.1	0.6	0.1	0.1	0.2	0.2	0.3
C _{16:0}	64.1	1.1	67.5	2.0	33.6	1.1	40.8	6.9
C _{16:1(n-7)}	0.5		0.4					0.4
C _{16:1(n-5)}	1.5	0.6	1.6	0.7	0.1	0.2	0.2	0.8
C _{18:0}	26.7	0.2	23.9	0.6	59.5	0.7	53.4	2.5
C _{18:1(n-9)}	4.1	43.2	3.6	40.0	5.2	22.7	2.9	24.2
C _{18:1(n-7)}	1.1	0.9	1.0	1.5		0.7	1.1	1.0
C _{18:2(n-6)}	0.8	36.1	0.6	32.3	1.1	22.8	0.5	18.5
C _{18:3(n-3)}		0.3		0.2		0.2		0.4
C _{20:4(n-6)}		8.7		5.5	0.2	27.2		8.3
C _{20:5(n-3)}				1.6				1.7
C _{22:4(n-6)}		0.5		0.3				
C _{22:5(n-6)}		1.3		0.4		3.6		0.5
C _{22:5(n-3)}		0.4		1.1				1.1
C _{22:6(n-3)}		5.7		12.6		17.1		33.4

Data from Cossignani, L. et al., *Ital. J. Food Sci.*, 3, 293, 1994.

TABLE 14.15

Fatty Acid Composition of the sn-1 and sn-2 Position Obtained from the Corresponding Lysophospholipid Fraction (lysophosphatidyl-cholines [lysoPC] and lysophosphatidylethanolamines [lysoPE]) after Phospholipase A₂ Hydrolysis; the Fatty Acid Composition of PC and PE Fraction Are Given for a Comparison (mol%)

	Fatty Acid Composition		Position sn-1 ^a		sn-2 ^b
	PC Fraction	PE Fraction	LysoPC	LysoPE	
C _{16:0}	40.27	23.04	70.49	36.18	9.8
C _{16:1(n-9)}	1.03	0.7	0.34	—	1.64
C _{18:0}	13.11	26.6	26.26	58.62	0.63
C _{18:1(n-9)}	27.1	19.17	2.52	2.88	48.65
C _{18:2(n-6)}	14.75	13.43	0.19	0.77	28.29
C _{18:3(n-3)}	0.26	0.38	0.1	0.36	0.41
C _{20:2(n-6)}	0.25	0.34	—	—	0.43
C _{20:3(n-6)}	0.18	0.42	—	—	0.31
C _{20:4(n-6)}	1.96	9.26	—	0.28	5.5
C _{22:4(n-6)}	—	1.06	—	—	0.25
C _{22:6(n-3)}	1.08	5.6	0.1	0.91	2.89

^a Fatty acid composition of the sn-1-position obtained from the corresponding lysophospholipid fraction (lysoPC and lysoPE) after phospholipase A₂ hydrolysis.

^b Fatty acid composition of the sn-2-position obtained as follows: $[\text{FFA}] = [2 \text{ PL}] - [\text{LysoPL}]$ where PL = total phospholipids.

Data from Amate, L., Ramirez, M., and Gil, A., *Lipids*, 34, 8, 1999.

14.3 CHOLESTEROL

14.3.1 INTRODUCTION

Among the sterols of the egg, which make up approximately 4% of egg lipids, cholesterol predominates. It occurs only in the egg yolk, mainly in a free form. Only 4 to 15% are esterified with FA (Chung et al., 1965). The FA compositions of the cholesteryl esters are shown in [Table 14.2](#).

The cholesterol values in individual eggs differ considerably, but on average the proportions of cholesterol in relation to the dry matter of the egg yolk are relatively constant. Currently, reliable tests indicate a ratio of 1.6% egg yolk to dry matter (Littmann-Nienstedt, 1996). In this ratio, the egg surpasses all other foods, so that a cholesterol content can be used qualitatively and quantitatively as an indicator for the amount of egg supplement in food.

Sterols accompanying cholesterol make up only approximately 4% of the total sterol content. Cholesterol, 7-cholestenol, and campesterol are found most frequently, but β -sitosterol, 24-methylencholesterol, desmosterol, and lanosterol could also be detected in very small amounts (Dresselhaus, 1974; [Table 14.16](#)).

TABLE 14.16
Attendant Sterols of Cholesterol in Egg Yolk

	% Related to Whole Sterols	mg/100 g Dry Matter	
		(1)	(2)
Cholestanol	1.10	26.5	9.7
7-Cholestenol	0.66	15.9	6.3
Campesterol	0.51	12.3	—
β -Sitosterol	0.23	5.5	6.7
24-Methylencholesterol	0.26	6.3	—
7-Dehydrocholesterol	0.32	7.7	6.3
Desmosterol	0.16	3.9	15.1
Lanosterol	0.11	2.7	3.1
Ergosterol	—	—	7.3
Total	3.89	93.8	64.5

(1) Dresselhaus, 1974; (2) Tu et al., 1970).

Tu et al. (1970) identified the same accompanying sterols as 1.8% of the total sterols. Estrogen steroids or other steroid sterols could not be detected. β -Sitosterol, which is found most frequently, obviously derives from vegetable feed.

In view of the unfavorable assessment of an increased cholesterol supply in the feed, tests were carried out to clarify the connection between the composition of the feed and the cholesterol content of the egg, and to seek possibilities for reducing the cholesterol content by means of feed supplements.

Supplementation with cholesterol increased the proportions of esterified cholesterol more than the amount of free cholesterol. The amount of esterified cholesterol increased up to 15% (Chung et al., 1965). However, at higher supplements of highly unsaturated oils (e.g., 30% maize oil) or surface-active agents such as Tween 80 or PC (each 5%), the amount of cholesterol in the egg yolk increased — obviously due to the improved absorption of the feed cholesterol (Chung et al., 1965).

The cholesterol content per gram decreased proportionally with increasing age of the hens, while the proportion of yolk increased. Since 1935, the laying capacity of a hen has increased from 120 to 257 eggs per year (Westermann, 1991). Due to this increased laying capacity, the cholesterol content has decreased by about 25% since the 1950s. Genetic selection programs (Hargis, 1988) achieved a reduction of only 7%, and other authors obtained results of 5 to 7%.

Westermann (1991) has given an overview of cholesterol contents and different reductive factors. More promising are procedures by which the cholesterol content can be reduced by approximately 60% by supercritical CO₂-extraction (Froning et al., 1990). This technical procedure is only successful with egg yolk powder, where the lipid content decreases by about 30%, and primarily in TAG. Due to this process, phospholipid contents are concentrated. With this method, it is possible also to remove neutral lipids. The cholesterol contents can be reduced by means of extraction procedures with different solvent mixtures, adsorption procedures, and by inclusion

complexes in cyclodextrins (Awad et al., 1997). Aihara et al. (1988) tested the decomposition of cholesterol by supplementing extracellular enzymes of “*Rhodococcus equi* no. 23.” Here, the authors achieved a decomposition of up to 90% at an incubation period of 48 h. In combination with cholesterol-oxidase and phospholipase C, the results were optimal; the phospholipase C was necessary to attack phospholipids at the surface of the LDL micelle. Oxidation products develop such as 4-cholesten-3-on, 1,4-androstadien-3,17-dion, and 4-androsten-3,17-dion. 4-Cholesten-3-on partially breaks down during the incubation period.

14.3.2 AUTOXIDATION OF CHOLESTEROL

During processing, not only are the unsaturated FA and the phospholipids of the egg influenced by autoxidation, but also the cholesterol. Apart from oxygen, the influences of light and a photosensitizer, a long storage period, and the addition of H_2O_2 are important. Riboflavin can serve as a photosensitizer; riboflavin occurs in substantial amounts in the egg, both in dried egg yolk and in pasta (Acker and Greve, 1963). However, under extreme conditions such as storage in the sunlight, there is formation of a cholesterol hydroperoxide and a hydroxy compound derived from it (7-hydroxycholesterol). Chicoye et al. (1968) confirmed these results and indicated the presence of additional oxidation products.

Oxidation products from cholesterol are of physiological significance. For example, 5 α -cholestan-5,6- α -epoxy-3- β -ol can be found in the skin after UV radiation and has been suggested to be involved in the development of skin cancer (Black and Chan, 1976). The compound is also thought to be involved in arteriosclerotic deposits (Benditt, 1977). *In vitro* studies show that 5 α -cholestan-5,6- α -epoxy-3- β -ol associates with DNA (Blackburn et al., 1979). 7 β -Hydroxycholesterol, and 7-ketocholesterol also have toxic properties. Smith (1981, 1987) gives an overview of the literature and reports between 60 and 80 identified oxidation products. The oxidized cholesterols are subdivided into primary and secondary product groups. Primary products are those that are oxidized at C₇ and in side chains from C₂₀ to C₂₅. Secondary oxycholesterols are mainly epoxides on C₅ and C₆, as well as cholestantriols. In some publications, oxidation products of the a-ring can be found on C₃ and C₄. [Figure 14.1](#) shows the corresponding structures. Even after 18 months of storage at 4°C, no cholesterol oxides could be detected by Pike and Peng (1985) in the egg shells.

In fresh eggs, only 7 α - and 7-ketocholesterol occur in small amounts (Nourooz-Zadeh, 1990). Both compounds can be found in the metabolism of cholesterol; they occur naturally in the egg yolk in concentrations of approximately 0.6 mg/kg.

During thermic processing (e.g., spray-drying for the production of egg yolk powder), but scarcely during preparation of egg dishes, oxidation products do develop, with primary oxidation products developing first. Up to 10% of the cholesterol can be oxidized during drying and storage under unfavorable conditions. Freezer storage (−20°C) leads to an increase in secondary oxidation products. Analysis of these compounds leads in part to faulty results, especially when cholesterol oxides are determined after saponification. In three spray-dried egg products, Fischer et al., (1985) found the amounts of oxycholesterol cited in [Table 14.17](#). In addition

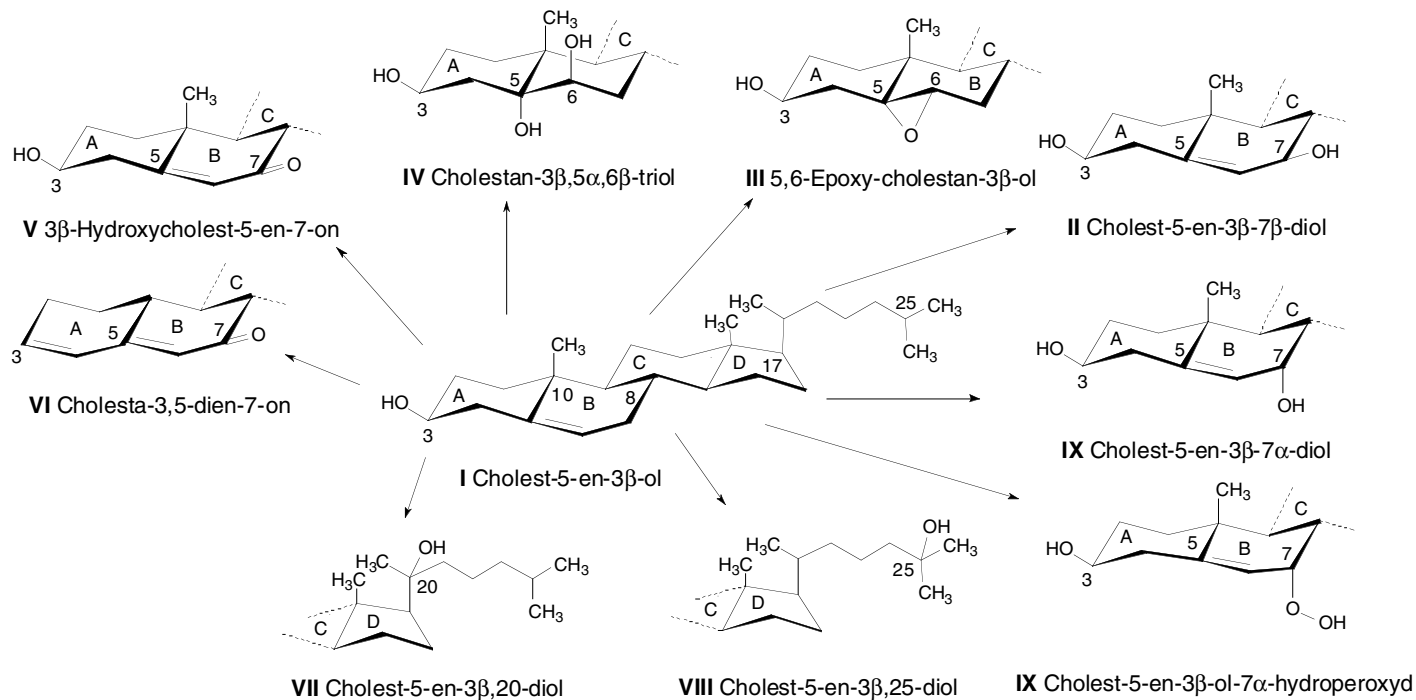


FIGURE 14.1 Cholesterol and oxidized cholesterol.

TABLE 14.17
Amounts of Oxycholesterols [μg/g] in Spray
Dried Egg Products

	Whole Egg Powder	Yolk Powder
Cholest-5-en-3β,7α-diol	8.6 ; 18.8 20.2	7.9 ; 8.3 13.0
Cholest-5-en-3β,7β-diol	7.6 ; 21.3 24.6	5.6 ; 7.1 14.0
5,6-Epoxy-cholestan-3β-ol (α- and β-Epoxyd)	5.8 ; 20.2 24.9	4.3 ; 5.9 8.7

Data from Fischer, K.-H., Laskawy, G., and Grosch, W., *Z. Lebensmittel. Unters. Forsch.*, 181, 14, 1985.

TABLE 14.18
Oxidized Cholesterol Derivates after Direct and Indirect
Heating

Sterols	Mixed Eggs (dried)	
	Direct Heating (μg/g)	Indirect Heating (μg/g)
7α-Hydroxycholesterol	7.0	1.8
7β-Hydroxycholesterol	18.5	1.5
Cholesterol-5α,6α-epoxyd	50.0	21.5
Cholesterol-5β,6β-epoxyd	37.4	1.9
25-Hydroxycholesterol	5.1	1.4
7-Ketocholesterol	37.0	2.0
5α-Cholestane-3β,5,6β-triol	13.0	11.6

Data from Missler, S.R., Wasilchuk, B.A., and Merritt, C. Jr., *J. Food Sci.*, 50, 595, 646, 1985.

TABLE 14.19**Oxidized Cholesterols in Fresh Yolk, and in Yolk Powder, Depending on Storage Time**

Product	Storage Time	Oxidized Cholesterols (in $\mu\text{g/g}$ egg lipid)							
		5 α ,6 α -epoxy	5 β ,6 β -epoxy	7-keto	7 α -hydroxy	7 β -hydroxy	20 α -hydroxy	25-hydroxy	5,6-dihydroxy
Fresh yolk	0	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
Yolk powder	6 month	1.3	6.5	3.5	2.2	2.5	0.8	n.n.	n.n.
Yolk powder	12 month	2.5	12.0	2.9	8.9	9.4	0.7	n.n.	tr.
Yolk powder ^a	8 years	9.4	dp.	5.7	27.5	46.8	6.6	10.4	27.6

^a partial unknown storage conditions.

n.n. = less than 0.2 ppm (not detectable); tr. = traces; dp. = disturbing peak, not evaluated.

Data from Nourooz-Zadeh, J. and Appelqvist, L.A., Cholesterol oxides in Swedish foods and food ingredients: fresh eggs and dehydrated egg products, *J. Food Sci.*, 52, 57, 1987.

to the three main products, traces of cholestan-3 β ,5 α ,6 β -triol and cholest-5-en-3 β ,25-diol have been identified in some samples.

