3 Lipids

3.1 Foreword

Lipids are formed from structural units with a pronounced hydrophobicity. This solubility characteristic, rather than a common structural feature, is unique for this class of compounds. Lipids are soluble in organic solvents but not in water. Water insolubility is the analytical property used as the basis for their facile separation from proteins and carbohydrates. Some lipids are surface-active since they are amphiphilic molecules (contain both hydrophilic and hydrophobic moieties). Hence, they are polar and thus distinctly different from neutral lipids. The two approaches generally accepted for lipid classification are presented in Table 3.1.

The majority of lipids are derivatives of fatty acids. In these so-called acyl lipids the fatty acids are present as esters and in some minor lipid groups in amide form (Table 3.1). The acyl residue influences strongly the hydrophobicity and the reactivity of the acyl lipids.

Some lipids act as building blocks in the formation of biological membranes which surround cells and subcellular particles. Such lipids occur in all foods, but their content is often less than 2% (cf. 3.4.1). Nevertheless, even as minor food constituents they deserve particular attention, since their high reactivity may strongly influence the organoleptic quality of the food.

Primarily triacylglycerols (also called triglycerides) are deposited in some animal tissues and organs of some plants. Lipid content in such storage tissues can rise to 15–20% or higher and so serve as a commercial source for isolation of triacylglycerols. When this lipid is refined, it is available to the consumer as an edible oil or fat. The nutritive/physiological importance of lipids is based on their role as fuel molecules (37 kJ/g or 9 kcal/g triacylglycerols) and as a source of essential fatty acids and vitamins. Apart from these roles, some other lipid properties are indispensable in food handling or processing.

Table 3.1. Lipid classification

A. Classification according to "acyl residue" characteristics	
I. Simple lipids (not saponifiable)	
Free fatty acids, isoprenoid lipids (steroids, carotenoids, monoterpenes), tocopherols	
II. Acyl lipids (saponifiable)	Constituents
Mono-, di-, triacylglycerols	Fatty acid, glycerol
Phospholipids (phosphatides)	Fatty acid, glycerol or sphingosine, phosphoric acid, organic base
Glycolipids	Fatty acid, glycerol or sphingosine, mono-, di- or oligosaccharide
Diol lipids	Fatty acid, ethane, propane, or butane diol
Waxes	Fatty acid, fatty alcohol
Sterol esters	Fatty acid, sterol
B. Classification according to the characteristics "neutral–polar"	
Neutral lipids	Polar (amphiphilic) lipids
Fatty acids ($>C_{12}$)	Glycerophospholipid
Mono-, di-, triacylglycerols	Glyceroglycolipid
Sterols, sterol esters	Sphingophospholipid
Carotenoids	Sphingoglycolipid
Waxes	
Tocopherols[a]	

[a] Tocopherols and quinone lipids are often considered as "redox lipids".

These include their melting behavior and the pleasant creamy or oily taste that is recognized by a receptor, which has recently been identified. Therefore, there are all together six taste qualities (cf. 8.6.1). – Fats also serve as solvents for certain

taste substances and numerous odor substances. On the whole, fats enrich the nutritional quality and are of importance in food to achieve the desired texture, specific mouthfeel and aroma, and a satisfactory aroma retention. In addition, foods can be prepared by deep frying, i. e. by dipping the food into fat or oil heated to a relatively high temperature.
The lipid class of compounds also includes some important food aroma substances or precursors which are degraded to aroma compounds. Some lipid compounds are indespensable as food emulsifiers, while others are important as fat- or oil-soluble pigments or food colorants.

3.2 Fatty Acids

3.2.1 Nomenclature and Classification

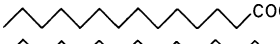
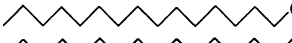
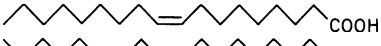
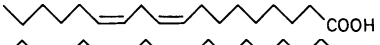
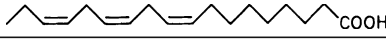
Acyl lipid hydrolysis releases aliphatic carboxylic acids which differ in chemical structure. They can be divided into groups according to chain length, number, position and configuration of their double bonds, and the occurrence of additional functional groups along the chains. The fatty acid distribution pattern in food is another criterion for differentiation.
Table 3.2 compiles the major fatty acids which occur in food. Palmitic, oleic and linoleic acids frequently occur in higher amounts, while the other acids listed, though widely distributed, as a rule occur only in small amounts (major vs minor fatty acids). Percentage data of acid distribution make it obvious that unsaturted fatty acids are the predominant form in nature.

Fatty acids are usually denoted in the literature by a "shorthand description", e. g. 18:2 (9, 12) for linoleic acid. Such an abbreviation shows the number of carbon atoms in the acid chain and the number, positions and configurations of the double bonds. All bonds are considered to be cis; whenever trans-bonds are present, an additional "tr" is shown. As will be outlined later in a detailed survey of lipid structure, the carbon skeleton of lipids should be shown as a *zigzag* line (Table 3.2).

3.2.1.1 Saturated Fatty Acids

Unbranched, straight-chain molecules with an even number of carbon atoms are dominant among the saturated fatty acids (Table 3.6). The short-chain, low molecular weight fatty acids ($< 14{:}0$) are triglyceride constituents only in fat and oil of milk, coconut and palmseed. In the free form or esterified with low molecular weight alcohols, they occur in nature only in small amounts, particularly in plant foods and in foods processed with the aid of microorganisms, in which they are aroma substances.
Odor and taste threshold values of fatty acids are compiled in Table 3.3 for cream, butter and cocoa fat. The data for cream and coconut fat indicate lower odor than taste threshold values of C_4- and C_6-fatty acids, while it is the reverse for C_8- up to C_{14}-fatty acids.
The aroma threshold increases remarkably with higher pH-values (Table 3.4) since only the undissociated fatty acid molecule is aroma active.

Table 3.2. Structures of the major fatty acids

Abbreviated designation	Structure[a]	Common name	Proportion (%)[b]
14:0		Myristic acid	2
16:0		Palmitic acid	11
18:0		Stearic acid	4
18:1(9)		Oleic acid	34
18:2(9,12)		Linoleic acid	34
18:3(9, 12, 15)		Linolenic acid	5

[a] Numbering of carbon atoms starts with carboxyl group-C as number 1.
[b] A percentage estimate based on world production of edible oils.

Table 3.3. Aroma threshold values (odor and/or taste) of free fatty acids in different food items

Fatty acid	Aroma threshold (mg/kg) in				
	Cream		Sweet cream butter[a]	Coconut fat	
	Odor	Taste		Odor	Taste[b]
4:0	50	60	40	35	160
6:0	85	105	15	25	50
8:0	200	120	455	> 1000	25
10:0	> 400	90	250	> 1000	15
12:0	> 400	130	200	> 1000	35
14:0	> 400	> 400	5000	> 1000	75
16:0	n.d.	n.d.	10,000	n.d.	n.d.
18:0	n.d.	n.d.	15,000	n.d.	n.d.

[a] Odor/taste not separated.
[b] Quality of taste: 4:0 rancid, 6:0 rancid, like goat, 8:0 musty, rancid, soapy, 10:0, 12:0 and 14:0 soapy
n.d.: not determined.

Table 3.4. Threshold values[a] of fatty acids depending on the pH-value of an aqueous solution

Fatty acids	Threshold (mg/kg) at pH		
	3.2	4.5	6.0
4:0	0.4	1.9	6.1
6:0	6.7	8.6	27.1
8:0	2.2	8.7	11.3
10:0	1.4	2.2	14.8

[a] Odor and taste.

Additive effects can be observed in mixtures: examples No. 1 and 2 in Table 3.5 demonstrate that the addition of a mixture of C_4-C_{12} fatty acids to cream will produce a rancid soapy taste if the capryl, capric and lauryl acid contents rise from 30 to 40% of their threshold value concentration. A further increase of these fatty acids to about 50% of the threshold concentration, as in mixture No. 3, results in a musty rancid odor.

Some high molecular weight fatty acids (>18:0) are found in legumes (peanut butter). They can be used, like lower molecular weight homologues, for identification of the source of triglycerides (cf. 14.5.2.3). Fatty acids with odd numbers of carbon atoms, such as valeric (5:0) or enanthic (7:0) acids (Table 3.6) are present in food only in traces. Some of these short-chain homologues are important as food aroma constituents. Pentadecanoic and heptadecanoic acids are odd-numbered fatty acids present in milk and a number of plant oils. The common name "margaric acid" for 17:0 is an erroneous designation. *Chevreul* (1786–1889), who first discovered that fats are glycerol esters of fatty acids, coined the word "margarine" to denote a product from oleomargarine (a fraction of edible beef tallow), believing that the product contained a new fatty acid, 17:0. Only later was it clarified that such margarine or "17:0 acid" was a mixture of palmitic and stearic acids.

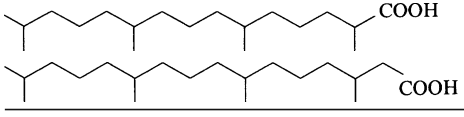
Branched-chain acids, such as iso- (with an isopropyl terminal group) or anteiso- (a secondary butyl terminal group) are rarely found in food. Pristanic and phytanic acids have been detected in milk fat (Table 3.6). They are isoprenoid acids obtained from the degradation of the phytol side chain of chlorophyll.