14.3.3 TECHNOLOGICAL DEPENDENCIES FOR THE FORMATION OF OXIDATION PRODUCTS OF CHOLESTEROL

These compounds develop spontaneously in the presence of oxygen, light, and high temperature. H₂O₂, which is partly added to the enzymatic decarbohydration process before drying, also represents a potential type of oxidation, so that mainly epoxides develop.

Antioxidants such as propylgallate, BHA, and BHT reduce the development of 5,6-epoxides during the spray-drying of egg yolk powder. During storage, the contents increase, for example, from 0 to 20 ppm after 7 months of storage. When H₂O₂ was added, a rapid increase in cholesterol-5,6-epoxides was noted (from 16 to 123 ppm). The amounts of oxidized cholesterol can be reduced significantly by the addition of antioxidants (Morgan and Armstrong, 1987; Li et al., 1996; Huber et al., 1995).

The amounts of cholesterol oxides in egg powder from eggs dried with direct procedures (i.e., by the use of burning gas) are obviously higher than from drying with indirect heating. Nitric oxides, which develop during the burning of organic material, are thought to stimulate the oxidation of the cholesterol (Missler et al., 1985), and N₂O₄ is proposed as the chief initiator for the oxidation process (Table 14.18).

Table 14.19 shows the presence of oxidized cholesterols as depending on the storage period. There is a significant increase in oxidation products in dried egg yolk during storage (Nourooz-Zadeh and Appelqvist, 1987).

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15 Modified Lipids and Fat Mimetics

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CONTENTS

- 15.1 Introduction
- 15.2 Naturally Modified Fats and Their Effects on Food Quality
 - 15.2.1 Modified Vegetable Oils
 - 15.2.2 Modified Fat by Feeding of Animals
- 15.3 Lipids Modified by Processing and Their Effects on Food Quality
 - 15.3.1 Blending
 - 15.3.2 Fractionation
 - 15.3.3 Hydrogenation
 - 15.3.4 Interesterification
- 15.4 Biotechnological Modification of Fat
 - 15.4.1 Enzymatic Modification of Fats and Oils
 - 15.4.2 Lipids from Genetically Modified Organisms
 - 15.4.3 Alternative Fats and Oils
- 15.5 Fat Mimetics and Their Effects on Food Quality
- References

15.1 INTRODUCTION

Widespread and improved fat modifications have produced a wider assortment of fat products, reduced production costs, and, most important, enabled nutritional requirements to be met. Nutrition experts generally recommend limiting the consumption of saturated fatty acids (FA) and *trans* isomers and increasing the consumption of unsaturated FA instead (Hammond, 2000a). From a nutritional point of view, vegetable oils are the healthiest. This does not mean that they meet all technological requirements (e.g., oxidative stability, melting point, and titer). To improve the oxidative stability, the produced oils should contain less polyenoic FA (PEFA) and larger amounts of oleic acid. Oils containing larger amounts of oleic acid are especially suited for frying and baking (Hammond, 2000a).

One way to modify soybean oil FA composition is to increase (in comparison with the traditional oil) the concentration of both stearic and palmitic acids. Oils

enriched with these acids crystallize in cool storage without the need for prior hydrogenation. In this way it is possible to obtain plastic fats at room temperature.

When modifying the FA composition in oils, a compromise must be reached. The oil should meet both the nutritional requirement for increased concentrations of PEFA and the technological requirements that facilitate the production of fat products of increased melting point and improved oxidative stability.

The redistribution of FA in triacylglycerols (TAG) is another possibility of fat modification. PEFA distribution in a TAG molecule determines its oxidative stability, melting point, and crystallization properties. Applying physical, chemical, and biotechnological methods modifies both the fat composition and properties. As far as physical methods are concerned, the following techniques have found practical application: fat fractionation in different temperature ranges, extraction with liquid CO₂ under supercritical conditions, or conventional extraction with organic solvents. Chemical methods include hydrogenation, chemical esterification, and transesterification. In addition to previously developed genetic methods involving culture procedures that promote desirable modification of vegetable oils, genetic engineering techniques are becoming increasingly more common. Geneticists have obtained transgenic oil plants, mainly soybean and rape (Kinney, 1996; Zou et al., 1997), and genetic modifications incorporate most of the earlier mentioned technological and nutritional requirements. However, ecological safety related to the cultivation of transgenic crops and the safety of genetically modified organism (GMO) fat consumers has not yet been determined.

Enzymatic methods are becoming increasingly more common in lipid modification; for example, in TAG hydrolysis or TAG interesterification. In addition, animal fats are modified with respect to FA composition, sterol presence, and physical properties. Genetic engineering techniques can be applied to the modifications done in the FA concentration and composition in, for example, milk, eggs and meat. Nutritional methods, which affect the FA composition and cholesterol concentration in animal lipids, are being perfected. Fat modifications promote the functional and protherapeutical food production for consumers representing different age, sex, and health state groups. Also, fat substitutes are used in functional foods and are primarily produced from chemically or enzymatically modified proteins or saccharides.

15.2 NATURALLY MODIFIED FATS AND THEIR EFFECTS ON FOOD QUALITY

15.2.1 MODIFIED VEGETABLE OILS

The methods for modification of FA composition and vegetable oil properties are chosen with regard to crop variety, crop strain, and location of crop cultivation. Selecting the varieties and strains of oil plants can produce oils of desirable technological and nutritional properties. Developments in breeding methods have produced new varieties and strains that produce modified and custom-designed oils. Such breeding methods brought about improved soybean, rape, sunflower, and flax (Hammond, 2000a). A new, doubly improved rapeseed variety was

introduced in the industry; this initiated widespread production of nutritionally valuable erucic acid-free oil. Also, new breeding methods have resulted in new soybean strains, the oil of which contains that contains 28% stearic acid, 17% palmitic acid, and a total concentration of the saturated acids of about 45% (Horejsi et al., 1994; Hartmann et al., 1996). Decreasing the linolenic acid and increasing the stearic acid resulted in soybean oil with enhanced oxidative stability (Kinney, 1996). A decrease in unsaturated FA in soybean oil for the benefit of increasing the saturated acids improves the oxidative stability, but it does not meet nutritional requirements. This oil modification direction has been justified practically as soybean oil enriched with saturated acids becomes a better raw material for the production of plastic fats (e.g., margarine). This oil enables the elimination or limitation of hydrogenated oils and a decrease in the level of FA *trans* isomers. Not all saturated FA are equally nutritionally objectionable. It was shown that the FA containing ten or less carbon atoms are less harmful for consumers (Wood, 1992).

New sunflower varieties were also obtained as the result of breeding. The modified sunflower oil contains less PEFA and more oleic acid. New varieties contain over 70% oleic acid and only 10% linolenic acid. The ratio of these acids is similar to that in olive oil. Such modified sunflower oils are especially suited for frying and baking and do not form any foreign flavors, which tend to occur when oils containing large amounts of PEFA are used for this purpose (Hammond, 2000a).

The previous examples show that modification of FA composition and of the physical and nutritional properties of oils can be obtained by breeding.

15.2.2 MODIFIED FAT BY FEEDING OF ANIMALS

Although nutrition specialists object to excessive consumption of animal fat, this component constitutes a considerable percentage in the global consumption balance. To improve its nutritional value, it is modified with respect to animal lipid composition and properties. The fat present in milk, eggs, and poultry meat is of particular interest. Nutritional modification of the amount and quality of cows' milk fat plays an important role and can be effectively performed. However, fat modification by feeding is limited due to the biohydrogenation in rumen, resulting in a lower supply of unsaturated FA from feed.

Despite the biohydrogenation of unsaturated acids in rumen, the administration of direct doses of vegetable and fish oils (as feed supplements) produces larger concentrations of unsaturated FA such as $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ in milk fat at the cost of saturated acids. To improve the effectiveness of milk fat modification and limit the biohydrogenation, animals are fed encapsulated fats in the form of for example, full oil seeds of flax, rape, vegetable oil preparations encapsulated in casein denatured with formaldehyde, FA calcium salts, or FA amides.

As the result of feeding cows encapsulated fat, desirable milk fat modification can be obtained so that the PEFA in fat are limited. A conjugated linoleic acid (CLA), also referred to as rumen acid (*cis*-9,*trans*-11-octadecadienoic acid), is a nutritionally important fat component. If present in the human diet, this acid prevents obesity,

acts as an anti-atheromatotic and anti-neoplastic compound, as well as stimulates the immune system. Its concentration in milk fat is determined by the feeding method. For example, its level can be increased twofold in milk in comparison to that produced in winter by letting cows graze on a pasture field. Milk products can also be enriched with CLA by introducing lactic bacteria into cow's diet. The same bacteria used in the production of fermented beverages consumed by cows would synthesize CLA in the rumen.

Perfecting the biosynthesis of fats using microorganisms has enabled the effective production of fungi and seaweed biomass as the source of n-3 FA (i.e., γ -linolenic acid (GLA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)). Animal feed supplemented with the above biomass produces increased amounts of these acids in the lipids of milk, eggs, and meat (Hridinka et al., 1996). The effect of feed composition on fat modification was shown in feeding pigs and chickens. Feed composition modification was shown to be highly effective in the modification of animal fat composition with respect to incorporation of essential PEFA. Enriching animal fat with, for example, oleic acid has a beneficial effect on the levels of cholesterol and nutritionally undesirable TGA in the blood plasma of consumers. In a study of feeding chickens (broilers), it was shown that the supplement of soybean oil to the feed (as the basic source of unsaturated FA) produced higher levels of linoleic acid in the lipids of leg muscles. To a smaller extent, this effect in poultry muscle lipids can be obtained by applying enriched (9% rapeseed oil and 2% soybean oil) feed for chickens. This resulted in an increase in the concentration of linolenic acid in the breast muscle (3.5%) and thigh muscle (6.2%) lipids (Milczak, 1999). Also, by adding vitamin E to the feed, such modification can be made even more effective. In the lipids of poultry fed diets enriched with vitamin E, the level of n-3 PEFA was higher than in the muscle lipids of chickens fed diets not supplemented with vitamin E. In nutritional modification of the poultry muscle lipid composition and properties, it is important to correctly select the fats to be incorporated into feed, their composition and properties, as well as to adjust the diet size to maximize the effect on the oxidative stability and sensory properties of meat that incorporates the modified lipids (Milczak, 1999; Szymczyk et al., 2001).

The nutritional modification of egg yolk lipids primarily involves an increase in the concentration of mono- and polyenoic fatty acids and a decrease in the cholesterol concentration. Feeding layer diets with increased concentrations of olive oil rich in C_{18:1} acid resulted in an increase of this acid in the egg yolk lipids. However, the best results in terms of an increase in the concentration of PEFA in egg yolk lipids were obtained by supplementing feed with fish oils or seaweed biomass rich in these acids.

Nutritional methods can also be applied to enriching egg yolk lipids with CLA by administering it in adequate doses with feed. However, the effectiveness of nutritional methods in the reduction of cholesterol in egg yolk lipids is low. The use of 3-hydroxy-3-methylglutaryl-CoA reductase for this purpose is more effective. In this way, the cholesterol level in egg yolk lipids can be decreased by 30%; however, this has consequences in the layers' productivity (Pisulewski et al., 2001; Pszczoła et al., 2000).

15.3 LIPIDS MODIFIED BY PROCESSING AND THEIR EFFECTS ON FOOD QUALITY

15.3.1 BLENDING

Blending vegetable oils of different origin or their combination with animal fats (e.g., with milk fat) is justified for technological, nutritional, and economic reasons. If the FA compositions of different vegetable oils are known, they can be combined in certain ratios to obtain products of improved composition and better functional properties (e.g., improved oxidative stability or shifted melting point). Blending sunflower oil with soybean, rapeseed, or corn oil can also serve this purpose (Hammond, 2000a). Similar effects can be obtained by combining soybean oil with palm olein, or flax oil with triolein.

To modify the physical and nutritional properties of milk fat, butter is enriched with vegetable oils such as sunflower, soybean, rapeseed, and palm oils. These oils can be added separately or in combination to the cream, or directly to the final product (Staniewski, 2000). In this way, milk fat can be enriched with essential PEFA that improve the nutritional value of the dairy products. At the same time, change in the unsaturated:saturated FA ratios results in favorable rheological modifications of butter-like dairy products. Supplementing cream before churning or butter with oils is also beneficial for standardization of rheological properties of butter-like products. The effectiveness of modifying physical properties, usually the rheological properties of butter, is primarily determined by the type of oil used (Shen et al., 2001; Staniewski, 2000).

The selection of oils and their proportions also determines the nutritional value and functional properties of margarine, which also consists of blended fats.

15.3.2 FRACTIONATION

Fat fractionation involves the separation of fat into different fractions, according to structure, molecular size, melting point, titer, and solubility in different solvents or volatility. The selection of the method and process conditions is based on knowledge of fat structure, its behavior in different temperature ranges, the mechanism of forming β and β' fat crystals that determine the melting point. Fats can be fractionated directly after melting or after dissolving in acetone, ethanol, or hexane. The composition of the fractions obtained with the use of solvents is different from the fraction composition obtained as the result of dry fractionation without solvents. Fractionation with organic solvents is less time-consuming, and the separation of crystals from liquid fractions is easy. The drawback of this method is that compounds that are responsible for flavor and odor are removed with solvents during the fat refining process.

The technology of milk fat fractionation does not use solvents, but rather crystallization from melted fat. Crystalline fractions of different chemical compositions and different physical properties are formed at controlled temperatures (Bhaskar et al., 1998). The results of fractionating are solid and liquid fractions with their melting points of 48°C and 25°C, respectively. The melting point of unfractionated fat was 41.6°C. The liquid fraction in comparison with the solid fraction had 17% more $C_{8:0}$ – $C_{10:0}$ FA, 11% less $C_{12:0}$ – $C_{16:0}$ FA, 32% less $C_{18:0}$ FA, but 41% more $C_{18:1}$, $C_{18:2}$,

and C_{18:3} FA. By applying the process of fractionation, products with varying physical properties and nutritional value can be obtained.

Progress in fat fractionation research has resulted in the use of extraction by CO₂ under supercritical conditions (Bhaskar et al., 1993). This method is applied to obtain milk fat fractions enriched with high-melting-point TGA with reduced amounts of cholesterol, vitamins, and some flavor- and odor-responsible components. After extraction with CO₂, the resulting fraction had a higher melting point and 2.5-fold more TGA, including C_{10:0} – C_{12:0} acids, than the initial anhydrous fat; the unsaturated:saturated acid ratios were 0.75 and 0.57 for the resulting fat and initial fat, respectively. This method allows the separation of fractions that differ with respect to their FA composition from milk fat. Solid fractions are enriched with TGA with a large number of carbon atoms that contain larger amounts of long-chain FA. The separated liquid fractions are enriched with low-molecular-weight TGA with large amounts of short-chain FA that play an important role in nutrition. Extraction with CO₂ also allows for the differentiation of the cholesterol amounts in the resulting fractions (Boudreau and Arul, 1993).

Another possibility for the modification of fat composition and physical properties (e.g., milk fat) is the application of molecular distillation. This method involves the evaporation of more volatile molecules and their condensation. After the process of milk fat distillation, fractions rich in short- and long-chain TGA and with reduced amounts of cholesterol are obtained. These fractions are used for the production of dietetic butter for people suffering from disturbances in fat assimilation (Staniewski, 2000).

15.3.3 HYDROGENATION

Fat hydrogenation, also referred to as fat hardening, is basically a process of vegetable oil modification. In this process, the double bonds of FA are saturated with hydrogen. This is a typical chemical process in which by using different catalysts and by changing process parameters, mainly temperature, it is possible to obtain products of wide variety of properties and FA composition. Also, the hydrogenation degree of the FA determines the product's properties. Via partial hydrogenation, it is possible to obtain liquid frying oils. Greater hydrogenation produces solid fats with a melting point of about 35°C. Such fats are used in the production of margarine, and confectionery and baking fats. Partially hydrogenated fats contain up to a few dozen percent *trans* isomers in their FA; and the final products based on such fats used for frying and baking contain from 6 to 37% of *trans* isomers.

Trans isomers always occur in oil-fried products because they are formed at high temperatures. The developments in fat hydrogenation with respect to process optimization and the selection of catalysts have resulted in a gradual reduction in FA *trans* isomers in partially hydrogenated fats. The result of complete hydrogenation, in which all bonds of unsaturated FA are saturated with hydrogen, are hardened fats that undergo further modification by interesterification.

Highly hydrogenated fats have lower concentrations of unsaturated FA, *trans* isomers and higher oxidative and thermooxidative stability than the initial oils. Their stability depends on the hydrogenation degree.

15.3.4 INTERESTERIFICATION

Interesterification is an interchange of acyl groups between either ester and alcohol (alcoholysis), ester and acid (acidolysis), or between two esters (proper esterification). Esterification is most widely applied to fat modification because it relocates FA in TAG of one or several fat components to be modified. In this process, acyl groups are repositioned both within TAG molecules (intramolecular esterification) and between different molecules (intermolecular esterification).

Interesterification alters the TAG structure and composition, while the natural structure of incorporated FA remains unchanged. The process is usually performed at temperatures below 100°C and requires alkali catalysts such as NaOH or CH₃ONa. Setting the temperature between 0 and 40°C can control the process so as to obtain a blend of TAG with different melting points. Modified fat products with custom-made functional properties can be obtained by selecting the proper reaction components, catalysts, and process temperatures. Frequently, interesterification is used for modifying blends of fats of different melting points, such as liquid oil and hard solid fat. Therefore, the selected temperature must be high enough to liquefy the solid fat as well. A liquid state of all reaction components facilitates an even distribution of particular FA within TAG and consequently produces a very advantageous structure resulting from a fine, strong crystal structure. Such modified fats are used in the production of margarine with a high PEFA content. A mere 18% interesterified fat concentration allows for incorporation of 82% of the oil in a margarine matrix, while the margarine itself remains solid. The application of interesterified fats facilitates the production of nutritionally valuable margarines and special-purpose confectionery fats, called shortenings.

The fact that the fine crystalline β' form prevails in the products containing interesterified fats also reduces the range of undesirable polymorphic changes in fats during their storage (Rousseau et al., 1998).

More often, lipolytic enzymes are replacing chemical catalysts in the interesterification process, as well as effectively regulating the process parameters.

15.4 BIOTECHNOLOGICAL MODIFICATION OF FAT

15.4.1 ENZYMATIC MODIFICATION OF FATS AND OILS

The application of biotechnological methods to enhance food components is necessary. Valuable reviews on biotechnological fat and oil modification have been written in recent years (Bornscheuer, 1999, 2000). Progress in the enzymatic modification of fats and oils has resulted from the knowledge of lipases gathered within the past 10 years (EC 3.1.1.3) and made commercial enzymes increasingly more available (Schmid and Verger, 1998; Woolley and Peterson, 1994).

Lipases are the basic tool for the biotechnological modification of fats and oils, and are used to obtain structured lipids (sTAG) (i.e., TAG of a defined chemical structure) having a modified qualitative and quantitative FA composition and a stereochemical structure different from that of the natural components. Lipases can synthesize sTAG having unsaturated acids in the *sn*-2-position. Such sTAG are

especially attractive when they contain PEFA such as DHA or EPA in the *sn*-2-position and in extreme positions medium-chain FA (M) – MLM type of sTAG.

The separation of monoacid TAG from among the sTAG group showed that *Candida antarctica* lipase (Novozyme™) synthesized EPA-enriched TGA in 98% yield.

The human milk fat substitute, “Betapol,” introduced on the market by the Loders Croklaan Company (Unilever) (Xu, 2000), is an example sTAG. This 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (OPO) is primarily obtained by the interesterification of tripalmitin with oleic acid, catalyzed by a lipase from *Rhizomucor miehei*.

Cocoa butter substitutes are also examples of sTAG and can be successfully synthesized by enzymes. One such substitute is synthesized from palm oil and stearic acid by *sn*-1,3 selective lipases from *Candida antarctica*, *Pseudomonas* sp.

Required sTAG are best obtained through enzymatic treatment of existing products or by TAG synthesis from glycerol and FA. They can be synthesized chemically or enzymatically as the result of esterification, acidolysis, alcoholysis, or interesterification (Lee and Akoh, 1998).

The market mainly offers low-calorie, chemically synthesized sTAG, including: Caperin (containing FA C_{8:0}, C_{10:0} and C_{22:0}) and Benefat (containing FA C_{2:0}-C_{4:0}, and C_{18:0}), as well as Neobee and Captex (Akoh, 1998). All these products are blends of different TAG. Due to the lack of stereoselectivity in the chemical process, these products are not chemically pure. Selective lipases appear to be the right tool; however, there are many factors that determine lipase selectivity, including some that remain unknown. Enzyme type, immobilization method, organic solvent type, and water activity, among others, determine the productivity and selectivity of the lipase catalyzing sTAG synthesis (Lee and Akoh, 1998).

A two-step enzymatic synthesis of sTAG seems to be a solution. The first step involves alcoholysis of appropriate TAG to obtain 2-mono-*sn*-acyloglycerol (2-MAG). In the second step after separation, this product is esterified with a FA (Figure 15.1). The method of two-step sTAG synthesis can be widely applied to the synthesis of different TAG. A key factor is to ensure adequate process conditions to obtain 2-mono-*sn*-acyloglycerol. The process must be carried out in such a way as to prevent migration of acyl groups and must be catalyzed by *sn*-1,3-selective lipases.

The wide interest in the recently discovered nutraceutical, CLA, has resulted in attempts to synthesize CLA-enriched sTAG (Garcia et al., 2000). Interestingly,

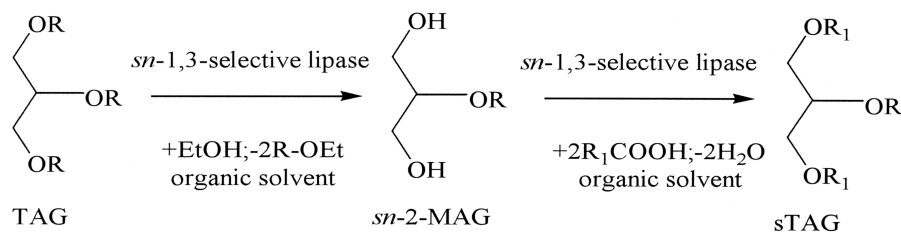


FIGURE 15.1 Two-step synthesis of structured TAG.

research into the esterification of vitamins and other antioxidative agents with PEFA is expected to produce oxidatively stable products.

There are still basic problems to be solved in order to successfully use the enzymatic method of sTAG synthesis. One of them is process scale-up. Further studies are also necessary to describe the nutritional and other properties of sTAG, including oxidative stability.

15.4.2 LIPIDS FROM GENETICALLY MODIFIED ORGANISMS

Conventional *in vitro* methods used to improve oil quality involve controlled plant cultivation and the selection of appropriate phenotypes. Canola seeds are the source of erucic acid-low oil and meal low in the undesirable sulfur-containing glucosinolates. Oil low in erucic acid is a classic example of oilseeds perfected by conventional means.

Desirable modifications of soybean and its oil obtained with conventional methods include a reduction in lipoxxygenase activity and of linolenic and palmitic acid concentrations to levels of 3.0 to 3.5% and 4.0 to 8.4%, respectively (MacKenzie, 1999). The previous modifications serve to reduce undesirable changes in oil flavor and odor that are partially determined by lipoxxygenase.

Commercially available Sunola and Saffola are improved sunflower and safflower oils produced by conventional breeding methods. These oils contain about 80% oleic acid and significantly reduced amounts of linoleic acid. Such an FA composition contributes to their high stability at high temperatures and makes them a good frying oil. Good-fry oil is a blend of high-quality frying oils based on a high oleic oil concentration with about 6% of sesame and/or rice bran oil added.

The *in vitro* methods also include chemical mutation techniques with the use of, for example, of N-ethyl-N-nitrosourea and ethylmethanesulfonate. These mutagenic agents enable the increase in the oleic acid concentration in *Brassica napus* seed oil to over 80% (MacKenzie, 1999) (Table 15.1).

The application of genetic engineering in perfecting the qualitative and quantitative properties of FA in oils has proved to be a difficult task. Knowledge of the metabolic paths of FA synthesis has been considerably enriched recently. Additionally, several new enzymes participating in seed oil biosynthesis, as well as new key genes that determine the synthesis of certain FA and TAG, have been discovered. Particular progress, however, was achieved in defining desaturases and related diiron-oxo proteins in plants. It appears that plants possess two main families of diiron-oxo enzymes: (1) those located in plastids, soluble acyl-acyl carrier protein (ACP) desaturases reacting with C₁₄, C₁₆, and C₁₈ saturated acyl-ACP substrates (class II of the diiron-oxo enzymes); and (2) membrane-bound desaturase using complex lipids or acyl-CoA as substrates (class III of the diiron-oxo enzymes) (Murphy, 1999). The characteristics of some desaturases have already enabled the application of the point mutation to obtain, for example, palmitoyl-ACP specific enzyme from Δ^9 stearoyl-ACP desaturase. It is only a matter of time before there is a rational method for the design of desaturases capable of controlling enzyme selectivity. Then, enzymatic processes will completely replace chemical synthesis and the insertion of conjugated double bonds will be possible without the use of chemicals.

TABLE 15.1**Examples of Transgenic and Mutant Plants with Modified FA Composition**

Species	Phenotype
Mutation	
Canola	Reduced linolenic acid in seed
Soybean	Reduced linolenic acid in seed, which is determined by temperature
Soybean	Increased oleic acid to 80% and reduced linolenic acid to <3%
Soybean	Increased palmitic acid to 17%
Soybean	Increased stearic acid to 30%
Transgene	
Soybean/n-3 desaturase antisense	Reduced linoleic acid in seed to <1.5%
Soybean and canola/n-3 desaturase antisense	Reduced linoleic acid and increased oleic acid in seed (>80%)
Rapeseed/MCFA-specific thioesterase and β -ketoacyl-ACP	Increased medium-chain FA (>50%)

Note: ACP = acyl carrier protein; MCFA = medium-chain fatty acid.

Information based on MacKenzie, S.L. 1999. *Molecular Biotechnology for Plant Food Production*, Paredes-Lopez, O., Ed., Basel: Technomic, 525–620; Kinney, A.J. 1994. *Curr. Opin. Biotechnol.*, 5, 144–151; Dehesh, K. et al. 2001. *Lipids, Fats, and Oils: Reality and Public Perception*, September 16–20, 2001, Berlin, Germany.

Transgenic oil crops containing large amounts of DHA, EPA, and GLA offer new opportunities. Plant desaturases with an amino-terminal cytochrome b_5 domain have been selected. These are enzymes that can participate in the introduction of a double bond into an acyl chain between an existing double bond and the carbonyl terminal (Murphy, 1999).

Modifications via genetic engineering techniques can lead to quantitative changes in the natural FA in a vegetable oil or can be used to introduce new, unnatural FA. In the modification of FA composition in vegetable oils, there is often the problem of reaching a compromise between obtaining the required product and solving crop cultivation problems (e.g., reduced crop yield, reduced resistance to diseases). Back-crossing appears to be a solution. It involves crossing a transgenic plant with a material possessing of agrotechnical properties.

Despite considerable progress in FA synthesis by transgenic oilseed plants, few genetically recombined commercial products are currently available. One of them is rapeseed oil modified through cloning lauroyl-specific acyl-ACP thioesterase from *Umbellularia californica* (California Bay) to *Brassica napus* (Del Vecchio, 1996). The resulting oil contains approximately 40% lauric acid. It seems that the only viable possibility of further increasing the lauric acid concentration to 50 or 60% is an additional cloning of the *sn-2* acyltransferase gene. An increase in the lauric acid concentration in the *sn-1* and *sn-3* positions induces,

among others, β oxidation and the glyoxylate cycle, leading to a reduction in the lauric acid concentration to the benefit of acetyl-CoA and sucrose. These additional treatments generate additional costs and, moreover, multiple cloning may cause gene expression instability.

The Calgene company has also produced the expression of an antisense gene encoding a *B. rapa* stearyl-ACP desaturase in both *B. napus* and *B. rapa*. This has led to the inhibition of oleic acid synthesis for the benefit of the stearic acid concentration in the oil of up to 40% (Knutzon et al., 1992).

The modification of vegetable oil composition involves, on the one hand, a reduction in the saturated FA concentration. This was achieved by DuPont, which reduced the concentration of these acids in soybean oil from 15% to less than 4% by suppressing the activity of a type II acetyl-ACP thioesterase (Kinney, 1996). The other direction of genetic modifications leads to an increase in the concentration of the unsaturated FA in oils. For example, greater unsaturated FA concentrations in canola seed oil (from 68% to 83%) were obtained by suppressing the activity of the Δ^{12} FA desaturase gene (Kinney, 1996).

Vegetable oil modification involves not only the FA qualitative and quantitative manipulations, but also the manipulation of components such as tocopherols and phytosterols. To fully characterize oils from genetically modified plants, these oils must be analyzed for their stereochemical structure, which determines several oil properties and the suitability of the oil for various purposes.

It is only a matter of time before new oils will be produced from genetically engineered plants. This, however, will require in-depth studies into the mechanisms of FA and oil synthesis, and especially into the interactions between particular stages of their synthesis and the controlling mechanisms.

15.4.3 ALTERNATIVE FATS AND OILS

For centuries, fat shortages have led to a wider interest in alternative lipid sources. Microbiological lipids received a lot of attention when they were found to be attractive due to their very desirable FA composition and the advantageous stereochemical structure of the TAG (Table 15.2).

Microbiological lipids are also attractive, thanks to their wide range and advantageous yield of microbial biomass in media supplemented with a wide range of substrates that are frequently waste products (Leman, 1997). Many oleaginous microorganisms have been characterized with respect to their lipid accumulation capacity. Oleaginous microorganisms are defined as those whose biomass contains over 20% lipids in dry matter.

The synthesis of microbial fat by bacteria is often ignored because the average fat concentration in dry biomass does not exceed 10%. However, there are strains of *Arthrobacter* sp., *Mycobacterium*, and *Corynebacterium* that are able to accumulate from 30 to 80% lipids in dry matter. Unfortunately, there are other problems related to low growth rates and yields of bacteria, lipid extraction, and the possible allergenicity and toxicity of the resulting lipids. Microalgae (e.g., *Botryococcus braunii* and *Chlorella pyrenoidosa*) serve as attractive sources of PEFA. Dry biomass fat can amount to as much as 85% (Kay, 1991). Moreover, microalgae are a very

TABLE 15.2
Lipids in Biomass of Lipid-Accumulating Organisms

Organism	Lipid Content (% w/w of Biomass)	Main FA (% of Total)
Chlorella vulgaris	39	C _{18:1} – 58
Apiotrichum curvatum	58	C _{18:1} – 44
Candida lipolytica	32–36	C _{18:2} – 51
Mucor ramannianus	56	C _{18:3} – 31
Aspergillus terreus	57	C _{18:2} – 40
Tolyposporium ehrenbergii	41	C _{18:1} – 81

Information based on Mukherjee, K.D. 1998. *Food Lipids: Chemistry, Nutrition, and Biotechnology*, Akoh, C.C. and Min, D.B., Eds., New York: Marcel Dekker, 589–640.

attractive source of EPA and DHA. For example, in oil from *Cryptocodinium cohnii*, the DHA concentration is about 40% (Gunstone, 1998). Lipid synthesis by microalgae is difficult to justify for economic reasons. Despite methods that decrease the cost of synthesis, microalgae require special growth conditions and costly and difficult methods for lipid separation.