Table 3.5. Odor and taste of fatty acid mixtures in cream

No.	Fatty acid mixtures of					Odor	Taste
	4:0	6:0	8:0	10:0	12:0		
	Concentration in % of aroma threshold[a]						
1	28	17	29	31	30	n.O.	n.T.
2	28	17	40	42	37	n.O.	rancid, soapy
3	28	17	52	53	45	musty, rancid	rancid, soapy
4	48	29	29	31	30	musty, rancid	n.T.
5	48	29	40	42	37	musty, rancid	rancid, soapy

[a] The concentration of each fatty acid is based on the threshold values indicated in Table 3.3 for odor for 4:0 and 6:0 and for taste for 8:0–12:0.
n.O. = no difference in odor from that of cream.
n.T. = no difference in taste from that of cream.

Table 3.6. Saturated fatty acids

Abbreviated designation	Structure	Systematic name	Common name	Melting point (°C)
A. Even numbered straight chain fatty acids				
4:0	$CH_3,(CH_2)_2COOH$	Butanoic acid	Butyric acid	− 7.9
6:0	$CH_3(CH_2)_4COOH$	Hexanoic acid	Caproic acid	− 3.9
8:0	$CH_3(CH_2)_6COOH$	Octanoic acid	Caprylic acid	16.3
10:0	$CH_3(CH_2)_8COOH$	Decanoic acid	Capric acid	31.3
12:0	$CH_3(CH_2)_{10}COOH$	Dodecanoic acid	Lauric acid	44.0
14:0	$CH_3(CH_2)_{12}COOH$	Tetradecanoic acid	Myristic acid	54.4
16:0	$CH_3(CH_2)_{14}COOH$	Hexadecanoic acid	Palmitic acid	62.9
18:0	$CH_3(CH_2)_{16}COOH$	Octadecanoic acid	Stearic acid	69.6
20:0	$CH_3(CH_2)_{18}COOH$	Eicosanoic acid	Arachidic acid	75.4
22:0	$CH_3(CH_2)_{20}COOH$	Docosanoic acid	Behenic acid	80.0
24:0	$CH_3(CH_2)_{22}COOH$	Tetracosanoic acid	Lignoceric acid	84.2
26:0	$CH_3(CH_2)_{24}COOH$	Hexacosanoic acid	Cerotic acid	87.7
B. Odd numbered straight chain fatty acids				
5:0	$CH_3(CH_2)_3COOH$	Pentanoic acid	Valeric acid	− 34.5
7:0	$CH_3(CH_2)_5COOH$	Heptanoic acid	Enanthic acid	− 7.5
9:0	$CH_3(CH_2)_7COOH$	Nonanoic acid	Pelargonic acid	12.4
15:0	$CH_3(CH_2)_{13}COOH$	Pentadecanoic acid		52.1
17:0	$CH_3(CH_2)_{15}COOH$	Heptadecanoic acid	Margaric acid	61.3
C. Branched chain fatty acids				
		2,6,10,14-Tetra-methyl-penta-decanoic acid	Pristanic acid	
		3,7,11,15-Tetra-methyl-hexa-decanoic acid	Phytanic acid	

3.2.1.2 Unsaturated Fatty Acids

The unsaturated fatty acids, which dominate lipids, contain one, two or three allyl groups in their acyl residues (Table 3.7). Acids with isolated double bonds (a methylene group inserted between the two cis-double bonds) are usually denoted as isolene-type or nonconjugated fatty acids.

The structural relationship that exists among the unsaturated nonconjugated fatty acids derived from a common biosynthetic pathway is distinctly revealed when the double bond position is determined by counting from the methyl end of the chain (it should be emphasized that position designation using this method of counting requires the suffix "ω" or "n"). Acids with the same methyl ends are then combined into groups. Thus, three family groups exist: ω3 (linolenic type), ω6 (linoleic type) and ω9 (oleic acid type; Table 3.7). Using this classification, the common structural features abundantly found in C_{18} fatty acids (Table 3.2) are also found in less frequently occurring fatty acids. Thus, erucic acid (20:1) occurring only in the mustard family of seeds (*Brassicaceae*, cf. 14.3.2.2.5), belongs to the ω9 group, arachidonic acid (20:4), occurring in meat, liver, lard and lipids of chicken eggs, belongs to the ω6 group, while the $C_{20}-C_{22}$ fatty acids with 5 and 6 double bonds, occurring in fish lipids, belong to the ω3 group (cf. 13.1.4.5 and 14.3.1.2).

Linoleic acid can not be synthesized by the human body. This acid and other members of the ω6 family are considered as essential fatty acids required as building blocks for biologically active membranes. α-Linolenic acid, which belongs to the ω3 family and which is synthesized only by plants, also plays a nutritional role as an essential fatty acid.

A formal relationship exists in some olefinic unsaturated fatty acids with regard to the position of the double bond when counted from the carboxyl end of the chain. Oleic, palmitoleic and myristoleic acids belong to such a Δ9 family (cf. Table 3.7); the latter two fatty acids are minor constituents in foods of animal or plant origin.

Unsaturated fatty acids with an unusual structure are those with one trans-double bond and/or conjugated double bonds (Table 3.7). They are formed in low concentrations on biohydrogenation in the stomach of ruminants and are consequently found in meat and milk (cf. 10.1.2.3). Such trans-unsaturated acids are formed as artifacts in the industrial processing of oil or fat (heat treatment, oil hardening). Since trans-fatty acids are undesirable, their content in German margarines has been lowered from 8.5% (1994) to 1.5% (1996) by improving the production process. Conjugated linoleic acids (CLA) are of special interest because they are attributed to have an anticarcinogenic effect. In fact, C_{18} fatty acids with two double bonds which differ in position and geometry belong to the group CLA. The occurrence of CLA in foods is shown in Table 3.8. Up to nine isomers have been identified in lipids and, apart from exceptions, 18:2 (9c, lltr) predominates (Table 3.8). Conjugated fatty acids with diene, triene or tetraene systems also occur frequently in several seed oils, but do not play a role in human nutrition. Table 3.7 presents, as an example, two naturally occurring acids with conjugated triene systems which differ in the configuration of one double bond at position 9 (cis, trans).

Unsaturated fatty acids emulsified in water taste bitter with a relatively low threshold value for α-linolenic acid (Table 3.9). Thus an off-taste can be present due to fatty acids liberated, as indicated in Table 3.9, by the enzymatic hydrolysis of unsaturated triacyl glycerides which are tasteless in an aqueous emulsion.

Table 3.8. Conjugated linoleic acids in food

Food	Total CLA[a] (g/kg fat)	18:2(c9,tr11) (% of CLA[a])
Milk	2–30	90
Butter	9.4–11.9	91
Cheese	0.6–7.1	17–90
Processed cheese	3.2–8.9	17–90
Ice cream	3.8–4.9	73–76
Sour cream	7.5	78
Yoghurt	5.1–9.0	82
Beef, roasted	3.1–9.9	60
Plant oils, marine oils	0.2–0.5	45

[a] CLA, conjugated linoleic acid.

Table 3.7. Unsaturated fatty acids

Abbreviated designation	Structure	Common name	Melting point (°C)
A. Fatty acids with nonconjugated cis double bonds			
	ω9-Family		
18:1 (9)	$CH_3-(CH_2)_7-CH{=}CH-CH_2-(CH_2)_6-COOH$	Oleic acid	13.4
22:1 (13)	$-(CH_2)_{10}-COOH$	Erucic acid	34.7
24:1 (15)	$-(CH_2)_{12}-COOH$	Nervonic acid	42.5
	ω6-Family		
18:2 (9, 12)	$CH_3-(CH_2)_4-(CH{=}CH-CH_2)_2-(CH_2)_6-COOH$	Linoleic acid	−5.0
18:3 (6,9,12)	$-(CH{=}CH-CH_2)_3-(CH_2)_3-COOH$	γ-Linolenic acid	
20:4 (5,8,11,14)	$-(CH{=}CH-CH_2)_4-(CH_2)_2-COOH$	Arachidonic acid	−49.5
	ω3-Family		
18:3 (9, 12, 15)	$CH_3-CH_2-(CH{=}CH-CH_2)_3-(CH_2)_6-COOH$	α-Linolenic acid	−11.0
20:5 (5,8,11,14,17)	$-(CH{=}CH-CH_2)_5-(CH_2)_2-COOH$	EPA[a]	
22:6 (4,7,10,13,16,19)	$-(CH{=}CH-CH_2)_6-CH_2-COOH$	DHA[a]	
	Δ9-Family		
18:1 (9)	$CH_3-(CH_2)_7-CH{=}CH-CH_2-(CH_2)_6-COOH$	Oleic acid	13.4
16:1 (9)	$CH_3-(CH_2)_5-$	Palmitoleic acid	0.5
14:1 (9)	$CH_3-(CH_2)_3-$	Myristoleic acid	
B. Fatty acids with nonconjugated trans-double bonds			
18:1 (tr9)	$CH_3-(CH_2)_7-CH^{tr}{=}CH-(CH_2)_7-COOH$	Elaidic acid	46
18:2 (tr9, tr12)	$CH_3-(CH_2)_4-CH^{tr}{=}CH-CH_2-CH^{tr}{=}CH-(CH_2)_7-COOH$	Linolelaidic acid	28
C. Fatty acids with conjugated double bonds			
18:2 (9, tr11)	$CH_3-(CH_2)_5-CH^{tr}{=}CH-CH^{c}{=}CH-(CH_2)_7-$ COOH		
18:3 (9, tr11, tr13)	$CH_3-(CH_2)_3-CH^{tr}{=}CH-CH^{tr}{=}CH-CH^{c}{=}CH-(CH_2)_7-COOH$	α-Eleostearic acid	48
18:3 (tr9, tr11, tr13)	$CH_3-(CH_2)_3-CH^{tr}{=}CH-CH^{tr}{=}CH-CH^{tr}{=}CH-(CH_2)_7-COOH$	β-Eleostearic acid	71.5
18:4 (9, 11, 13, 15)[b]	$CH_3-CH_2-(CH{=}CH)_4-(CH_2)_7-COOH$	Parinaric acid	85

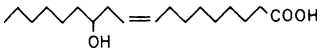
[a] EPA: Eicosapentanoic acid, DHA: Docosahexanoic acid.
[b] Geometry of the double bond was not determined.