One of the key achievements in expanding the pool of the available long-chain PEFA (DHA and EPA) is the isolation of the gene encoding synthesis of Δ^5 -desaturase from the yeast *Mortierella alpina* (Michaelson et al., 1998). Obtaining PEFA from microbial biomass is justified for economic reasons. Moreover, microbiological oil is cholesterol-free and not contaminated with heavy metals or pesticides.

GLA synthesized by the *Mucor javanicus* strain was commercially available in Great Britain as oil of Javanicus. Unfortunately, despite the relatively high GLA concentration of 15 to 18%, this product could not resist the competition of cheaper sources of GLA: evening primrose, borage, and black currant oils. In Japan, oil from *Mortierella isabellina* is a commercially available GLA source.

Mortierella alpina is one of the most valuable sources of PEFA. Many firms use microorganisms to synthesize oil with over 40% arachidonic acid. Controlling the synthesis of certain PEFA by *Mortierella alpina* became possible after the obtaining mutants with varied desaturase activity (Certik et al., 1998). By modifying the culture conditions for this fungus, the PEFA composition could be considerably altered. For example, a reduction in temperature and supplementing the culture liquid with GLA stimulated the synthesis of EPA.

By ensuring the appropriate growth conditions for the microorganisms, it is possible to obtain microbiological cocoa butter-like fat (cocoa butter substitutes). This can be achieved by supplementing the culture liquid with, for example, FA whose increased concentration in the resulting microbial oil is desired. The stearic acid concentration in bio-oil can also be increased by inhibition of the stearic to oleic acid conversion by repressing the Δ^9 -desaturase gene.

Microbiological lipid synthesis undoubtedly offers vast opportunities with respect to the syntheses of PEFA and special TAG such as cocoa butter substitutes. However, the problems related to production economics and the production of bio-oils with optimal compositions must be solved first.

15.5 FAT MIMETICS AND THEIR EFFECTS ON FOOD QUALITY

It is very difficult to replace fat in food due to the very important function it plays in the human body. Fat is a concentrated energy source, a vitamin source and medium, in addition to providing structural functions. Fat mimetics must meet high requirements. These products must have fat-like properties such as flavor and structure, but low energy, small cholesterol contents, and the ability to substitute 90% of the fat from a diet.

Fat mimetics in food can have different functions: exchange agents, stabilizers, gel-forming components, thickening components, consistency agents, and humidity retainers; and they can have the role of factors responsible for flavor and odor. It is sometimes impossible to replace fat from food with a single fat substitute; therefore, there is a wide range of fat substitutes with varying chemical compositions and functional, sensory, and nutritional properties.

Fat mimetics are primarily made of proteins from egg, milk, soybean, and wheat gluten. Protein processing technology includes the process of micro-encapsulation, involving shaking combined with controlled heating. It produces microscopic forms of coagulated protein positioned around the deformed protein molecules. Such fat substitutes of protein origin added to food contribute to the fat-like consistency and co-form the fat-like flavor sensations. These substitutes are completely digestible and their caloric value falls between 4 and 16 kJ/g. They are used in the production of sauces, mayonnaise, dietetic butters, ice creams, yogurts, pastries, and confectioneries.

Another group of fat substitutes uses saccharides such as starch, cellulose, saccharose, inulin, fibrulin, and maltodextrin obtained from potato, corn, wheat, or tapioca starch. The saccharide fat substitutes in food act as thickening agents, emulsion stabilizers, and humidity-retaining components. Most of them are low energy or energy-free products (e.g., microcrystalline cellulose).

Fat mimetics used in industry are macromolecules similar to conventional fats and oils with respect to their physical and chemical properties. They are chemically synthesized or extracted from traditional fats and combined with other components such as saccharose. Commercially available carbohydrate fat substitutes include DDA (fat ester of malonic and alkyl-malonic acids) and Olean (Olestra, a mixture of octa-, hepta-, and hexa-esters of saccharose).

The advantages of fat mimetics are their high substitution ratio — they can substitute traditional fat in a 1:1 ratio — and their high heat stability, which is important during the processes of cooking, baking, and frying. The growing demand for functional food facilitates production growth, and thus the use of fat substitutes in the food industry.

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16 Frying Fats

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CONTENTS

- 16.1 Introduction
- 16.2 Frying Media
 - 16.2.1 The “Ideal” Frying Medium
 - 16.2.2 Types of Oils Used for Frying
- 16.3 Deep-Frying
 - 16.3.1 Introduction
 - 16.3.2 Physical Changes
 - 16.3.3 Chemical Reactions
 - 16.3.4 Interactions between the Frying Oil and Food Components
 - 16.3.5 Desirable and Undesirable Changes due to Deep-Fat Frying
 - 16.3.6 Chips
- 16.4 Quality of Industrial Frying Oils and Fats
 - 16.4.1 Introduction
 - 16.4.2 The Importance of Fatty Acid Composition
 - 16.4.3 Specifications
 - 16.4.4 Stabilization of Frying Oils with Synthetic and Natural Antioxidants
- 16.5 Evaluation of Deterioration and Assessment of the Quality of the Used Oil
 - 16.5.1 Introduction
 - 16.5.2 Traditional and Standardized Methods
 - 16.5.3 Quick Tests
 - 16.5.4 Total Polar Compounds
 - 16.5.5 Polymerized TAG
 - 16.5.6 Other Approaches
 - 16.5.7 Prediction of Heat Stability
- 16.6 Nutritional and Health Aspects
 - 16.6.1 Introduction
 - 16.6.2 The Intake of Fat
 - 16.6.3 Macronutrients, Metals, and Water-Soluble Vitamins
 - 16.6.4 Frying Oils as Sources of Natural Antioxidants
 - 16.6.5 Classes of Compounds of Toxicological Concern
- 16.7 Conclusion
- References

16.1 INTRODUCTION

Frying is one of the most widely used practices in the preparation and manufacture of food. There is an increased consumption of fried food today and this is related to the preference of modern consumers for convenience food, the growth of the snack food industry, and the increasing number of fast-food restaurants and take-away outlets.

During frying, the oil is continuously exposed to high temperatures in the presence of air and moisture. Under such conditions, many complex reactions occur; these reactions can be classified as oxidation, polymerization, and hydrolysis. Some of these reactions result in the desirable flavor, color, and texture of the fried food, while others are undesirable from the perspectives of quality, nutrition, and toxicology.

One of the factors that contribute significantly to the quality of the final product of frying is the frying medium. Its degradation depends on the fatty acid (FA) composition and initial state, as well as the presence of prooxidants or antioxidants. Although most of the modifications are generally known, it is difficult to foresee the rate of fat degradation because of the high number of factors involved. These factors are not only linked to the medium but also to the frying process itself (temperature, duration of heating, mode of heating, rate of oil turnover) or to the food subjected to heating.

Frying in shallow fat is called pan-frying or sautéing. Shallow frying means that the oil is used only once and its role as a heat transfer medium is minor. Its action is mainly to prevent sticking to the pan or to provide glaze.

Deep-frying uses enough fat to cover the food. The oil is hot enough to seal the surface of the food to form a crust. Deep-fat frying is the most widely used method domestically, industrially, and in catering outlets.

Roasting in an oven where temperatures may be as high as 220°C can also be considered frying (Bognar, 1998).

When acceptable frying practices are applied and the oil is discarded regularly, the oil remaining in the fried food retains its nutritive value and a significant portion of the liposoluble vitamins. The fried product retains water-soluble vitamins, minerals, and other nutrients to a greater extent than in other cooking methods.

Frying is one of the oldest methods of food preparation. It is believed that it was used as early as 1600 BC by the ancient Egyptians. Most probably, it was discovered by roasting a piece of meat in a pot over a hot fire. The fat would be rendered from the meat, the moisture would boil away, and the result would be pan-fried meat. In the Old Testament, rules for sacrifices make a distinction between bread “baked in the oven” and bread cooked in the “griddle” or “in the pan.” Pliny (Gaius Plinius Secundus, 27–79 A.D.) recorded a prescription that required eggs steeped in vinegar and then fried in oil. Authors in the Middle Ages (Chaucer and Cervantes) described in detail the process of cooking in oil (Morton, 1998).

In the modern era, the practice of frying has both declined and increased. Two important steps in the technological advancement of frying were the development of continuous fryers and the introduction into the food market of partially fried French fries (par-fries). The industrial production of the latter led to further

improvement of frying equipment. The consumption of fried food is increasing today, due to the preference of modern consumers for convenience food. Modern frying practices have contributed to the production of better products that combine sensory quality, color, crust, and texture with reduced loss of nutritive value.

16.2 FRYING MEDIA

16.2.1 THE “IDEAL” FRYING MEDIUM

It is difficult to give an accurate definition of the perfect frying medium because there are so many applications and so many factors that affect the frying process. Some general guidelines and characteristics of a good frying oil are:

- A deep-frying medium should be selected not only on the basis of a good Rancimat value, but also on a specified FA composition.
- To avoid polymerization, the frying medium should not contain too much linolenic acid (several countries set limits as low as 2% for linolenic acid).
- High levels of saturated FA (palmitic and stearic) should be avoided because of their effect on the sensory properties of the fried food.
- Blends should be avoided, unless they are properly examined and found not to cause foaming (blends of long-chain fatty with short-chain FA oils are known for their foaming effect).

16.2.2 TYPES OF OILS USED FOR FRYING

The fatty materials used for frying can be unhydrogenated fats and oils or hydrogenated products specifically prepared. Liquid frying fats are made from (1) refined, bleached, and deodorized oils such as palm olein, corn oil, sunflower oil, soybean oil, safflower oil, cotton seed oil, peanut oil, rapeseed oil, and canola oil; and (2) fractionated hydrogenated oils. In Mediterranean countries, olive oil in its various forms (virgin, refined, olive residue oil) is also used. Solid fats are palm stearine, coconut fat, palm kernel oil, lard, tallow, and various shortenings with different melting points and solid fat indices. Beef dripping fat is also used because it provides a distinctive flavor to potato chips.

A high intake of saturated and *trans*-FA from frying fats is considered nutritionally undesirable. To reduce the intake of such FA, products of genetic modification such as high oleic sunflower oil, low linolenic sunflower, modified canola, and other oils have been proposed as good alternatives to partially hydrogenated oil (Dobarganes et al., 1993; Warner and Mounts, 1993). In manipulating the FA composition to increase the monounsaturated FA content, changes in the level of natural antioxidants, which may accompany FA alteration, should be taken into consideration. Normand and co-workers (2001) demonstrated that the stability of modified canola oil is affected far more by the rate of tocopherol degradation than changes in FA composition. This finding may explain why only a small improvement was found by a number of researchers who compared the frying stability of regular and modified oils (Petukhov et al., 1999).

16.3 DEEP-FRYING

16.3.1 INTRODUCTION

Deep-frying follows defined principles of heat and mass transfer. Convective heat transfer from the hot oil and conductive heat transfer through the food are the driving forces that lead to the chemical and other changes in both the oil and the food. Mass transfer includes dehydration (evolution of steam), distillation (volatile materials), extraction, and leaching from the food to the oil. The physical changes and chemical reactions during deep-fat frying are briefly described next.

16.3.2 PHYSICAL CHANGES

Steam escapes from the surface of the oil and also removes volatile compounds. An increase in volatiles causes a lowering of the smoke point. The moisture creates a blanket above the oil that minimizes contact between atmospheric oxygen and food. The formation of steam prevents the oil from getting into the interior of the fried product. Thus, the temperature of the food is always 100°C, regardless of the temperature of the oil.

The formation of polymers leads to an increase in viscosity. The various lipids that can leach into the frying oil change the properties and the performance of the frying oil. Colored lipids solubilized in the oil contribute to the darkening. Phospholipids are emulsifiers. Traces of liposoluble metal compounds may act as prooxidants. Liposoluble vitamins and phenolic compounds are antioxidants. Volatile compounds (e.g., from fish or onions) contribute to off-flavors.

Oil penetration depends on the shape of food, its textural properties, porosity, the viscosity of the frying medium, and the temperature and duration of frying. Increased viscosity results in larger volumes of absorbed oil. Food that is high in initial fat content does not absorb oil. On the contrary, fat is leached from the food into the oil.

16.3.3 CHEMICAL REACTIONS

At frying temperatures (160 to 200°C), the moisture escaping from the food hydrolyzes triacylglycerols (TAG) to FA, glycerol, and mono- and diacylglycerols.

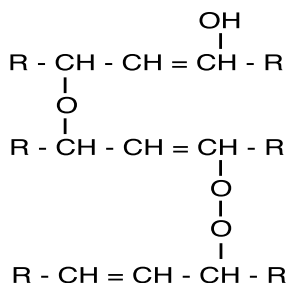
Oxidation causes the formation of hydroperoxides and conjugated compounds, which by cleavage give aldehydes, alcohols, ketones, lactones, acids, esters, and hydrocarbons. Radical mechanisms lead to the formation of dimers, other oligomers, and oxidized TAG. The latter have one or more acyl group with an extra oxygen (hydroxy, keto, epoxy derivatives). Other oxidation products are TAG with short-chain fatty acyl and *n*-oxo fatty acyl groups.

The volatiles in the frying oil increase in the beginning, but then decrease as frying continues. The most important volatiles for the quality of frying oil are saturated aldehydes C₆-C₉, enals (e.g., 2-decenal), dienals (e.g., 2,4-heptadienal), and hydrocarbons (hexene, hexane, heptane, octane, nonane, and decane). The presence of volatile oxidation products formed during the frying process was discussed by Perkins (1996) and Nawar (1998).

The formation of nonvolatile decomposition products is due to the oxidation and polymerization of unsaturated FA. These products act as catalysts in the radical

reactions and accelerate deterioration. Oils heated at intervals have higher degradation rates than oils heated continuously. Aldehydes affect the flavor of deep-fried food. *2-Trans-4-trans*-decadienal contributes to a pleasant flavor while other aldehydes produce unpleasant aromas. *Trans-4,5-epoxy-trans-2-decenal* indicates a prolonged period of heating (Belitz and Grosch, 1999).

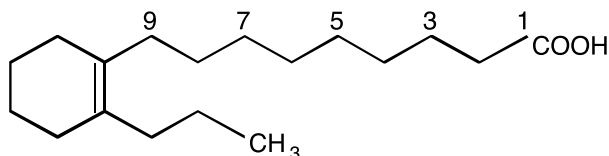
Dimers and other oligomers are good markers of oil degradation and their levels are regulated in many countries. The polymers are usually divided into polar dimers, nonpolar dimers, and oligomers having a higher molecular weight than dimers. The structures of oxygenated polymers are not fully elucidated because of the heterogeneity of this class of compounds (Dobarganes and Marquez-Ruiz, 1996; Gasparoli, 1998; Mahungu et al., 1999). The structure of an oxidation polymer with an ether and a peroxide bond has been presented by Belitz and Grosch (1999):



In the absence of air, hydrolysis, isomerization, and polymerization are the main reactions. Carbon-carbon bonds link the thermal dimers and trimers. A Diels-Alder reaction is proposed for their formation but dimerization of unsaturated FA is also explained by a cationic mechanism (Gertz et al., 2000). This cationic mechanism is probably related to the formation of cyclic monomeric FA. Polymerization is one of the causes of viscosity increase. It also causes gumming.