Table 3.9. Taste of unsaturated fatty acids emulsified in water

Compound	Threshold (mmol/l)	Quality
Oleic acid	9–12	bitter, burning, pungent
Elaidic acid	22	slightly burning
Linoleic acid	4–6	bitter, burning, pungent
Linolelaidic acid	11–15	bitter, burning, scratchy
γ-Linolenic acid	3–6	bitter, burning, pungent
α-Linolenic acid	0.6–1.2	bitter, burning, pungent, like fresh walnut
Arachidonic acid	6–8	bitter, repugnant off-taste

3.2.1.3 Substituted Fatty Acids

Hydroxy Fatty Acids. Ricinoleic acid is the best known of the straight-chain hydroxy fatty acids. Its structure is 12-OH, 18:1 (9). It is an optically active acid with a D(+)-configuration:

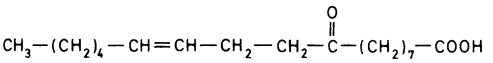


(3.1)

Ricinoleic acid is the main acid of castor bean oil, comprising up to 90% of the total acids. Hence, it can serve as an indicator for the presence of this oil in edible oil blends.

D-2-Hydroxy saturated 16:0 to 25:0 fatty acids with both even and odd numbers of carbons in their chains occur in lipids in green leaves of a great number of vegetables. γ- or ∂-Lactones are obtained from 4- and 5-hydroxycarboxylic acids (C_8 to C_{16}) by the elimination of water. ∂-Lactones have been found in milk fat and fruits. They are very active aroma components (cf. 5.3.2.3).

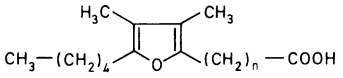
Oxo Fatty Acids. Natural oxo (or keto) acids are less common than hydroxy acids. About 1% of milk fat consists of saturated (C_{10}–C_{24}) oxo fatty acids, with an even number of carbon atoms, in which the carbonyl group is located on C-5 to C-13: One of 47 identified compounds of this substance class has the following structure:



(3.2)

Furan Fatty Acids. These occur in fish liver oil in a range of 1–6% and up to 25% in some freshwater fish. Furan fatty acids are also part of the minor constituents of some plant oils and butter (Table 3.10). They are also present in fruits (lemon, strawberry), vegetables (cabbage, potato) and mushrooms (champignons).

Two of these acids have the following formulas



I: n = 8; II: n = 10 (3.3)

Photooxidation (cf. 3.7.2.1.4) of these acids can deteriorate especially the quality of soybean oil.

Substituted fatty acids are also derived by autoxidation or enzymatic peroxidation of unsaturated fatty acids, which will be dealt with in more detail in 3.7.2.3 and 3.7.2.4.1.

Table 3.10. Examples for the occurrence of furan fatty acids I and II

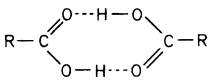
Oil	Concentration (mg/kg) I[a]	II[a]
Soya oil	120–170	130–230
Wheat germ oil	100–130	105–150
Rapeseed oil	6–16	7–20
Corn oil	8–11	9–13
Butter	13–139	24–208
Leaves of the tea shrub[b]	50	713
Green tea[b]	4	80–100
Black tea[b]	10	159
Spinach[b]	86	733

[a] I: 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid.
II: 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (Formula 3.3).
[b] Values based on dry weight.

3.2.2 Physical Properties

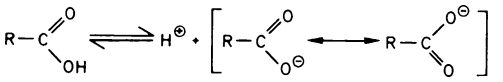
3.2.2.1 Carboxyl Group

Carboxylic acids have a great tendency to form dimers which are stabilized by hydrogen bonds:



(3.4)

The binding energy of the acid dimer dissolved in hexane is 38 kJ/mole. Also, the fatty acid molecules are arranged as dimers in the crystalline lattice (cf. Fig. 3.2).
The acidic character of the carboxyl group is based on proton dissociation and on the formation of the resonance-stabilized carboxylate anion:



(3.5)

The pK_s values for the C_2–C_9 short-chain acid homologues range from 04.75–4.95. The pK_s of 7.9 for linoleic acid deviates considerably from this range. This unexpected and anomalous behavior, which has not yet been clarified, is clearly illustrated in the titration curves for propionic, caprylic and linoleic acids recorded under identical conditions (Fig. 3.1).

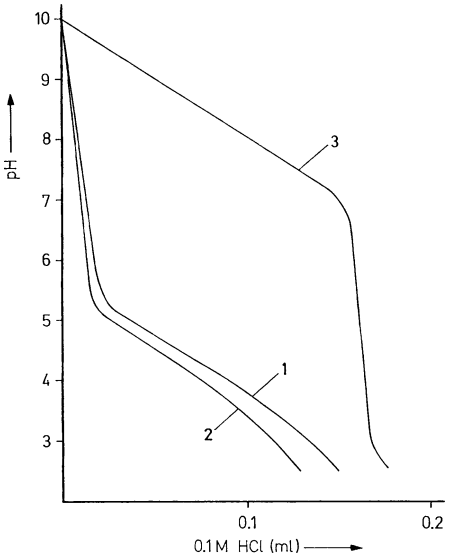
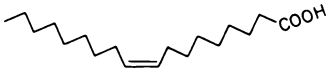


Fig. 3.1. Fatty acid titration curves (according to *Bild* et al., 1977). Aqueous solutions (0.1 mol/l) of Na-salts of propionic (1), caprylic (2) and linoleic acids (3) were titrated with 0.1 mol/l HCl

3.2.2.2 Crystalline Structure, Melting Points

Melting properties of fats depend on the arrangement of the acyl residues in the crystal lattice in addition to other factors attributed solely to the structure of triglycerides.
Calculations of the energy content of the carbon chain conformation have revealed that at room temperature 75% of the C–C bonds of a saturated fatty acid are present in a fully staggered *zigzag* or "trans" conformation and only 25% in the energetically slightly less favorable skew conformation.
The unsaturated fatty acids, because of their double bonds, are not free to rotate and hence have rigid kinks along the carbon chain skeleton. However, a molecule is less bent by a trans than by a cis double bond. Thus, this cis-configuration in oleic acid causes a bending of about 40°:

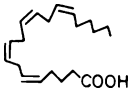


(3.6)

The corresponding elaidic acid, with a trans-configuration, has a slightly shortened C-chain, but is still similar to the linear form of stearic acid:

(3.7)

The extent of molecular crumpling is also increased by an increase in the number of cis double bonds. Thus, the four cis double bonds in arachidonic acid increase the deviation from a straight line to 165°:



(3.8)

When fatty acids crystallize, the saturated acids are oriented as depicted by the simplified pattern

in Fig. 3.2. The dimer molecular arrangement is thereby retained. The principal reflections of the X-ray beam are from the planes (c) of high electron density in which the carboxyl groups are situated. The length of the fatty acid molecule can be determined from the "main reflection" site intervals (distance d in Fig. 3.2). For stearic acid (18:0), this distance is 2.45 nm.

The crystalline lattice is stabilized by hydrophobic interaction along the acyl residues. Correspondingly, the energy and therefore the temperature required to melt the crystal increase with an increased number of carbons in the chain.

Odd-numbered as well as unsaturated fatty acids can not be uniformly packed into a crystalline lattice as can the saturated and even-numbered acids. The odd-numbered acids are slightly interfered by their terminal methyl groups.

The consequence of less symmetry within the crystal is that the melting points of even-numbered acids (C_n) exceed the melting points of the next higher odd-numbered (C_{n+1}) fatty acids (cf. Table 3.6).

The molecular arrangement in the crystalline lattice of unsaturated fatty acids is not strongly influenced by trans double bonds, but is strongly influenced by cis double bonds. This difference, due to steric interference as mentioned above, is reflected in a decrease in melting points in the fatty acid series 18:0, 18:1 (tr9) and 18:1 (9). However, this ranking should be considered as reliable only when the double bond positions within the molecules are fairly comparable. Thus, when a cis double bond is at the end of the carbon chain, the deviation from the form of a straight extended acid is not as large as in oleic acid. Hence, the melting point of such an acid is higher. The melting point of cis-2-octadecenoic acid is in agreement with this rule; it even surpasses the 9-trans isomer of the same acid (Table 3.11).

The melting point decreases with an increasing number of isolated cis-double bonds (Table 3.11). This behavior can be explained by the changes in the geometry of the molecules, as can be seen when comparing the geometric structures of oleic and arachidonic acid.

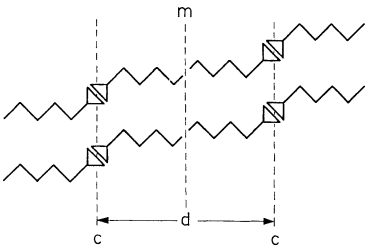


Fig. 3.2. Arrangement of caproic acid molecules in crystal (according to *Mead* et al., 1965). Results of a X-ray diffraction analysis reveal a strong diffraction in the plane of carboxyl groups (c) and a weak diffraction at the methyl terminals (m): d: identity period

3.2.2.3 Urea Adducts

When urea crystallizes, channels with a diameter of 0.8–1.2 nm are formed within its crystals and can accomodate long-chain hydrocarbons. The stability of such urea adducts of fatty acids parallels the geometry of the acid molecule. Any deviation from a straight-chain arrangement brings about weakening of the adduct. A tendency to form inclusion compounds decreases in the series 18:0 > 18:1 (9) > 18:2 (9, 12).

A substitution on the acyl chain prevents adduct formation. Thus, it is possible to separate branched or oxidized fatty acids or their methyl esters from the corresponding straightchain compounds on the basis of the formation of urea adducts. This principle is used as a method for preparative-scale enrichment and separation of branched or oxidized acids from a mixture of fatty acids.

Table 3.11. The effect of number, configuration and double bond position on melting points of fatty acids

Fatty acid		Melting point (°C)
18:0	Stearic acid	69
18:1 (tr9)	Elaidic acid	46
18:1 (2)	cis-2-Octadecenoic acid	51
18:1 (9)	Oleic acid	13.4
18:2 (9, 12)	Linoleic acid	−5
18:2 (tr9, tr12)	Linolelaidic acid	28
18:3 (9, 12, 15)	α-Linolenic acid	−11
20:0	Arachidic acid	75.4
20:4 (5,8,11,14)	Arachidonic acid	−49.5

3.2.2.4 Solubility

Long-chain fatty acids are practically insoluble in water; instead, they form a floating film on the water surface. The polar carboxyl groups in this film are oriented toward the water, while the hydrophobic tails protrude into the gaseous phase. The solubility of the acids increases with decreasing carbon number; butyric acid is completely soluble in water.

Ethyl ether is the best solvent for stearic acid and other saturated long-chain fatty acids since it is sufficiently polar to attract the carboxyl groups. A truly nonpolar solvent, such as petroleum benzine, is not suitable for free fatty acids.

The solubility of fatty acids increases with an increase in the number of cis double bonds. This is illustrated in Fig. 3.3 with acetone as a solvent. The observed differences in solubility can be utilized for separation of saturated from unsaturated fatty acids. The mixture of acids is dissolved at room temperature and cooled stepwise to $-80\,^{\circ}C$. However, the separation efficiency of such a fractional crystallization is limited since, for example, stearic acid is substantially more soluble in acetone containing oleic acid than in pure acetone. This mutual effect on solubility has not been considered in Fig. 3.3.

3.2.2.5 UV-Absorption

All unsaturated fatty acids which contain an isolated cis double bond absorb UV light at a wavelength close to 190 nm. Thus, the acids can not be distinguished spectrophotometrically.

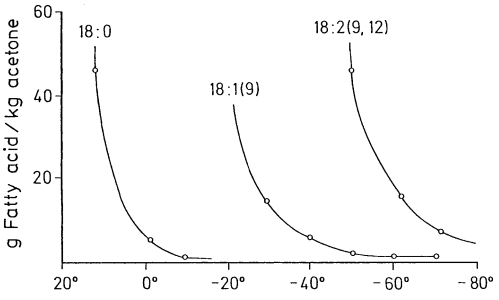


Fig. 3.3. Fatty acid solubility in acetone (according to *Mead* et al., 1965)

Conjugated fatty acids absorb light at various wavelenths depending on the length of conjugation and configuration of the double bond system. Figure 3.4 illustrates such behavior for several fatty acids. See 3.2.3.2.2 for the conversion of an isolene-type fatty acid into a conjugated fatty acid.

3.2.3 Chemical Properties

3.2.3.1 Methylation of Carboxyl Groups

The carboxyl group of a fatty acid must be depolarized by methylation in order to facilitate gas chromatographic separation or separation by fractional distillation. Reaction with diazomethane is preferred for analytical purposes. Diazomethane is formed by alkaline hydrolysis of N-nitroso-N-methyl-p-toluene sulfonamide.

The gaseous CH_2N_2 released by hydrolysis is swept by a stream of nitrogen into a receiver containing the fatty acid solution in ether-methanol (9:1 v/v). The reaction:

$$R-COOH + CH_2N_2 \longrightarrow R-COOCH_3 + N_2 \qquad (3.9)$$

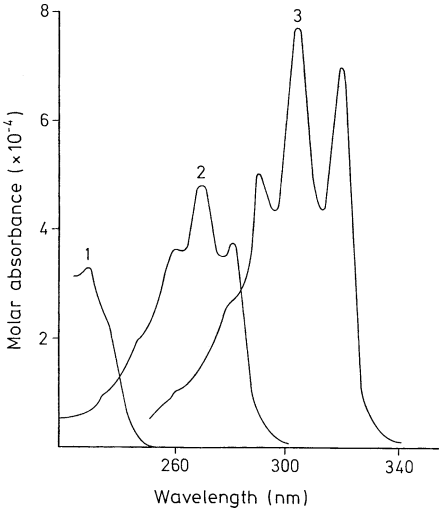


Fig. 3.4. Electron excitation spectra of conjugated fatty acids (according to *Pardun*, 1976). 1 9,11-isolinoleic acid, 2 α-eleostearic acid, 3 parinaric acid

proceeds under mild conditions without formation of by-products. Further possibilities for methylation include: esterification in the presence of excess methanol and a *Lewis* acid (BF_3) as a catalyst; or the reaction of a fatty acid silver salt with methyl iodide:

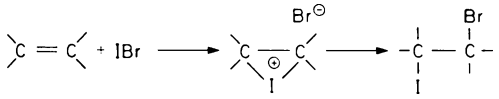
$$R-COOAg + CH_3l \longrightarrow R-COOCH_3 + AgI, \tag{3.10}$$

3.2.3.2 Reactions of Unsaturated Fatty Acids

A number of reactions which are known for olefinic hydrocarbons play an important role in the analysis and processing of lipids containing unsaturated fatty acids.

3.2.3.2.1 Halogen Addition Reactions

The number of double bonds present in an oil or fat can be determined through their iodine number (cf. 14.5.2.1). The fat or oil is treated with a halogen reagent which reacts only with the double bonds. Substitution reactions generating hydrogen halides must be avoided. IBr in an inert solvent, such as glacial acetic acid, is a suitable reagent:

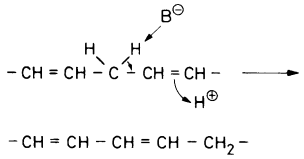


(3.11)

The number of double bonds is calculated by titrating the unreacted IBr reagent with thiosulfate.

3.2.3.2.2 Transformation of Isolene-Type Fatty Acids to Conjugated Fatty Acids

Allyl systems are labile and are readily converted to a conjugated double bond system in the presence of a base (KOH or K-tertbutylate):

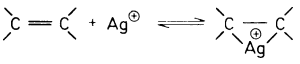


(3.12)

During this reaction, an equilibrium is established between the isolene and the conjugated forms of the fatty acid, the equilibrium state being dependent on the reaction conditions. This isomerization is used analytically since it provides a way to simultaneously determine linoleic, linolenic and arachidonic acids in a fatty acid mixture. The corresponding conjugated diene, triene and tetraene systems of these fatty acids have a maximum absorbance at distinct wavelengths (cf. Fig. 3.4). The assay conditions can be selected to isomerize only the naturally occurring cis double bonds and to ignore the trans fatty acids formed, for instance, during oil hardening (cf. 14.4.2).

3.2.3.2.3 Formation of a π-Complex with Ag^+ Ions

Unsaturated fatty acids or their triacylglycerols, as well as unsaturated aldehydes obtained through autoxidation of lipids (cf. 3.7.2.1.5), can be separated by "argentation chromatography". The separation is based on the number, position and configuration of the double bonds present. The separation mechanism involves interaction of the π-electrons of the double bond with Ag^+ ions, forming a reversible π-complex of variable stability:

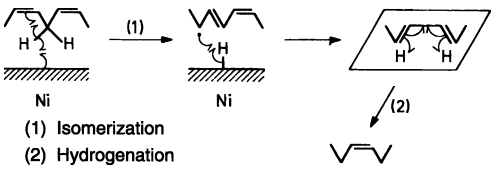


(3.13)

The complex stability increases with increasing number of double bonds. This means a fatty acid with two cis double bonds will not migrate as far as a fatty acid with one double bond on a thin-layer plate impregnated with a silver salt. The R_f values increase for the series 18:2 (9, 12) < 18 :1 (9) < 18:0. Furthermore, fatty acids with isolated double bonds form a stronger Ag^+ complex than those with conjugated bonds. Also, the complex is stronger with a cis- than with a trans-configuration. The complex is also more stable, the further the double bond is from the end of the chain. Finally, a separation of nonconjugated from conjugated fatty acids and of isomers that differ only in their double bond configuration is possible by argentation chromatography.