Cyclic products are important from a health perspective because they are of some toxicological concern, and there are a lot of studies on metabolic processes and their ingestion (Mahungu et al., 1999; Sebedio and Chardigny, 1996). Monomeric cyclic FA are formed from linolenic, linoleic, and oleic acids during heating of vegetable oils at temperatures above 200°C. Because these temperatures are reached in the deodorization step of refining, refined bleached and deodorized oils may also contain cyclic monomers (Christi and Dobson, 2000; Henon et al., 2001). During cyclization, one double bond is lost. Monocyclic monoenoic and dienoic FA derive from linolenic and linoleic acids. Cyclization of oleic acid produces saturated FA. Structures that have been identified include C₁₈ disubstituted cyclohexenyl dienes with one double bond in the ring and the other in the side chain containing the carboxyl group or the aryl group (10,15-cyclization) and similar cyclopentenyl dienes (11,15- or 10,14-cyclization). Linoleic acid produces various isomers containing a cyclopentenyl ring at positions C₈-C₁₂, C₁₀-C₁₄, C₁₃-C₁₇, and C₅-C₁₀. From oleate, 5- and 6-membered saturated ring compounds are formed (Le Quere and Sebedio, 1996; Mahungu et al., 1999; Christi and Dobson, 2000). Cyclic FA monomers are practically absent in virgin olive oil and their level is not signif-

icantly increased after repeated fryings (i.e., no more than 1 $\mu\text{g/g}$) (Romero et al., 2000). A cyclohexenyl FA formed during frying has the following structure:

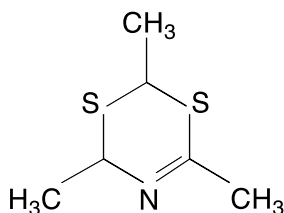


Trans isomers are formed during frying, whether at home or in the industry (Chardigny et al., 2001). Such geometrical isomers are also present in refined oils, especially physically refined oils, because of the high temperatures applied. The major isomers found in both cases are 9,12-*cis-trans*- and 9,12-*trans-cis*-octadecadienoic acids, followed by conjugated *cis-trans*-, *trans-cis*-, and *trans-trans* dienes. Several isomeric trienes are formed from linolenic acid during thermal treatment of oil. Grandgirard and co-workers (1984) identified di-*trans* and mono-*trans* isomers such as 18:3 9c, 12c, 15t; 18:3 9t, 12c, 15c; 18:3 9c, 12t, 15c; and 18:3 9c, 12t, 15t. Determination of the geometric and positional isomers is tedious work because of the complexity of the mixture and the lack of reference materials. A collaborative study supported by the European Union Commission has been performed (Buchgraber et al., 2001). The objective of this study was the inter-comparison of methods for the determination of *trans*-FA in edible oils and the certification of three materials. One of the reference materials is for *trans*-FA formed by heating either during refining or frying.

16.3.4 INTERACTIONS BETWEEN THE FRYING OIL AND FOOD COMPONENTS

Maillard reactions between components of the fried food (sugars and amino acids) are responsible for the golden color of fried products. Lipid oxidation products also contribute to the main reactions. Reactive oxidation products include aldehydes, epoxides, hydroxyketones, and dicarbonylic compounds, which react with lysine, proline, and other amino acids.

Some of the typical fried flavors are due to degradation products of the frying oil. Other compounds contributing to the flavor originate from the food itself. Flavor compounds are also formed by interactions of lipid oxidation products and food components. A typical example is 2,4,6-trimethyl-1,3,5-dithiazine (see structure), a major product of reaction of 2,4-decadienal with cysteine (Pokorný, 1999):



Saccharides participate in Maillard reactions. Polysaccharides form a compact film on the surface in the beginning of frying and produce a crispy crust. The level of dietary fiber and resistant starch may change, but not significantly.

Numerous flavor compounds are formed by caramelization, Maillard or Strecker reactions, or the oxidation of phenolic compounds and terpenes. Some classes of flavor compounds found in frying foods are furan derivatives, pyrrole derivatives, pyrazines, thiols, sulfides, aldehydes, and aromatic compounds from phenol oxidation (Pokorný, 1999).

16.3.5 DESIRABLE AND UNDESIRABLE CHANGES DUE TO DEEP-FAT FRYING

Desirable changes that occur during deep-frying include:

- Adds color, flavor, and texture to the fried food
- Provides economy and speed of cooking
- Provides consistent quality of the final product
- Causes lower losses of nutrients and water-soluble vitamins in relation to those caused by other cooking methods (the proteins, carbohydrates, and minerals are almost fully retained)
- Improves digestibility and bioavailability of nutrients in the digestive system
- Improves the hygienic quality of the food by inactivation of microorganisms
- Causes reduction of fat in animal high-fat foods and nutritionally improves FA profile

Undesirable changes due to frying include:

- A higher content of fat in the final product
- Unfavorable changes in the sensory properties when prolonged heating is applied
- Increased oxidation and polymerization products in abused oils
- Loss of heat- and oxidation-susceptible vitamins

16.3.6 CHIPS

Deep-fat fried food such as hot chips, chicken, and fish are convenience foods in many countries. These products are high in fat and they have been implicated in studies relating fat consumption to certain diseases (e.g., cardiovascular disease, obesity, and some forms of diabetes and cancer).

To obtain lower-fat, higher-quality chips, good deep-frying techniques are needed. An important factor is the size and shape of the chips. Thick-cut chips (larger than 12 mm in diameter) absorb less fat than thinner chips. Cracks and rough surfaces increase fat absorption. Chips may have a 6 to 20% fat content, while crisps may have a fat content as high as 35%. Other important factors in

addition to cut thickness are the heat balance and the volume of frying oil. Heat balance is the ability of the frying system to maintain a constant temperature (Mehta and Swinburn, 2001).

16.4 QUALITY OF INDUSTRIAL FRYING OILS AND FATS

16.4.1 INTRODUCTION

For a better performance of the oil to be used in deep-frying, several factors should be taken into consideration. These factors include (1) The nature of the frying oil and the presence of minor constituents (antioxidants or prooxidants), (2) the specifications of the oil before use, (3) the rate of oil turnover, and (4) the formation of detrimental materials during the frying operation.

16.4.2 THE IMPORTANCE OF FATTY ACID COMPOSITION

Saturated FA, such as palmitic and stearic acids, are stable toward oxidation and polymerization. Because they have a high melting point, they add structure to certain products. However, due to this characteristic the appearance of a fried food may be adversely affected (e.g., waxy mouth-feel, dry surface of stored fried food). Monoenoic FA, primarily oleic acid, are considered to be beneficial from a health standpoint. Frying oils rich in such FA do not add to the structure; they are stable against oxidation and provide a light taste. Polyenoic FA (PEFA) deteriorate more rapidly than monoenoic FA and the shelf life of products fried in oils rich in these acids is shorter. Oxidation products formed from PEFA vary widely, depending on the structure of the FA and the relative concentration of linoleic and linolenic acids. The percentage of linolenic acid in heated oils should be very low.

16.4.3 SPECIFICATIONS

A frying oil with a high FA content will smoke progressively at a lower temperature. Free fatty acid (FFA) content increases rapidly if heating is poorly controlled; if the frying rate is insufficient to remove water from the food; and, of course, if the turnover rate is kept above certain hours.

The main requirements for a high-quality frying oil are: FFA: 0.05 to 0.1%; peroxide value: 0.5 to 1 mEq/kg; moisture: <0.05%; color: red max. 3, yellow max. 30 (Lovibond cell 5.25 inch); smoke point: >220°C; taste and flavor: bland.

Foaming is a safety hazard. If excessive foaming occurs, the heating source must be switched off to avoid the risk of fire. Foaming may be due to oil breakdown products, contact with metals, and traces of detergents from the cleaning of fryers. Lecithins and mono- or diacylglycerols also cause foaming. An effective antifoaming agent is dimethylpolysiloxane. Proteinaceous residues have to be removed by filtration or skimming because they give rise to unpleasant flavors. Debris from the fried food is usually responsible for such residues, although traces may be also found in unrefined oils such as cold pressed oils or beef drippings used in frying.

Copper, iron, and manganese catalyze the decomposition of hydroperoxides to radicals. Copper is a strong prooxidant, almost ten times more active than iron. During the frying operation, contamination of the oil with such metals should be avoided. Otherwise, a premature decomposition will take place, causing unpleasant rancid notes in the product.

Alkali contaminant materials (ACM) may be due to the fried food and the degradation or to residues left in the oil after refining. ACM are traces of soaps that result in an increase in the pH. Bromothymol blue is used to determine the presence of ACM. In restaurant frying operations when the discard point is reached, ACM levels may be as high as 40 mg/kg (expressed as sodium oleate).

16.4.4 STABILIZATION OF FRYING OILS WITH SYNTHETIC AND NATURAL ANTIOXIDANTS

Various researchers have suggested that modifications or the addition of various compounds or mixtures can protect frying oils against thermal and oxidative deterioration. However, there is a lack of agreement among researchers on the effectiveness of antioxidants in retarding deterioration of oils during frying. This is partly due to the fact that the use of actual frying conditions to study antioxidant effectiveness is not always easy, and extrapolation of data on the action of antioxidants from storage or heating experiments in model systems may be misleading. The rate of the initiation reactions of autoxidation is very high at frying temperatures and antioxidants, usually added at levels of 0.01 to 0.02%, are rapidly decomposed. In addition, degradation reactions take place; these are not encountered when the oil is autoxidized at room temperature. Certain additives, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are lost through volatilization and the desired level of the additive is not maintained during the frying operation.

Among the various synthetic antioxidants used for the protection of frying oils against thermal oxidation, the most extensively studied are BHA, BHT, *t*-butylhydroquinone (TBHQ), and propylgallate (PG). The addition of TBHQ has been claimed to have good “carry-through” effect into potato chips, deep-fried in oil containing 0.02% antioxidant. Other substances that have been tested for their effect on the stability of frying oils include silicone oil; secondary antioxidants such as citric acid, EDTA, tartaric acid, and ascorbyl palmitate; and polymeric antioxidants (Anoxomer).

In the past three decades, there has been an increasing trend toward natural ingredients in food and many attempts have been made to replace chemicals in the stabilization of lipids. The emphasis placed on natural antioxidants is due to toxicity concerns about some synthetic antioxidants, but mainly to findings that relate active ingredients in the diet and radical mechanisms in the human body.

Researchers interested in the properties and occurrence of natural antioxidants concentrate on vitamins E and C, carotenoids, phenolic acids, flavonoids, sesame lignins, phytosterols, extracts from the leaves of the plants belonging to the *Lamiaceae* family, oryzanols, tea leaf extracts, phosphatides, olive oil phenols, squalene, and propolis. Barrera-Arellano et al. (1999), Boskou (1999), Blekas and Boskou

(1999), Kochhar (2000), Koketsu and Satoh (1997), Zandi and Gordon (1999), and Irwandi et al. (2000) have discussed the effect of natural antioxidants on the stability of frying oils.

16.5 EVALUATION OF DETERIORATION AND ASSESSMENT OF THE QUALITY OF THE USED OIL

16.5.1 INTRODUCTION

The methods used to determine the effect of heat on frying oils can be divided into two categories. The first category includes relatively simple standardized methods such as color, peroxide value, anisidine value, FFA, foam, smoke point, viscosity, and/or quick tests based on dielectric constant measurements and color indicators. The methods in this category are convenient for routine analysis. The second category includes more sophisticated methods that are based on gas-liquid or high-performance liquid chromatographic techniques or other, more or less complex procedures necessary to obtain more information about specific hydrolytic or thermal oxidation products. Furthermore, sensory analyses of the fried products can be used to evaluate the suitability of the frying oils. This is carried out by panelists who examine the color, crispness, flavor, and overall acceptance of chips fried in the oil.

16.5.2 TRADITIONAL AND STANDARDIZED METHODS

The development of FFA parallels, to a certain extent, other degradative changes but the measurement alone does not correlate well with the quality of the frying oil.

The iodine value is a measure of unsaturation. A decrease in the iodine value is consistent with a decrease in the number of double bonds in a heated oil as it becomes oxidized.

The color of the oil becomes darker during frying. The change in color can be measured with a Lovibond tintometer. For a quick assessment of color, comparison kits are available. As in the case of FFA, color alone does not express the quality of the used oil.

The peroxide value is a normal oxidation index, which has to be carefully used because at high temperatures, hydroperoxide decomposition may be more rapid than formation; thus, degradation is not reflected by this index.

The smoke point is an important characteristic for restaurants in deciding when to change frying oil. This value depends on the development of volatiles.

Excessive foam in the oil during frying is a nuisance and, in extreme cases, a safety hazard that necessitates changing of the oil. Foaming is due to viscosity changes, emulsifiers, and stabilizers formed from the oil and the food being fried. The foam height test is not standardized. During frying, the viscosity increases because of the formation of high-molecular-weight compounds. Oil with an increased viscosity is absorbed more readily by the food and the food becomes soggy and greasy. Viscosity is regulated in Belgium; the frying oil should be discarded if the viscosity reaches 27 mPa.s at 50°C.

The anisidine value measures aldehydes, which are not easily decomposed. Thus, the test to determine the anisidine value is more meaningful than that to determine the peroxide value.

The totox value is defined as $2 \times \text{Peroxide value} + \text{Anisidine value}$. The induction period is usually measured by the Rancimat apparatus and expressed in hours. The induction period of unused oils is approximately 10 to 15 Rancimat hours. This period can be reduced to 1 to 2 hours in 1 or 2 days of commercial frying.

Conjugated dienes and trienes measure the shift of double bonds. The shift is measured by the absorbences at 232 and 268 nm. Similar to the peroxide value, the absorbance at 232 nm measures the level of reactive intermediates. $E_{1\text{cm}}^{1\%}$ values, which are very low in unheated oils, may become as high as 15 within a few days of commercial frying. $E_{1\text{cm}}^{1\%}$ values at 268 nm follow changes in the iodine value, viscosity, PEFA, and polymer content.

The carbonyl value measures secondary oxidation products and is determined by the 2,4-dinitrophenylhydrazine method. Initial carbonyl values are very low but they can increase to 100 or 150 mEq/kg when the oil is heated.

Refractive index provides a simple means to monitor the deterioration of frying oils. This parameter is usually measured at 40°C.

Quantitation of FA composition by gas-liquid chromatography of methyl esters is useful for the monitoring of unsaturated FA. Losses of PEFA follow the increase in total polar compounds. Near the rejection point (27% TPC), 10 to 15% losses of PEFA can be observed.

16.5.3 QUICK TESTS

Quick tests can be based on chemical reactions or physical properties. The chemical quick tests used in industry measure color, foam, or FFA. These tests are helpful when the initial compositions of oil and fried food are the same and the frying conditions do not change.

The two most important quick tests based on physical parameters measure the changes in viscosity and dielectric constant. Several studies have been published for the marketed sensors and the correlation to total polar material. The advantages and drawbacks of these measurements have been discussed by Dobarganes and Marquez-Ruiz (1998) and Gertz (2000).

16.5.4 TOTAL POLAR COMPOUNDS

Quantitation of total polar compounds (TPC) is the basis of legislation in many countries for the control of used frying fats. The method is gravimetric and based on the adsorption of polar compounds onto a silica gel column. Limits up to 27% have been set for TPC in countries where frying oils are regulated. There are approved methods (IUPAC, AOAC) for TPC determinations. TPC can also be determined with Sep-Pak cartridges. An alternative technique uses the Iatroscan thin-layer chromatography/flame ionization system (Sebedio et al., 1987).

There are some serious drawbacks to the methods used to determine TPC. Cyclic FA monomers are not eluted in the polar fraction. These alteration products may be

present both in the polar and nonpolar fractions. FA and partial triglycerides are eluted in the polar fraction but can be either naturally present or due to hydrolysis. Samples with similar total levels of alteration cannot be differentiated in relation to the type of degradation (hydrolytic or thermal oxidation). To overcome some of these difficulties, analysis of methyl esters instead of the oils has been suggested.