3.2.3.2.4 Hydrogenation

In the presence of a suitable catalyst, e. g. Ni, hydrogen can be added to the double bond of an acyl lipid. This heterogeneous catalytic hydrogenation occurs stereo selectively as a cis-addition. Catalyst-induced isomerization from an isolene-type fatty acid to a conjugated fatty acid occurs with fatty acids with several double bonds:



(3.14)

Since diene fatty acids form a more stable complex with a catalyst than do monoene fatty acids, the former are preferentially hydrogenated. Since nature is not an abundant source of the solid fats which are required in food processing, the partial and selective hydrogenation, just referred to, plays an important role in the industrial processing of fats and oils (cf. 14.4.2).

3.2.4 Biosynthesis of Unsaturated Fatty Acids

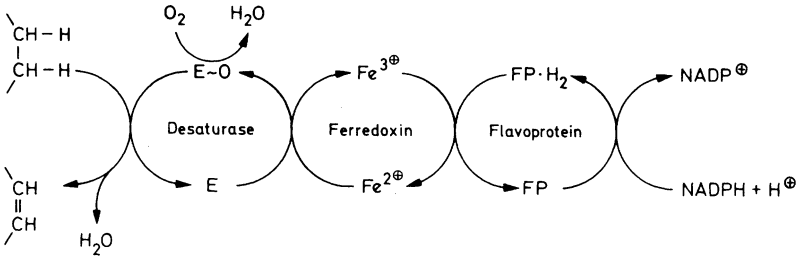
The biosynthetic precursors of unsaturated fatty acids are saturated fatty acids in an activated form (cf. a biochemistry textbook). These are aerobiocally and stereospecifically dehydrogenated by dehydrogenase action in plant as well as animal tissues. A flavoprotein and ferredoxin are involved in plants in the electron transport system which uses oxygen as a terminal electron acceptor (cf. Reaction 3.15).

To obtain polyunsaturated fatty acids, the double bonds are introduced by a stepwise process. A fundamental difference exists between mammals and plants. In the former, oleic acid synthesis is possible, and, also, additional double bonds can be inserted towards the carboxyl end of the fatty acid molecule. For example, γ-linolenic acid can be formed from the essential fatty acid linoleic acid and, also, arachidonic acid (Fig. 3.5) can be formed by chain elongation of γ-linolenic acid. In a diet deficient in linoleic acid, oleic acid is dehydrogenated to isolinoleic acid and its derivatives (Fig. 3.5), but none of these acquire the physiological function of an essential acid such as linoleic acid.

Plants can introduce double bonds into fatty acids in both directions: towards the terminal CH_3-group or towards the carboxyl end. Oleic acid (oleoyl-CoA ester or β-oleoyl-phosphatidylcholine) is thus dehydrogenated to linoleic and then to linolenic acid. In addition synthesis of the latter can be achieved by another pathway involving stepwise dehydrogenation of lauric acid with chain elongation reactions involving C_2 units (Fig. 3.5).

3.3 Acylglycerols

Acylglycerols (or acylglycerides) comprise the mono-, di- or triesters of glycerol with fatty acids (Table 3.1). They are designated as neutral lipids. Edible oils or fats consist nearly completely of triacylglycerols.



(3.15)

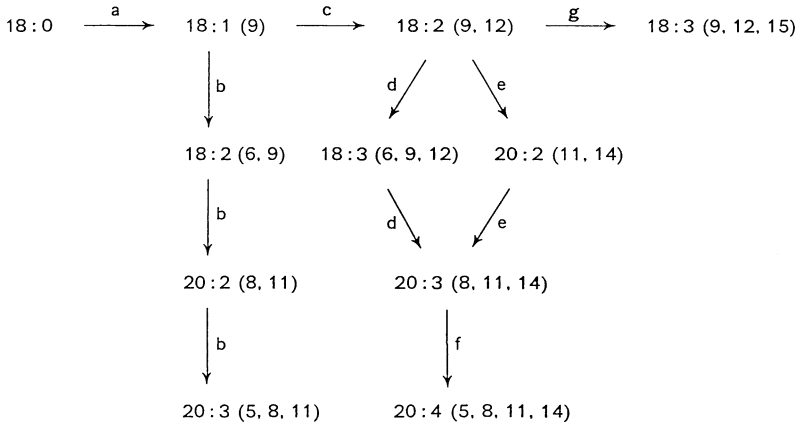


Fig. 3.5. Biosynthesis of unsaturated fatty acids. Synthesis pathways: a, c, g in higher plants; a, c, g and a, c, d, f in algae; a, b and d, f (main pathway for arachidonic acid) or e, f in mammals

3.3.1 Triacylglycerols (TG)

3.3.1.1 Nomenclature, Classification, Calorific Value

Glycerol, as a trihydroxylic alcohol, can form triesters with one, two or three different fatty acids. In the first case a triester is formed with three of the same acyl residues (e. g. tripalmitin; P_3). The mixed esters involve two or three different acyl residues, e. g., dipalmito-olein (P_2O) and palmito-oleo-linolein (POL). The rule of this shorthand designation is that the acid with the shorter chain or, in the case of an equal number of carbons in the chain, the chain with fewer double bonds, is mentioned first. The Z number gives the possible different triacylglycerols which can occur in a fat (oil), where n is the number of different fatty acids identified in that fat (oil):

$$Z = \frac{n^3 + n^2}{2} \tag{3.16}$$

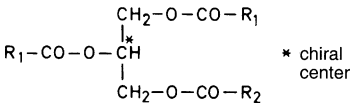
For $n = 3$, the possible number of triglycerols (Z) is 18. However, such a case where a fat (oil) contains only three fatty acids is rarely found in nature. One exception is Borneo tallow (cf. 14.3.2.2.3), which contains essentially only 16:0, 18:0 and 18:1 (9) fatty acids.

Naturally, the Z value also takes into account the number of possible positional isomers within a molecule, for example, by the combination of POS, PSO and SOP. When only positional isomers are considered and the rest disregarded, Z is reduced to Z′:

$$Z' = \frac{n^3 + 3n^2 + 2n}{6} \tag{3.17}$$

Thus, when $n = 3$, $Z' = 10$.

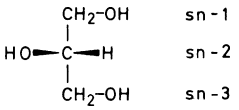
A chiral center exists in a triacylglycerol when the acyl residues in positions 1 and 3 are different:



(3.18)

In addition enantiomers may be produced by 1-monoglycerides, all 1,2-diglycerides and 1,3-diglycerides containing unlike substituents.

In the stereospecific numbering of acyl residues (prefix sn), the L-glycerol molecule is shown in the *Fischer* projection with the secondary HO-group pointing to the left. The top carbon is then denoted C-1. Actually, in a *Fischer* projection, the horizontal bonds denote bonds in front and the vertical bonds those behind the plane of the page:



(3.19)

For example, the nomenclature for a triacylglycerol which contains P, S and O:

sn-POS = sn-1-Palmito-2-oleo-3-stearin .

This assertion is only possible when a stereospecific analysis (cf. 3.3.1.4) provides information on the fatty acids at positions 1, 2 and 3. rac-POS = sn-POS and sn-SOP in the molar ratio 1:1, i. e. the fatty acid in position 2 is fixed while the other two acids are equally distributed at positions 1 and 3.

POS = mixture of sn-POS, sn-OPS, sn-SOP, sn-PSO, sn-OSP and sn-SPO

The physiological calorific value of TGs depends on the fatty acid composition. In the case of TGs with fatty acids of medium chain length (6–10 C atoms), the calorific value decreases from 9 to 7 kcal/g and in the case of asymmetric TGs, e. g., a combination of 2:0, 3:0 or 4:0 with 18:0, it decreases to 5 kcal/g. These special TGs, which are available only synthetically, are classified as fat substitutes (cf. 8.16).

Table 3.12. Triacylglycerols and their polymorphic forms

Compound	Melting point (°C) of polymorphic form		
	α	β′	β
Tristearin	55	63.2	73.5
Tripalmitin	44.7	56.6	66.4
Trimyristin	32.8	45.0	58.5
Trilaurin	15.2	34	46.5
Triolein	−32	−12	4.5–5.7
1,2-Dipalmitoolein	18.5	29.8	34.8
1,3-Dipalmitoolein	20.8	33	37.3
1-Palmito-3-stearo-2-olein	18.2	33	39
1-Palmito-2-stearo-3-olein	26.3	40.2	
2-Palmito-1-stearo-3-olein	25.3	40.2	
1,2-Diacetopalmitin	20.5	21.6	42.3

3.3.1.2 Melting Properties

TG melting properties are affected by fatty acid composition and their distribution within the glyceride molecule (Table 3.12).

Mono-, di- and triglycerides are polymorphic, i. e. they crystallize in different modifications, denoted as α, β′ and β. These forms differ in their melting points (Table 3.12) and crystallographic properties.

During the cooling of melted acylglycerols, one of the three polymorphic forms is yielded. This depends also on the temperature gradient chosen. The α-form has the lowest melting point. This modification is transformed first into the β′-form upon heating and then into the β-form. The β-form is the most stable and, hence, also has the highest melting point (Table 3.12). These changes are typically monotropic, i. e. they proceed in the order of lower to higher stability.

Crystallization of triglycerides from a solvent system generally yields β-form crystals.

X-ray analysis as well as measurements by Raman spectroscopy revealed that saturated triglycerols in their crystalline state exist in a "chair form" (Fig. 3.6a): The "tuning fork" configuration for the β′-modification was not verified. The different properties of the three forms are based on the crystallization in different systems.

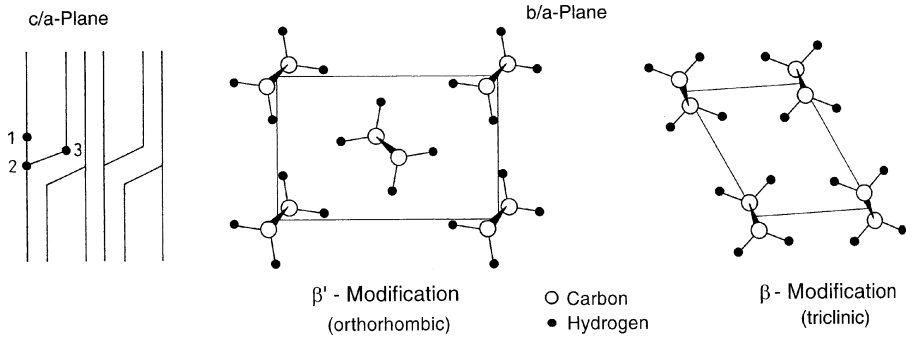


Fig. 3.6. Arrangement of the β′- and β-form of saturated triacylglycerols in the crystalline lattice (Cartesian coordinates a, b, c)

Table 3.13. Crystallization patterns of edible fats or oils

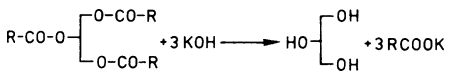
β-Type	β′-Type	β-Type	β′-Type
Coconut oil	Cottonseed oil	Peanut	
Corn germ oil	Butter	Sunflower oil	Whale oil
Olive oil	Palm oil	Lard	
Palm seed oil	Rapeseed oil		

α-form: hexagonal system; the melting point is relatively low, since areas of the methyl ends are freely arranged as in liquid crystals.
β′-form: (Fig. 3.6b): orthorhombic system; the carbon chains are perpendicular to each other.
β-form: (Fig. 3.6c): triclinic system; parallel arrangement of the carbon chains.
Unsaturated fatty acids interfere with the orderly packing of molecules in the crystalline lattice, thereby causing a decrease in the melting point of the crystals.
TG such as 1,3-diaceto-palmitin, i. e. a triglyceride with one long and two short-chain fatty acids, exists in the exceptionally stable α-form. Since films of such TGs can expand by 200 to 300 times their normal length, they are of interest for application as protective coating for fat-containing foods. In edible fats and oils, more than the three mentioned polymorphic forms can be present, e. g., 4–6 forms are being discussed for cocoa butter. In order to classify fats and oils, that form is used that is predominant after solidification (Table 3.13).

3.3.1.3 Chemical Properties

Hydrolysis, methanolysis and interesterification are the most important chemical reactions for TGs.

Hydrolysis. The fat or oil is cleaved or saponified by treatment with alkali (e. g. alcoholic KOH):

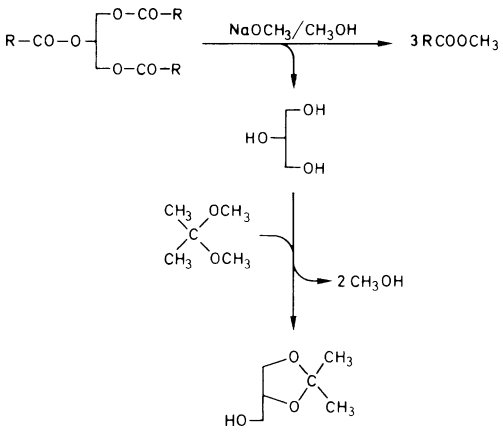


(3.20)

After acidification and extraction, the free fatty acids are recovered as alkali salts (commonly called soaps). This procedure is of interest for analysis of fat or oil samples. Commercially, the free fatty acids are produced by cleaving triglycerides with steam under elevated pressure and temperature and by increasing the reaction rate in the presence of an alkaline catalyst (ZnO, MgO or CaO) or an acidic catalyst (aromatic sulfonic acid).

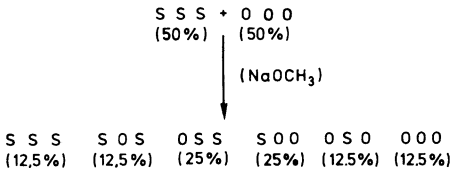
Methanolysis. The fatty acids in TG are usually analyzed by gas liquid chromatography, not as free acids, but as methyl esters. The required transesterification is most often achieved by Na-methylate (sodium methoxide) in methanol and in the presence of 2,2-dimethoxypropane to bind the released glycerol. Thus, the reaction proceeds rapidly and quantitatively even at room temperature.

Interesterification. This reaction is of industrial importance (cf. 14.4.3) since it can change the physical properties of fats or oils or their mixtures without altering the chemical structure of the fatty acids. Both intra- and inter-molecular acyl residue exchanges occur in the reaction until an equilibrium is reached which depends on the structure and composition of the TG molecules. The usual catalyst for interesterification is Na-methylate.
The principle of the reaction will be elucidated by using a mixture of tristearin (SSS) and triolein (OOO) or stearodiolein (OSO). Two types of interesterification are recognized:



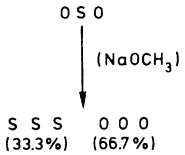
(3.21)

a) A single-phase interesterification where the acyl residues are randomly distributed:



(3.22)

b) A directed interesterification in which the reaction temperature is lowered until the higher melting and least soluble TG molecules in the mixture crystallize. These molecules cease to participate in further reactions, thus the equilibrium is continuously changed. Hence, a fat (oil) can be divided into high and low melting point fractions, e. g.:



(3.23)

3.3.1.4 Structural Determination

Apart from identifying a fat or oil from an unknown source (cf. 14.5.2), TG structural analysis is important for the clarification of the relationship existing between the chemical structure and the melting or crystallization properties, i. e. the consistency.

An introductory example: cocoa butter and beef tallow, the latter used during the past century for adulteration of cocoa butter, have very similar fatty acid compositions, especially when the two main saturated fatty acids, 16:0 and 18:0, are considered together (Table 3.14). In spite of their compositions, the two fats differ significantly in their melting properties. Cocoa butter is hard and brittle and melts in a narrow temperature range (28–36 °C). Edible beef tallow, on the other hand, melts at a higher temperature (approx. 45 °C) and over a wider range and has a substantially better plasticity. The melting property of cocoa butter is controlled by the presence of a different pattern of triglycerols: SSS, SUS and SSU (cf. Table 3.14). The chemical composition of Borneo tallow (Tenkawang fat) is so close to that of cocoa butter that the TG distribution patterns shown in Table 3.14 are practically indistinguishable. Also, the melting properties of the two fats are similar, consequently, Borneo tallow is currently used as an important substitute for cocoa butter. Analysis of the TGs present in fat (oil) could be a tedious task, when numerous TG compounds have to be separated. The composition of milk fat is particularly complex. It contains more than 150 types of TG molecules.

Table 3.14. Average fatty acid and triacylglycerol composition (weight-%) of cocoa butter, tallow and Borneo tallow (a cocoa butter substitute)

	Cocoa butter	Edible beef tallow	Borneo tallow[a]
16:0	25	36	20
18:0	37	25	42
20:0	1		1
18:1 (9)	34	37	36
18:2 (9,12)	3	2	1
SSS[b]	2	29	4
SUS	81	33	80
SSU	1	16	1
SUU	15	18	14
USU		2	
UUU	1	2	1

[a] cf. 14.3.2.2.3

[b] S: Saturated, and U: unsaturated fatty acids.

The separation by HPLC using reverse phases is the first step in TG analysis. It is afforded by the chain length and the degree of unsaturation of the TGs. As shown in Fig. 3.7 the oils from different plant sources yield characteristic patterns in which distinct TGs predominate.

TGs differing only in the positions of the acyl residues are not separated. However, in some cases it is possible to separate positional isomeric triglycerols after bromination of the double bonds because triglycerols with a brominated acyl group in β-position are more polar compared to those in α-position.

The separation capacity of the HPLC does not suffice for mixtures of plant oils with complex triglycerol composition. Therefore it is advisiable to perform a preseparation of the triglycerols according to their number of double bonds by "argentation chromatography" (cf. 3.2.3.2.3).