16.5.5 POLYMERIZED TAG

Polymer TAG comprise an important fraction of polar compounds for reliable monitoring of the changes in the oil during frying. This fraction is determined by size-exclusion high-performance liquid chromatography, and there are approved IUPAC and AOAC methods.

16.5.6 OTHER APPROACHES

Sanchez-Muniz and Sanchez-Montero (1999) have proposed a completely different approach based on the hydrolysis of thermally oxidized oils by pancreatic lipase. According to these authors, more work is needed in this direction to better understand the major stages of the digestion of the oxidized oil and the role of minor components (sterols, phenols), and to better assess the level of alteration at which lipase is completely inhibited.

Gloria and Aguilera (1998) proposed a differential scanning calorimetry (DSC) technique to follow changes in the thermal characteristics of commercial frying oils during heating. DSC tracings indicate that heating of the oil results in a progressive shift of the crystallization peak at 43 to 48°C to lower temperatures and reduced enthalpies of crystallization. These characteristics are well correlated with increases in viscosity, polar compounds, and color.

Recently, Xu (2000) developed a new quick spectrophotometric method to evaluate deep-frying oil quality. The oil samples are scanned from 350 to 650 nm. Each type of oil is found to show systematic changes in spectrophotometric absorbance; these changes are quite evident between 470 and 500 nm. According to Xu, there is a strong correlation between the absorbance and total polar constituents and the method offers itself as a reliable routine test for assessing frying oil quality.

More recently, Deyawatee and co-investigators (2001) studied the deterioration of soybean oil during the frying of potato chips by Fourier transform infrared (FTIR) spectroscopy. The investigators claim that the decrease in ester linkages of TAG and in the iodine value, and the increase in *trans* unsaturation and formation of unsaturated aldehydes, as measured by FTIR, correlate well with the chemical methods for monitoring deterioration. FTIR spectroscopy combined with a partial least squares approach was also used by Che Man and Setiowaty (1999) to predict the anisidine value. This method depends on the concentration of 2-alkenals; 2,4-dienals; and to a lesser extent saturated aldehydes. The method is proposed to replace the laborious wet-chemical method. It is based on the influence of the carbonyl group on the C–H frequency (range, 2747 to 2619 cm⁻¹) and the carbonyl region (1715 to 1763 cm⁻¹).

16.5.7 PREDICTION OF HEAT STABILITY

In a recent report, Gertz and co-workers (2000) proposed a new method to estimate the oxidative stability at elevated temperatures (OSET). The fatty material is heated at 170°C for 2 h after the addition of water-conditioning silica gel. The polymeric TAG (mainly nonpolar dimers and oligomers) are then measured using size-exclusion HPLC and the OSET value calculated.

16.6 NUTRITIONAL AND HEALTH ASPECTS

16.6.1 INTRODUCTION

There are many studies in the scientific literature that examine the nutritional value and safety of heated oils. Some of the most important aspects of the problem are thoroughly discussed in reviews by Clark and Serbia (1991), Sebedio and Char-digny (1996), Marquez-Ruiz and Dobarganes (1996), Paul and Mittal (1997), and Billek (2000).

It is now established that some compounds formed during heating under non-realistic conditions have antinutritional properties. These compounds might inhibit enzymes and the absorption of nutrients, destroy vitamins, oxidize lipids, and/or potentially cause gastric distress or mutations. When overheated oils or fractions containing degradation products were fed to experimental animals, loss of weight, delay in growth, diarrhea, skin problems, growth of liver, ulcer, and other detrimental effects were observed. These harmful degradation products are absent or present in minute quantities when good frying practices are applied. From the plethora of reviews and articles found in the literature, it is reasonable to assume that when proper monitoring of discarding is practiced, the formation of toxic substances is of no practical significance. A moderate consumption of fried food prepared under normal culinary practices is completely safe. Fats and oils reach harmful levels of degradation long after they become unacceptable from the point of view of flavor. This excludes possible risks for the consumer. Furthermore, the regulated or suggested limits for total polar compounds, oxidized FA, and TAG oligomers add additional security. However, many scientists and regulators remain concerned about the possible detrimental effect of oils that are used in frying. Undoubtedly, a better knowledge of changes occurring during frying and metabolism of heated fats can help in the selection of oils and food materials with minimal fat decomposition, minimal delivery of fat calories, and a healthful balance of FA.

16.6.2 THE INTAKE OF FAT

The intake of too much saturated fat, too many calories, and too much cholesterol from animal fats used in frying is a problem already addressed by many medical and nutritional bodies. The public is now fully aware of the risks in relation to atherosclerosis and other diseases. The growing consumer demand for healthful fats has caused a switch from animal fat to vegetable frying oils; this trend is now general among fast-food industries and catering outlets, and also in domestic applications. According to Varela and Ruiz-Roso (1998), the fat intake can be improved through

frying. For example, when lean meat is fried in an oil rich in monoenoic FA, saturated FA pass into the frying oil and there is an increase in monoenoic FA and a decrease in saturated FA in the fried meat.

16.6.3 MACRONUTRIENTS, METALS, AND WATER-SOLUBLE VITAMINS

From a nutritional point of view, frying is beneficial relative to other cooking methods (e.g., boiling or stewing) because the contents of proteins, saccharides, and minerals are nearly fully retained (Bognar, 1998). Vitamins B₁, B₂, B₆, and C are better retained than in boiling, steaming, or stewing. The absorption and metabolism of elements (Ca, P, Fe, Cu) and their nutritive utilization do not change significantly (Vaquero, 1998). The nutritive value of proteins is not significantly affected, as indicated by net protein utilization (NPU) values obtained for fried fish and other products (Henry, 1998; Pokorný, 1999). Changes in the digestibility of proteins, losses of amino acids, and alterations in dietary fiber and resistant starch are of little nutritional importance.

An advantage of deep-frying is that temperatures within the food do not exceed the temperature of the steam under the crust. Frying time, on the other hand, is short in relation to other cooking procedures. Therefore, fried foods provide important nutrients. Only a small loss of protein availability can be observed in the outer layer of the fried food where the oxidation and hydrolytic products from the oil are absorbed on protein and saccharides (Pokorný et al., 2001).

16.6.4 FRYING OILS AS SOURCES OF NATURAL ANTIOXIDANTS

Important natural antioxidants in frying oils are tocopherols and tocotrienols. Other antioxidants, such as derivatives of *p*-hydroxybenzoic and hydroxycinnamic acid, hydroxytyrosol, and lignans, are also present in certain oils (Boskou, 1999; Carlos-Espin et al., 2000).

Tocopherols are primarily antioxidants that are consumed by reaction with lipid radicals. At frying temperatures, tocopherol losses depend on the type of oil, the nature of the tocochromanol mixture, and the presence of more active primary antioxidants. It has been demonstrated that a significant α -tocopherol equivalent remains after repeated fryings in many frying oils. However, it is not clear if α -tocopherol is least stable in relation to the β -, γ -, and δ -homologs, and the relevant literature is somewhat conflicting. In 1998, Simmone and Eitenmiller reported that α -tocopherol is the most stable during simulated frying in soybean oil, corn oil, and palm olein compared to the β -, γ -, and δ -homologs and tocotrienols. Tocopherol stability can be increased if frying takes place in a low oxygen atmosphere (Fujisaki et al., 2000) or in the presence of more active primary antioxidants. The retention of tocopherols in heated oils is evidenced by the resistance of the oils to the loss of their radical scavenging activity, as determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical test at 515 nm. A practical application of this stability is the delivery of liposoluble vitamins. The antioxidant activity of tocopherols and tocotrienols provides protection against thermal oxidation of added retinyl palmitate. An

increased level of this ester in oils and fried food indicates that the addition of retinyl palmitate to frying oils can be useful for delivery of vitamin A activity (Simmone and Eitenmiller, 1998).

Other antioxidants retained in heated oils enhance the stability of α -tocopherol and reduce losses in free radical scavenging activity. Such antioxidants include sinapic acid in rapeseed oil (Carlos-Espin et al., 2000); sesamol, sesamol dimers, and sesaminol in sesame seed oil; and hydroxycinnamic acids and hydroxytyrosol in olive oil (Boskou, 1999; Pellegrini et al., 2001).

16.6.5 CLASSES OF COMPOUNDS OF TOXICOLOGICAL CONCERN

In a used medium, the distinguishable fractions include intact TAG, volatile breakdown products, nonvolatile breakdown products, polar TAG, polar polymers, and nonpolar polymers. The research work carried out thus far indicates that among the various products formed during frying, those capable of creating adverse physiological conditions in experimental animals and considered harmful to humans include cyclic FA monomers, polymeric compounds, and oxidation products.

There is increasing interest in the oxidation products of sterols because of the possible adverse effects on human health. The bulk of the literature, however, focuses on cholesterol oxidation products. There is rather scarce evidence concerning phytosterols, and especially phytosterols oxidized during deep-fat frying, despite the fact that vegetable oils are now primarily used for frying. Cholesterol oxidation products have been investigated for possible mutagenic, cytotoxic, and carcinogenic activities. It is also believed that oxysterols inhibit cholesterol biosynthesis and cause arterial damage, although it is rather certain that at the levels normally encountered in the diet, acute effect on humans cannot be seen. The formation of oxidized sterols in frying fats and health implications have been reviewed by Dutta et al. (1996).

Carcinogenic substances have been found in drippings, charred pieces of batter, and overheated pan-fried foods. Their content in deep-fried food is very low. Chemically, such substances are heterocyclic compounds belonging to the imidazopyridines, imidazoquinolines, and imidazoquinoxalines (Pokorný, 1999). Another class of compounds exhibiting mutagenic properties are aldehydes, such as 4-hydroxynonenal and 4-hydroxyhexenal (Marquez-Ruiz and Dobarganes, 1996).

Polar compounds and TAG oligomers are the basis of legislation regarding the discard of frying oil in many European countries. Some of these countries (Italy, Belgium, France, and others) have set a limit of 25% for polar compounds, while Austria, Germany, and the Netherlands have a limit of 27%. With regard to TAG oligomer content, limits ranging from 10 to 16% have been established.

In a recent report, Sanchez-Muniz and Bastida (2001) demonstrated that the two cut points for polar compounds and TAG polymers are not correlated in the same manner in various oils. Certain compounds defined and determined as polar, such as acylated glycerols, are not necessarily products of alterations due to heating. On the other hand, the polymers formed are of some toxicological concern and express more precisely the level of changes in the heated oil. The authors stressed the need to unify criteria in the different countries and propose a single upper limit of 10%

for TAG oligomers, instead of having two limits (i.e., one for the total polar compounds and the other for the oligomers).

16.7 CONCLUSION

Frying oils and fried food are becoming more important in the diet, owing to the increased consumption of fried food and number of convenience food restaurants and take-away outlets that serve food that is prepared via frying. Oils and fats are rich sources of essential FA and other nutrients. When used for frying over prolonged periods, they deteriorate by darkening in color, smoking, and foaming. Their biological value decreases because unsaturated FA and important phytonutrients, or other minor constituents, are partially or wholly lost. However, when good frying practices are applied, frying oils can retain a significant part of their initial nutritive value and still carry vitamins and functional ingredients. It must also be stressed that, over the years, a lot of information has accumulated concerning the chemical changes in frying oils. This information can be used to improve the quality of frying food and the lipid intake profile by defining more strictly the point at which an oil is no longer fit for use, and by extending regulations to the oil absorbed by the food. Further research is required to identify areas of limited information and to better understand the role of oxidation retardants from natural sources, the interactions between frying oil and food components, and the effect of preprocessing techniques and post-frying practices.

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17 Lipid–Protein and Lipid–Saccharide Interactions

Jan Pokorný and Anna Kołakowska

CONTENTS

- 17.1 Introduction
- 17.2 Lipid–Protein Complexes in Raw Materials
 - 17.2.1 Dispersible Lipoproteins
 - 17.2.2 Lipoprotein Membranes
- 17.3 Interactions during the Processing and Storage of Food
 - 17.3.1 Interactions during Food Storage and Technological Operations at Ambient Temperature
 - 17.3.2 Changes during Cooking and Frying
 - 17.3.3 Changes by Oxidation Reactions of Lipoproteins
- 17.4 Effect of Lipid–Protein Interactions on Functional Properties and Quality of Food
 - 17.4.1 Effect of Lipid–Protein Interactions on the Nutritional Value
 - 17.4.2 Effect of Lipid–Protein Interactions on the Sensory Value, Including Texture
- 17.5 Lipid–Saccharide Interactions
 - 17.5.1 Introduction
 - 17.5.2 Natural Lipid–Starch Complexes
 - 17.5.3 Complex Formation during Starch Processing
 - 17.5.3.1 Starch Contribution
 - 17.5.3.2 Effects of Lipid Type on Interactions
 - 17.5.3.3 Interactions of Fish Lipids with Starch
 - 17.5.4 Interaction Effects on Quality
 - 17.5.4.1 Starch
 - 17.5.4.2 Lipids

References

17.1 INTRODUCTION

Foods and food raw materials primarily consist of hydrophilic substances compatible with the aqueous medium of plant and animal tissues. Lipids, lipophilic vitamins, terpenes, and other nonpolar water-insoluble substances should thus be emulsified or converted into hydrophilic substances by formation of complexes with other food constituents. The most important complex-forming food components are proteins and carbohydrates — or, more correctly stated, saccharides.

17.2 LIPID–PROTEIN COMPLEXES IN RAW MATERIALS

17.2.1 DISPERSIBLE LIPOPROTEINS

Lipoproteins are the most common form of lipid binding in both animal and plant products (LeMeste and Davidou, 1995). They are, essentially, emulsions in which nonpolar lipids and other nonpolar substances are located in the center of such a particle, held together by hydrophobic and other physical forces. Lipoproteins are surrounded by a layer of natural emulsifiers, such as phospholipids or sterols. Their hydrophobic chains are oriented inside the oil droplet, while their polar groups are oriented toward the outer aqueous phase. Due to relatively low hydrophilic–lipophilic balance (HLB) value, such emulsions are not very stable. The polar groups are stabilized by binding to a layer of hydrated protein, mostly by multiple hydrogen bonds. Outer polar groups of such a lipoprotein molecule are loosely bound to surrounding water molecules — again through hydrogen bonds. Because of their polar character, lipoproteins are easily dispersed in water. Lipoproteins in plant storage organs, mostly in seeds or in pericarp, and in animal adipose tissues may be tightly packed in the respective cells. Other lipoproteins are present in the blood of animal tissues. Lipoproteins carrying fatty acids (FA) contain proteins different from those of storage lipoproteins.

Ratios of the lipid and the protein moieties may be very different (Figure 17.1.A compared with 17.1.B). Lipoproteins can be classified according to their specific gravity, as determined by centrifugation (Perkins, 1993). The best-known lipoproteins are those of human blood plasma, which can be divided into very low-density (VLDL), low-density (LDL), and high-density (HDL) lipoproteins. The higher the lipid content, the lower the specific density. The HDL has the highest affinity for the aqueous phase. Similar types of lipoproteins are also present in animal tissues. In plants, lipids are not transported in the organism; thus, lipoproteins are only present in the seeds, pericarp, or germ.