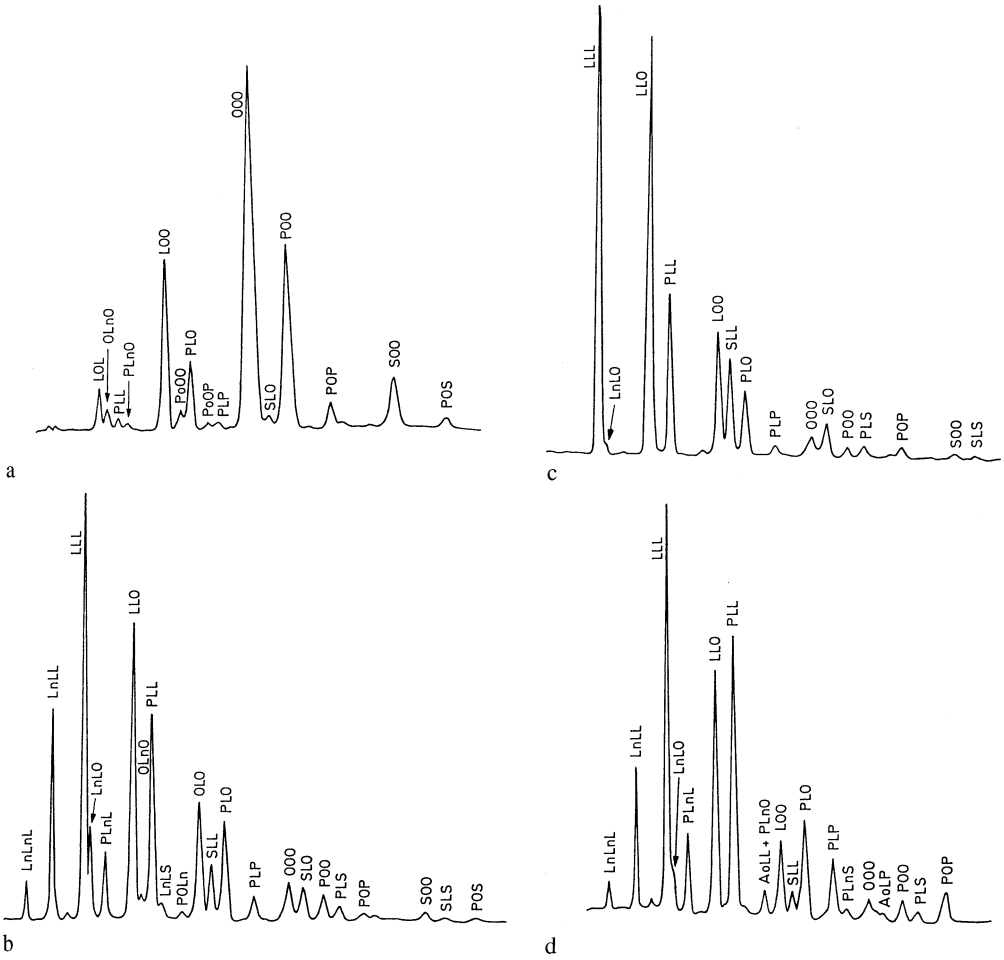


Fig. 3.7. Composition of triacylglycerols present in edible fats or oils as determined by HPLC. **a** Olive oil, **b** soybean oil, **c** sunflower oil, **d** wheat germ oil. Fatty acids: P palmitic, S stearic, O oleic, L linoleic, Ln linolenic, Ao eicosanoic

Various hypotheses have been advanced, supported by results of TG biosynthesis, to predict the TG composition of a fat or oil when all the fatty acids occurring in the sample are known. The values calculated with the aid of the *1,3-random-2-random* hypothesis agree well with values found experimentally for plant oil or fat. The hypothesis starts with two separated fatty acid pools. The acids in both pools are randomly distributed and used as such for TG biosynthesis. The primary HO-groups (positions 1 and 3 of glycerol) from the first pool are esterified, while the secondary HO-group is esterified in the second pool. The proportion of each TG is then determined (as mole-%):

$$\beta\text{-XYZ(mol-\%)} = 2 \cdot \left[\begin{matrix}\text{mol-\%} \times \text{in} \\ \text{1,3-Position}\end{matrix}\right] \cdot \left[\begin{matrix}\text{mol-\%Y in} \\ \text{2-Position}\end{matrix}\right] \cdot \left[\begin{matrix}\text{mol-\%Z in} \\ \text{1,3-Position}\end{matrix}\right] \cdot 10^{-4} \tag{3.24}$$

The data required in order to apply the formula are obtained as follows: after partial hydrolysis of fat (oil) with pancreatic lipase (cf. 3.7.1.1), the

Table 3.15. Triacylglycerol composition (mole-%) of a sunflower oil.
A comparison of experimental values with calculated values based on a 1,3-random-2-random hypothesis

Triacylglycerol[a]	Found	Calculated	Triacylglycerol[a]	Found	Calculated
β-StOSt	0.3	0.5	β-OStL	0.5	0.2
β-StStO	0.2	trace	β-OOL	8.1	6.5
β-StOO	2.3	1.6	β-OLO	3.1	4.2
β-OStO	0.1	trace	β-StLL	13.2	14.0
β-StStL	0.3	0.2	β-LStL	1.3	0.3
β-StLSt	2.2	1.7	β-OLL	20.4	21.9
OOO	1.3	1.2	β-LOL	8.4	8.7
β-StOL	4.4	4.2	LLL	28.1	28.9
β-StLO	4.0	5.3	Others	0.9	0.9

St: Stearic, O: oleic, and L: linoleic acid.
[a] Prefix β: The middle fatty acid is esterified at the β- or sn-2-position, the other two acids are at the sn-1 or sn-3 positions.

fatty acids bound at positions 1 and 3 are determined. The fatty acids in position 2 are calculated from the difference between the total acids and those acids in positions 1 and 3.

Table 3.15 illustrates the extent of agreement for the TG composition of sunflower oil obtained experimentally and by calculation using the 1,3-random-2-random hypothesis. However, both approaches disregard the differences between positions 1 and 3. In addition, the hypothesis is directed to plants, of which the fats and oils consist of only major fatty acids.

Stereospecific Analysis. Biochemically, the esterified primary OH-groups of glycerol can be differentiated from each other; thus, the determination of fatty acids in positions 1, 2 and 3 is possible. The reactions carried out are presented in Fig. 3.8. First, the TG (I) is hydrolyzed under controlled conditions to a diacylglycerol using pancreatic lipase (cf. 3.7.1.1). Phosphorylation with a diacylglycerol kinase follows. The enzyme reacts stereospecifically since it phosphorylates only the 1,2- or (S)- but not the 2,3-diglycerol. Subsequently, compound I is hydrolyzed to a monoacylglycerol (III). The distribution of the acyl residues in positions 1, 2 and 3 is calculated from the results of the fatty acid analysis of compounds I, II and III.

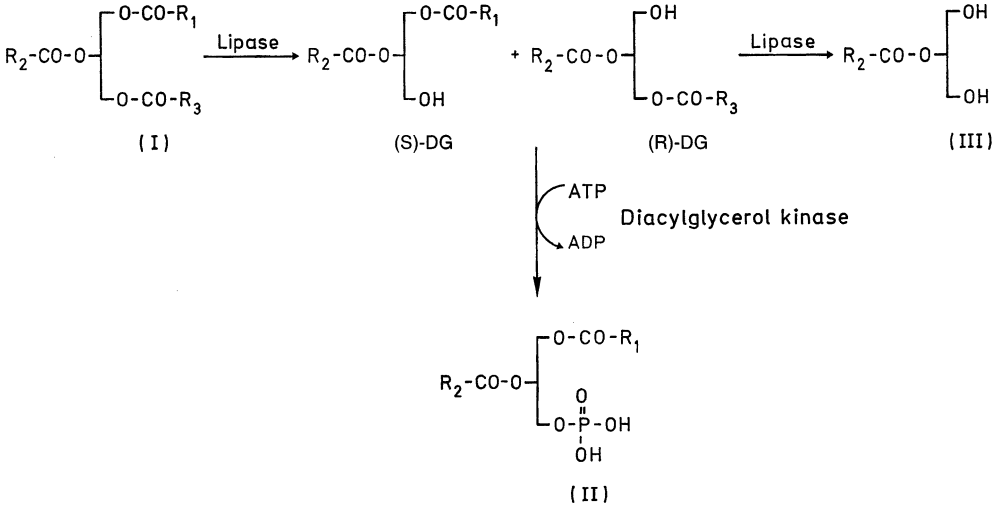


Fig. 3.8. Enzymatic stereospecific analysis of triacylglycerols

Alternatively, the stereo-specific analysis can be carried out chemically. The TGs are partially hydrolyzed in the presence of ethyl magnesium bromide. The resulting diacylglycerols are isolated and their OH groups converted to urethane with (S)-1-(1-naphthyl)ethylisocyanate. The sn-1,3- and the diastereomeric sn-1,2- and 2,3-di-acylglycerol urethane derivatives are separated in a subsequent HPLC step. The fatty acid analysis of the urethanes show the distribution of the acyl residues in positions 1, 2 and 3.

Individual TGs or their mixtures can be analyzed with these procedures. Based on these results (some are presented in Table 3.16), general rules for fatty acid distribution in plant oils or fats can be deduced:

- The primary HO-groups in positions 1 and 3 of glycerol are preferentially esterified with saturated acids.
- Oleic and linolenic acids are equally distributed in all positions, with some exceptions, such as cocoa butter (cf. Table 3.16).
- The remaining free position, 2, is then filled with linoleic acid.

Results compiled in Table 3.16 show that for oil or fat of plant origin, the difference in acyl residues between positions 1 and 3 is not as great as for TGs of animal origin (e. g., chicken egg). Therefore, the 1,3-random-2-random hypothesis can provide results that agree well with experimental findings.