17.2.2 LIPOPROTEIN MEMBRANES

Another form of lipid binding is the formation of lipid–protein membranes. Their function is to separate two aqueous media from one another. Therefore, they consist of a double layer of polar lipids, primarily phospholipids and sterols. Their hydrocarbon chains are oriented toward the aqueous phase, and the polar groups toward a layer of hydrated protein, which, in turn, is bound to polar groups of another layer of oriented lipid molecules (Figure 17.1.C). The intermediary hydrated protein layer

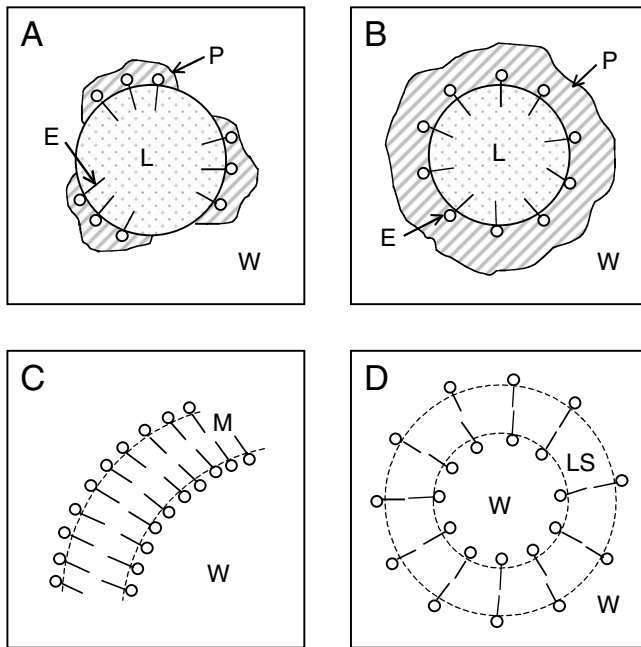


FIGURE 17.1 Types of lipoproteins. A = low-density lipoproteins; B = high-density lipoproteins; C = lipid membrane; D = liposome; E = emulsifier (polar lipid); L = lipid phase; LS = liposome; M = lipid membrane; P = protein; W = water phase.

thus attracts the outer lipid layers and maintains them in the specific structure. The membrane is thus hydrophobic from both sides. In the case of liposomes, there exists only a single lipid layer (Figure 17.1.D).

In animal tissues, intercellular lipoprotein membranes separate two cells of the same tissue, and other membranes separate intracellular particles from the cytoplasm. In plant tissues, cell walls are constructed from polysaccharides, but lipoprotein membranes protect intracellular particles in the same way as in animal tissues. Every membrane contains orifices enabling passage of hydrophilic material or gases through the membrane because such transport is necessary for the metabolism.

17.3 INTERACTIONS DURING THE PROCESSING AND STORAGE OF FOOD

17.3.1 INTERACTIONS DURING FOOD STORAGE AND TECHNOLOGICAL OPERATIONS AT AMBIENT TEMPERATURE

During food storage either at ambient temperature or under refrigeration, lipoproteins do not change appreciably in the beginning, unless the material becomes dehydrated by water evaporation. Dehydration naturally destroys hydrogen bonds, and thus lipoproteins become unstable. Disturbing the native structure of protein molecules changes their susceptibility to interaction. During prolonged storage, lipoproteins as

well as free lipids are oxidized, especially if the lipid fraction is polyunsaturated. The reaction proceeds even under refrigeration or frozen storage. Structure does play a role; for example, texturized minced fish is more stable than conventional frozen minced fish on storage of 6 to 8 months at -20°C (Kořakowska and Szczygieski, 1994).

Another phenomenon involves the input of mechanical energy. When cream is converted to butter during churning, the reaction proceeds by destroying the original dispersion character of the cream (see [Chapter 13](#)).

17.3.2 CHANGES DURING COOKING AND FRYING

At higher temperatures, the protein moiety of lipoproteins undergoes denaturation (Anton et al., 2000), which affects the structure and the surface properties. Lipids and phospholipids are liberated from their complexes with proteins so that they become better available for further reactions (e.g., extraction or oxidation). In technological and culinary operations, fats and oils or their emulsions are often added to the material, they are mechanically dispersed, and hydrogen bonds between lipid and protein molecules are formed or rearranged. Other weak physical forces also play a role. The final effect depends on the particular technology employed and the composition of the food.

During frying, water is released from food, being replaced by frying oil (see [Chapter 16](#)). In surface layers, lipoproteins are destroyed to high degree by water loss, but frying oil is usually not bound to protein or only to a small degree by hydrogen or hydrophobic bonds, depending on the degree of its degradation.

17.3.3 CHANGES BY OXIDATION REACTIONS OF LIPOPROTEINS

Food lipids are oxidized on storage or by heating, primarily following the free radical mechanism (see [Chapter 8](#)). Radicals formed in the process can react both with lipids and proteins (Saeed et al., 1999). Both reactions start at the same time (Soyer and Hultin, 2000). In the reaction with proteins, dimers or even higher oligomers of polypeptide chains are formed.

The reaction of radicals with unsaturated FA of the lipid moiety results in the formation of hydroperoxides, where the hydroperoxy group is located near the center of the hydrocarbon chain. Lipid hydroperoxides are very reactive, even at ambient temperature, especially in the presence of water (Kouřimská et al., 1993). Via secondary reactions, the hydroperoxide group is converted into different other oxygen-containing groups, all of which could react with a reactive group of proteins ([Table 17.1](#)); the reactivities of the hydroperoxides are higher than those of the secondary oxidation products in the case of meat proteins (Janitz et al., 1990). The number of potential combinations is thus quite high (Pokorný and Janíček, 1975). Additional hydrogen bonds between the lipid and protein moieties are formed, which reduce the solubility of the product.

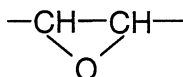
Covalent bonds between lipid and protein moieties are also produced. For example, in frozen bream, 35% total lipids were bound to protein through covalent bonds (Kořakowska et al., 1995). The imine bonds formed between a lipidic carbonyl group

TABLE 17.1**Functional Groups of Oxidized Lipids and Proteins Likely to React in Lipid-Protein Interactions**

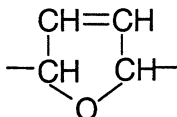
Functional Groups of Oxidized Lipids	Functional Groups of Proteins
Hydroperoxide group – OOH	Amine group – NH ₂
Keto group =C=O	Amide group – CONH ₂
Aldehyde group – CHO	Thiol group – CH ₂ SH
Epoxy group ^a	Sulfide group – CH ₂ –S–CH ₃
Dihydrofuran group ^b	Disulfide group – CH ₂ –S–S–CH ₂ –
Peroxide group ^c	Peptide bound – CO–NH–CH ₂ –
Ketol group ^d	Hydroxyl group – CH ₂ OH
Hydroxy group =CHOH	<i>p</i> -Phenol group ^e
Carboxyl group – COOH	Indole group ^f

Note:

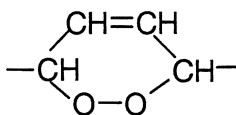
a = epoxy group



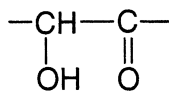
b = dihydrofuran group



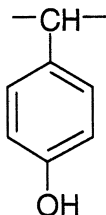
c = peroxide group



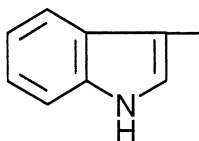
d = ketol group



e = *p*-phenol group



f = indole group



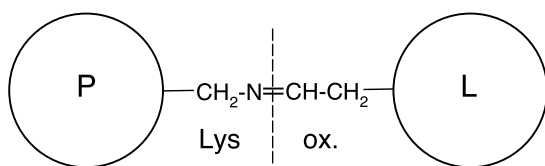


FIGURE 17.2 Example of protein–lipid covalent bonds. L = lipid phase; Lys = residue of bound lysine; ox. = residue of a carbonyl chain of oxidized lipid; P = protein.

and an amine group of the protein moiety (primarily lysine) are typical examples of covalent bond formation (Figure 17.2). Macromolecular pigments were formed in model experiments of (E)-4,5-epoxy-(E)-2-heptenal and lysine (Hidalgo and Zamora, 1993). Imines and other derivatives containing unsaturated bonds are polymerized and result in the formation of insoluble substances.

Another pathway to polymeric products is the cross-linking of lipid or polypeptide chains with bifunctional compounds, such as dicarbonylic lipid oxidation products (e.g., malonaldehyde) or even with monofunctional carbonylic derivatives such as hexanal. The amount of lipids bound by protein may be quite high; for example, 3.4 to 3.6 g oxidized methyl oleate was bound to 1 g bovine serum albumin in model experiments (Seguchi, 1986). Oxidized sterols and terpenes react in a similar way under analogous conditions (Volfová et al., 1998).

Complexes of oxidized lipids with proteins are cleaved with proteolytic enzymes, such as pepsin, trypsin, or chymotrypsin, only slowly and incompletely; thus, their consumption may give rise to digestion problems. In the past two decades, they have received great attention in human medicine because they are precursors of atherosclerotic plaques (Kromhout, 1999).

Complexes of oxidized lipids with proteins show moderate antioxidant activity, such as those with carnosine or histidine (Decker et al., 2001). Therefore, small amounts of oxidized lipids may be beneficial for oxidative stability (Alaiz et al., 1998).

Phospholipids contribute to the formation of complexes with oxidized lipids because they contain, in addition to oxidized FA chains, primary amine groups, quaternary amine groups, and hydroxyl, phosphate, and other polar groups.

The participation of these reactions in nonenzymic browning should be mentioned (Pokorný, 1981). The browning is often accompanied by the formation of fluorescing substances (Liang, 1999). Pyrroles formed in reactions of oxidized lipids with proteins are important precursors of both brown and fluorescing compounds (Zamora et al., 2000). The resulting products of oxidized lipids with proteins are yellow, red, or brown in color (Pokorný et al., 1974). The color intensity increases rapidly with increasing unsaturation of the original lipid fraction, and correlates with loss of primary amine groups (Pokorný et al., 1975). The character of brown products is similar to that of melanoidins from Maillard reactions (Hidalgo et al., 1999). Because fish lipids are highly unsaturated (see Chapter 13), their interaction products are dark brown. Melanoidins partially lose nitrogen by retro-aldolization, and the remaining pigments become increasingly soluble in the lipidic phase (Pokorný et al., 1990). They form stains on the surface of white fish muscle, which

are objectionable from the standpoint of the appearance of frozen fish. Similar problems sometimes occur with stored poultry. On storage of apples, scald-like stains are produced by reactions of membrane lipids with amino acids (Burmeister and Dilley, 1995).

17.4 EFFECT OF LIPID–PROTEIN INTERACTIONS ON FUNCTIONAL PROPERTIES AND QUALITY OF FOOD

17.4.1 EFFECT OF LIPID–PROTEIN INTERACTIONS ON THE NUTRITIONAL VALUE

Natural lipoproteins are easily digestible because they are enzymatically hydrolyzed nearly completely before absorption in the intestines. Their nutritional value is equal to those of the respective lipids and proteins bound in lipoproteins, when consumed separately.

On the contrary, the nutritional value of oxidized lipid–protein interaction products is substantially lower than that of the original lipoproteins. The main reason is the lower digestibility; most covalent bonds formed in the interactions are not attacked by proteases under the conditions of digestion. The 6-amino group of bound lysine is particularly sensitive to interactions with carbonylic oxidation products (Janitz et al., 1990), and the resulting imine bonds substantially reduce the lysine availability. Lysine losses correlate with the increase in fluorescence. Other amino acids, such as tyrosine, tryptophan, and methionine, are also partially converted into unavailable products. Interaction products may be allergenic even when allergenic proteins have reacted (Doke et al., 1989).

The impaired solubility is also important. In stored frozen fish, the decrease in the extractability and solubility of proteins correlated with the amounts of oxidized lipids bound to protein (Sikorski and Kołakowska, 1996). Similar results were obtained with sardine and megrim (Careche and Tejada, 1990). The functionality of muscle foods is damaged by reactions with oxidized lipids (Xiong and Decker, 1995).

17.4.2 EFFECT OF LIPID–PROTEIN INTERACTIONS ON THE SENSORY VALUE, INCLUDING TEXTURE

Fats and oils are added to food raw materials during culinary operations, among other reasons, to improve the flavor and texture of the food. The surface properties of lipoproteins formed, and even of the lipid films and droplets, assist in retaining gases during dough fermentation, thus improving the volume, softness, appearance, and acceptability of many bakery products. During chewing of high fat foods, fats are emulsified into a relatively thick fluid. Consumers perceive the viscosity as highly acceptable and appreciate the texture of such a product.

On the contrary, the interaction products of oxidized lipids with food proteins are not easily moistened and emulsified, and result in deterioration of the texture after food ingestion. The functional properties of beef proteins are deteriorated by

oxidized lipids (Farouk and Swan, 1998). The feeling of the chewed morsel is rated lower because of the lower viscosity, which is due to a higher content of insoluble fractions. The content of salt-soluble proteins decreased proportionally to increasing lipid peroxidation in frozen fish (Sarma et al., 1998). Fortunately, they are never present in such a high amount to become an important factor in human food. The only exception may be dried food products because oxygen can easily penetrate to lipids and lipoproteins even in lower layers, and the oxidation products can then react with proteins. The resulting condensation products affect the dispersibility after reconstituting the original food.

Oxidized lipoproteins have an unfavorable effect on the appearance of light-colored stored food (see [Section 17.3.3](#)); but after roasting or frying, the surface turns brown anyway due to browning reactions. The reddish-brown color is not perceived as a negative phenomenon in the finished meal.

17.5 LIPID–SACCHARIDE INTERACTIONS

17.5.1 INTRODUCTION

Lipid–saccharide interactions predominantly involve interactions between lipids and starches. They take place, under natural conditions, in plant materials and occur during processing of those materials and in mixtures containing both lipids and starches. The formation of such complexes is the goal of numerous technological procedures (e.g., dough kneading, prolongation of bread shelf life, microencapsulation technology, manufacture of edible starch-based film). Lipid–starch interactions can both hamper and facilitate processing and can affect the properties of both starch and lipids (Ho and Izzo, 1992; Friedman, 1995; Ross, 1995; Tomasik and Schilling, 1998).

17.5.2 NATURAL LIPID–STARCH COMPLEXES

Difficulties encountered during attempts to extract lipids, related to the necessity of granule destruction, have been responsible for earlier opinions that perhaps there are no natural complexes, the complexes emerging during starch gelatinization (Morrison and Coventry, 1989).

Naturally, small amounts of lipid–starch complexes are present in native starch. They may occur as (Friedman, 1995; Tomasik, 2000):

1. Inclusion complexes inside the amylose helix and eventually short helices of amylopectin branches
2. Surface sorption complexes
3. Complexes in which the lipids occupy the capillaries between starch granules ([Figure 17.3](#))

An amylose chain twists around a lipid molecule to form a regular helix. The inside of the helix is hydrophobic, with most of hydroxy groups of glucose units remaining outside the helix. In this way, the crystallographic V-pattern of starch is

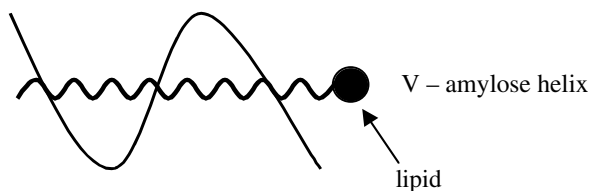


FIGURE 17.3 Amylose–lipid inclusion complex.

formed. It has a single, instead of a double, helix, and a so-called collapsed or compact helix. The helix segments are stabilized by the Van der Waals, hydrophobic, and hydrogen forces and may form complexes, having very special structures, with lipids. Three categories of lipids are known to form complexes with amylose: free FA, lysophospholipids, and monoacylglycerols. The internal diameter of the amylose helix, consisting of six glucose residues per turn, is 0.45 nm. In monoacylglycerols and phospholipids, it is only the FA chain that can penetrate the helix because hydrophilic groups are too voluminous to fit into the helix. The presence of double bonds restricts the possibility of inclusion in the helix (Linden and Lorient, 1999). In the non-waxy cereal starch (barley), 21% of the amylose occurs as complexes with lipids; these are complexes involving saturated lipids (Morrison et al., 1993). In potato starch, the monoacyl with saturated FA to amylose ratio in a complex was found to be 1:10. Cereal starches contain large amounts of lysophospholipids and free FA. Most of the lipids in wheat starch are lysophospholipids; lysophosphatidylcholine also accounts for 76 to 90% of lipids. Their extraction requires destruction of the starch granule structure and indicates the presence of complexes with amylose.

Included lipids stabilize the structure of starch and affect its properties. Most likely, amylopectin also forms natural included complexes in the form of short helices in the branching part of the molecule (Friedman, 1995; Tomasik, 2000).

Surface sorption complexes (starch surface lipids) are complexes of lipids bound to the surface of the granules. These are primarily triacylglycerols (TAG), followed by free FA, glycolipids, and phospholipids. The surface lipid present depends on the origin of the starch (Vasanthan and Hoover, 1992). These lipids can be extracted with cold solvents (Barnes, 1987; Tomasik, 2000), but it cannot be ruled out that such an extract will also contain non-starch lipids that are absorbed onto the surface of starch granules during isolation procedures (Morrison and Coventry, 1989; Vasanthan and Hoover, 1992).

17.5.3 COMPLEX FORMATION DURING STARCH PROCESSING

17.5.3.1 Starch Contribution

In addition to the already-present complexes, further lipid–starch interactions can occur during starch processing. Starch lipids can be complexed, and surface lipids can complex with substances covering lipids from the outside.

Heating a starch suspension results in the formation of amylose–lipid complexes, followed by their dissociation, and by restructuring of the complex once the sus-

pension has cooled down. Reheating of such starch will lead to various polyform starches (Hibi et al., 1990).

Complexing ability depends on the amylose level, its binding to amylopectin, and on lipid composition. Among starches, the least able to form amylose–lipid complexes are potato starches, which contain less amylose, protein, and lipids than cereal starches. Wasserman and Meste (2000) found no amylose–lipid (stearic acid) inclusion complex during heating and cooling of a potato starch and lipid suspension, but they did report the observation that the lipids adhered to the starch granules.

It is commonly believed that it is amylose that forms complexes with lipids, but complexation, which occurs concomitantly with the endothermic gelatinization, has been identified and provided evidence of the existence of amylopectin–lipid interactions (Eliasson, 1994). Apparent starch amylose content correlated well with enthalpies of amylose–lipid complexes (Villwock et al., 1999). Lipids are most likely to complex with amylopectin to the extent they do with amylose. The interaction depends on the amylose:amylopectin ratio. If amylose is present, the first interaction is that between amylose and lipids, followed by a lipid–amylopectin interaction. This is a mixture of B- and V-pattern starches. The best complexes are formed by 100% amylopectin; poorer complexes occur in a 50% solution due to the presence of amylose (Gudmudsson and Eliasson, 1990).

The outer branches of the amylopectin molecule assist in the formation of a helical inclusion complex with suitable lipids (Gudmudsson and Eliasson, 1990). Interactions depend on the starch origin. Mutant maize and mutant rice produce amylopectin with branched chains having about 15 residues rather than the normal 10 to 13 glucose residues. Instead of a normal A-pattern, mutant cereals gave B-patterns (Imberty et al., 1991).

17.5.3.2 Effects of Lipid Type on Interactions

Lipid–starch interactions involve monoacylglycerols with long-chain saturated FA. Interaction increases linearly with increasing monoacylglycerol chain length (Huang and White, 1993). The (C:18) acids were observed to interact both with amylose-containing rice starch and with waxy starch. The 18:0 complexing was more efficient than that produced by shorter-chain saturated and unsaturated FA (Guraya et al., 1997). The phospholipids transesterified with polyenoic FA retarded the gelatinization and retrogradation of starch during storage to a much greater extent than less-unsaturated phospholipids (Siswoyo and Morita, 2000). No significant difference was observed between gels formed (potato starch) in the presence of 1- or 2-monopalmitin and 1- or 2-monoolein (Milqvist et al., 1994). However, as demonstrated by the latter authors, starch-lipid complexing was more efficient with the 2-isomer (monolaurin) than with the 1-isomer (monolaurin).

Most of the data on lipid–starch interactions was derived during studies on dough making and extrusion, wherein the material studied contained, in addition to starch, proteins, also easily interacting with lipids.

As a result of cornmeal extrusion, two thirds of lipids are bound (they are not amenable to hexane extraction). The data suggest that lipid–carbohydrate or three-way lipid–carbohydrate–protein interactions may be responsible for the

lipid binding observed. An interaction may involve either: (1) a physical entrapment or encapsulation mechanism, or (2) a molecular-level interaction. Lipid type should have no effect on the entrapment mechanism because the spaces in which lipids are entrapped would not be the size of the single-lipid molecules, and therefore the small differences in size between TAG, diacylglycerols (DAG), and a FA should have no effect (Ho and Izzo, 1992). TAG will interact predominantly by hydrophobic bonding, Van der Waals force (i.e., they will involve the entrapment mechanism), while DAG and free FA will interact by hydrogen bonding in addition to hydrophobic bonding (Chu et al., 1995; Liu et al., 1997). FA molecules have the ability to form inclusion complexes, which may be a mechanism for them to interact with amylose (Ho and Izzo, 1992). Thus, FA bond with starch more efficiently than TAG. This is a direct molecular-level interaction during extrusion between FA and amylose or amylopectin fractions of cornmeal. The bonding involved is hydrogen bonding or hydrophobic interaction by helical inclusion complex formation or, most likely, a combination of the two. Amylose-FA complexes were produced with FA and monoacylglycerols, but not with TAG (Mecier, 1980, after Aretz et al., 1992). Monoglycerides are complexed much more readily than are TAG (Nestl et al., 1989, after Aretz et al., 1992). The amount of unsaturated lipids complexed relative to saturated lipids is very small (Meuser et al., 1985, after Aretz et al., 1992).

DAG can interact with starch by physical entrapment or by molecular-level hydrogen bonding. More DAG than TAG and less DAG than FA are bonded. Glycolipids do not bond during extrusion (Ho and Izzo, 1992)

Guzman et al. (1992) demonstrated that two thirds of free lipids in cornmeal were bonded during extrusion cooking at 50 to 60°C. Further heating did not result in bonding, while heating at 120 to 125°C decreased the amount of lipid bonding. With respect to polar lipids, two thirds were bonded in raw cornmeal, the remaining one third bonding after extrusion (Guzman et al., 1992).

After adding lipids to an aqueous amylose-amylopectin mixture, interaction was found to depend on the lipid type: unsaturated monoglycerides > lecithin > TAG. TAG hardly produced any interactions. Complex stability was found to increase with chain length and to decrease with increasing unsaturation. Such was the case for complexes with both amylose and amylopectin (Gudmudsson and Eliasson, 1990). However, Bienkiewicz and Kołakowska (2001a, 2001b) obtained different results.

17.5.3.3 Interactions of Fish Lipids with Starch

Mechanical mixing of fish lipids with amylose resulted in half the lipids bonding with starch (Figure 17.4). Most of the bonded lipids were strongly (covalently?) bonded. Microwaving caused still more intense lipid bonding. On the other hand, freezing of the previously heated samples was observed to produce bonding of more than 90% of lipids with amylose. Freezing itself also brought about very strong bonding between lipids and amylose, the effect being stronger than that produced by heating.

Interaction of fish lipids with amylopectin was easier and faster; as early as during mixing, almost all the lipids (95%) were bonded with very strong bonds.

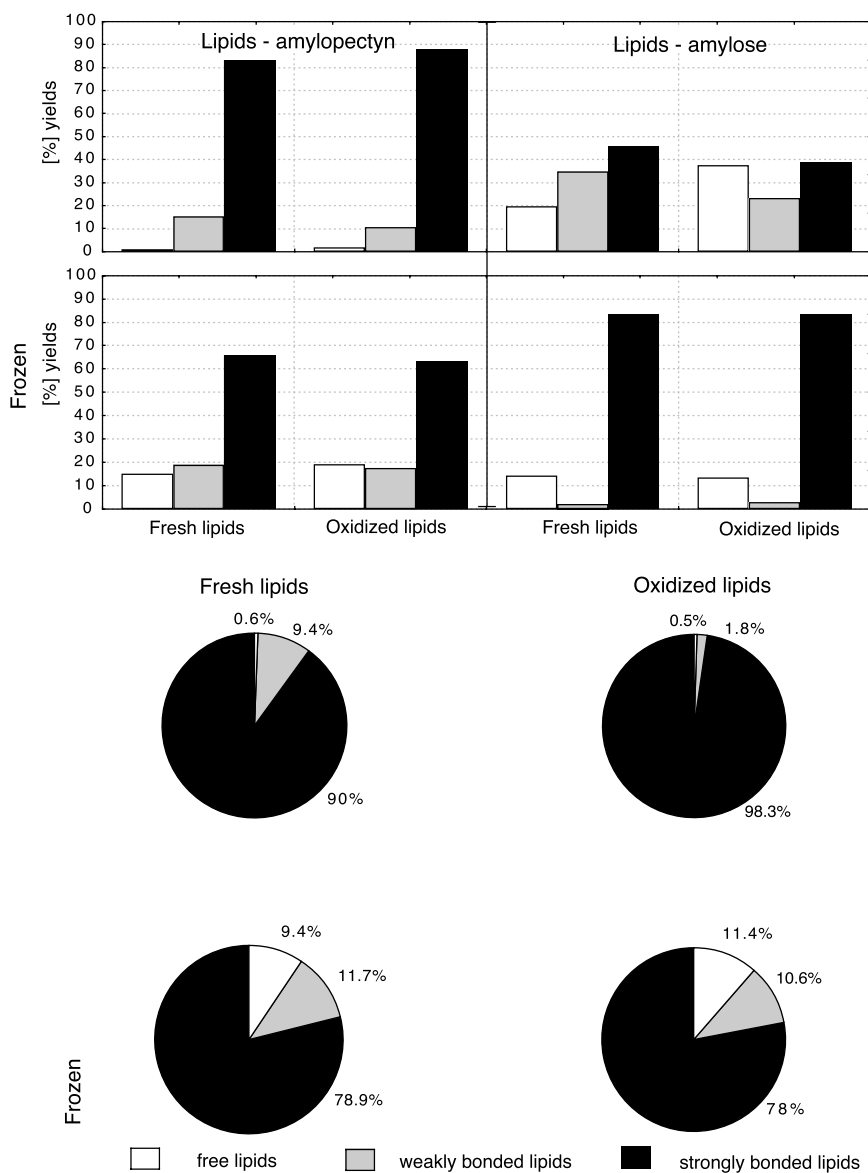


FIGURE 17.4 Fish lipid–starch interaction. Yields [%] of lipids (upper) and PUFA (bottom) from lipid–starch system, by selective extraction. (Adapted from Bienkiewicz, G. and Kołakowska, A. 2001. Fish lipids–amylopectin starch interactions, *21st Nordic Lipid Symposium Lipidforum*, Bergen, June 5–8, 2001; Bienkiewicz, G. and Kołakowska, A. 2001. Fish lipids–amylose interactions, *24th World Congr. International Society for Fat Research (ISF)*, Berlin, September 16–20, 2001, 53.)

However, further treatment (heating and additional freezing) did not intensify the interactions, contrary to the effect observed in fish lipids–amylose complexes, but resulted in weakening the bonds and in the release of lipids (Bienkiewicz and Kołakowska, 2001b). Consequently, complexes containing amylopectin are much less stable than those involving amylose. Particularly unstable amylopectin complexes were formed with oxidized lipids, while the most stable complexes were those with BHA-protected lipids, which suggests mainly hydrophobic interaction in lipid–amylopectin complexes.

The more unsaturated FA, the easier and stronger they bond with starch, both with amylose and amylopectin, provided they are not oxidized. The most efficient and most stable interactions are those produced by polyenoic FA from BHA-protected lipids. Docosahexaenoic acid was preferentially bonded. Oxidized lipids do not interact readily.

In summary, interaction with amylopectin occurs more readily than with amylose but the bonds are less stable in the former and may disintegrate during heating and freezing; perhaps only the entrapped complexes are formed. On the other hand, interaction with amylose starch proceeds less readily, but the complexes are enhanced by processing.

17.5.4 INTERACTION EFFECTS ON QUALITY

17.5.4.1 Starch

Amylose–lipid complexes affect a number of physical and chemical properties of starch (Nierle et al., 1990; Morrison et al., 1993; Shamekh et al., 1998). Complexing reduces swelling of starch granules, interferes with amylase access to them, and increases the gelatinization temperature (Morrison et al., 1993; Wilska-Jeszka, 1994; Fortuna et al., 1999; Becker et al., 2001).

Lipid–starch complexing affects starch digestibility (Shamekh et al., 1998). Most authors contend that lipid–starch complexes reduce starch digestibility, but opinions differ. Amylose–lipid complexes were found to significantly reduce the α -amylase *in vitro* availability (Holm et al., 1985; Guraya et al., 1997). Most likely, some of the lipid–starch complexes involve resistant starch. However, available data suggest that they are considerably more digestible *in vivo*, although at a lower rate than free amylose (Holm et al., 1983). Lysophospholipid complexes with amylose of barley were easily hydrolyzed (Shamekh et al., 1998). The resistance to enzymes (amylases) is not due to complexation with lipids (Siljestrom, 1989). Formation of resistant starch is competitively affected by the complexation of amylose with lipids in barley (Szczodrak and Pomeranz, 1992).

After digestion with amylase, cornmeal extrudates released most of the bonded lipids, both polar and nonpolar (Ho and Izzo, 1992).

Complexes of free FA and lysophosphatidylcholine (LPC) with amylose were easily broken down by glucoamylase as hydrolysis proceeded, except in the case of unsaturated FA, which evidenced a strong resistance to the enzyme (Kitahara et al., 1997). During a 72-h long hydrolysis, the degree of hydrolysis was 4.8 and 22%

for LPC and linolenic acid, respectively (potato amylase with LPC and FA, hydrolysis with glucoamylase) (Kitahara et al., 1996).

There is good correlation between the complexing power of amylose and the anti-staling effect. This is the effect of emulsifiers. The mechanism is not known, but is operative at various stages of bread making. During mixing, monoacylglycerols are distributed throughout the dough and can be adsorbed to the surface of starch granules, which reduces starch swelling on cooking. During cooling, an amylose–monoacylglycerol inclusion complex is formed and precipitation — coagulation inside starch granules — occurs, thus making amylopectin chain reorganization difficult. Because bread staling involves amylopectin crystallization, the complexes, or lipids, counteract staling (Linden and Lorient, 1999).

17.5.4.2 Lipids

Bonding of lipids protects them from oxidation; this was evidenced, for example, by that fact that no n-3 polyenoic FA losses were incurred during extrusion of chum salmon muscle with 10% wheat flour (at up to 160°C) (Suzuki et al., 1988). Also, oxidation was observed only among free lipids during cornmeal extrusion (Guzman et al., 1992) and dough mixing (Daniels et al., 1970). Interaction of fish lipids with amylose and amylopectin (at a ratio of 1:1) did not protect weakly bonding lipids (extractable with chloroform–methanol), the bonds being most probably the hydrogen bonds, from oxidation during mixing and frozen storage (Bienkiewicz, 2001). It is, however, difficult to tell if a strong bonding of lipids by starch protected them from oxidation because oxidation could not be assayed in the largest, non-extractable fraction. There is no reason to fear that interaction with starch can mask lipid oxidation, as is the case in lipid–protein interactions, because less oxidized lipids than fresh lipids were bonding and the bonds were weaker. Presumably, the oxidation process, once started, can facilitate lipid interaction, which is taken advantage of in baking by the use of lipoxxygenase; free radical trapping takes place during extrusion (Strauss et al., 1992). One also has to bear in mind that this is true in systems containing at least three potentially interacting components: lipids, starch, and protein. This is also the case with extrudates, antioxidant properties of complexes (Mastrocola et al., 2000), and texture-affecting preparations (Warner et al., 2001).

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