The fatty acid pattern in animal fats is strongly influenced by the fatty acid composition of animal feed. A steady state is established only after 4–6 months of feeding with the same feed composition. The example of chicken egg (Table 3.16) indicates that positions 1 and 3 in triglycerides of animal origin show much greater variability than in fats or oils of plant origin. Therefore, any prediction of TG types in animal fat should be calculated from three separate fatty acid pools (*1-random-2-random-3-random* hypothesis).

The specific distribution of saturated fatty acids in the triglycerols of fats and oils of plant origin serves as an evidence of *ester oils*.

Ester oils are produced by esterification of glycerol with purified fatty acids obtained from olive oil residues. In this case the saturated acyl groups are equally distributed between all three positions of the glycerol molecule, whereas

Table 3.16. Results of stereospecific analysis of some fats and oils[a]

Fat/Oil	Position	16:0	18:0	18:1 (9)	18:2 (9,12)	18:3 (9,12,15)
Peanut	1	13.6	4.6	59.2	18.5	–
	2	1.6	0.3	58.5	38.6	–
	3	11.0	5.1	57.3	18.0	–
Soya	1	13.8	5.9	22.9	48.4	9.1
	2	0.9	0.3	21.5	69.7	7.1
	3	13.1	5.6	28.0	45.2	8.4
Sun-	1	10.6	3.3	16.6	69.5	–
flower	2	1.3	1.1	21.5	76.0	–
	3	9.7	9.2	27.6	53.5	–
Olive	1	15.2	2.9	68.6	11.0	–
	2	2.5	0.6	81.0	14.6	–
	3	19.6	5.2	62.6	9.4	–
Palm	1	60.1	3.4	26.8	9.3	–
	2	13.3	0.2	67.9	17.5	–
	3	71.9	7.6	14.4	3.2	–
Cocoa	1	34.0	50.4	12.3	1.3	–
	2	1.7	2.1	87.4	8.6	–
	3	36.5	52.8	8.6	0.4	–
Chicken	1	68.2	6.0	12.4	2.3	–
egg	2	4.8	0.3	60.8	31.3	–
	3	8.9	7.7	69.4	5.4	–

[a] Values in mol%. In order to simplify the Table other fatty acids present in fat/oil are not listed.

in olive oil saturated acyl groups are attached to position 1 and 3. As proof, the amount of 2-MG containing palmitic acid is determined after hydrolysis of the triglycerols with a lipase (pancreas). Values above 2% are indicative of an adulteration of the olive oil with an ester oil.

The positional specific distribution of palmitic acid is unfavorable for the use of fats and oils of plant origin in infant food, as this acid is liberated by lipolysis in the gastric tract. Palmitic acid then forms insoluble salts with Ca^{2+}-ions from the food, possibly resulting in severe bilious attacks. The fatty acids of human milk consist of up to 25% of palmitic acid; 70% are bound to the 2-position of the triglycerols. During lipolysis 2-monopalmitin is formed that is easily resorbed.

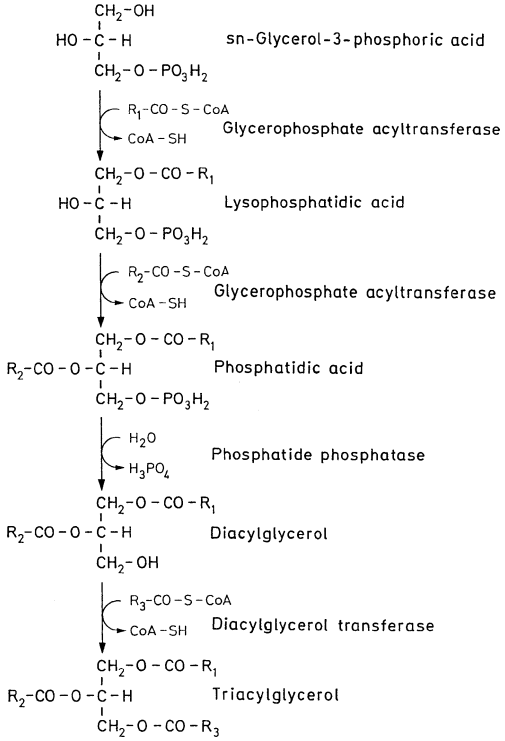


Fig. 3.9. Biosynthesis of triacylglycerols

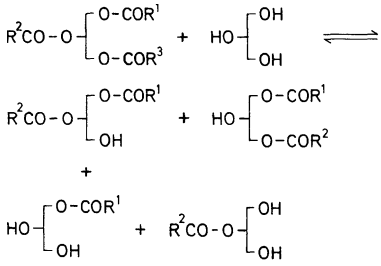
3.3.1.5 Biosynthesis

A TG molecule is synthesized in the fat cells of mammals and plants from L-glycerol-3-phosphate and fatty acid-CoA esters (Fig. 3.9). The L-glycerol-3-phosphate supply is provided by the reduction of dihydroxy acetone phosphate by NAD^+-dependent glycerol phosphate dehydrogenase. The dihydroxy acetone phosphate originates from glycolysis.

The lipid bodies (oleosomes, spherosomes) synthesized are surrounded by a membrane and are deposited in storage tissues.

The TG fatty acid composition within a plant species depends on the environment, especially the temperature. A general rule is that plants in cold climates produce a higher proportion of unsaturated fatty acids. Obviously, the mobility of TGs is thus retained. In the sunflower (cf. Fig. 3.10), this rule is highly pronounced; whereas in safflower, only a weak response to temperature variations is observed (Fig. 3.10).

els may be increased by the action of hydrolases during food storage or processing. MG and DG are produced commercially by fat glycerolysis (200 °C, basic catalyst)



(3.25)

From the equilibrium (cf. Formula 3.25) that contains 40–60% MG, 45–35% DG and 15–5% TG, the MG are separated by distillation under high vacuum. The amount of 1-MG (90–95%) is predominant over the amount of 2-MG.

3.3.2 Mono- and Diacylglycerols (MG, DG)

3.3.2.1 Occurrence, Production

The occurrence of MG and DG in edible oils or fats or in raw food is very low. However, their lev-

3.3.2.2 Physical Properties

MG and DG crystallize in different forms (polymorphism; cf. 3.3.1.2). The melting point of an ester of a given acid increases for the series 1,2-DG < TG < 2-MG < 1,3-DG < 1-MG:

	Melting Point (°C) β-form
Tripalmitin	65.5
1,3-Dipalmitin	72.5
1,2-Dipalmitin	64.0
1-Palmitin	77.0
2-Palmitin	68.5

MG and DG are surface-active agents. Their properties can be further modified by esterification with acetic, lactic, fumaric, tartaric or citric acids. These esters play a significant role as emulsifiers in food processing (cf. 8.15.3.1).

3.4 Phospho- and Glycolipids

3.4.1 Classes

Phospho- and glycolipids, together with proteins, are the building blocks of biological membranes. Hence, they invariably occur in all foods of animal and plant origin. Examples are compiled in Table 3.17. As surface-active compounds, phospho- and glycolipids contain hydrophobic moieties (acyl residue, N-acyl sphingosine) and hydrophilic portions (phosphoric acid, carbohydrate). Therefore, they are capable of forming orderly structures (micelles or planar layers) in aqueous media; the bilayer structures are found in all biological membranes. Examples for the composition of membrane lipids are listed in Table 3.18.

3.4.1.1 Phosphatidyl Derivatives

The following phosphoglycerides are derived from phosphatidic acid. Phosphatidyl choline or lecithin (phosphate group esterified with the

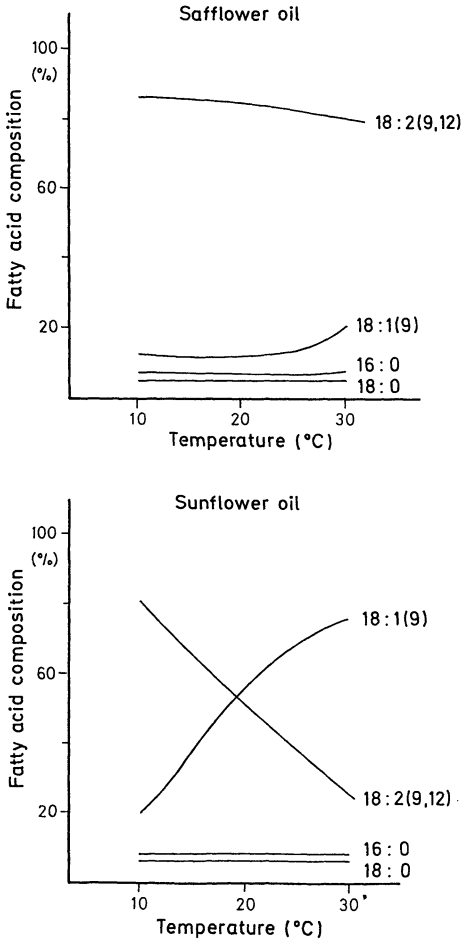


Fig. 3.10. The effect of climate (temperature) on the fatty acid composition of triacylglycerols

Table 3.17. Composition of lipids of various foods[a]

	Milk	Soya	Wheat	Apple
Total lipids	3.6	23.0	1.5	0.088
Triacylglycerols	94	88	41	5
Mono-, and diacylglycerols	1.5		1	
Sterols	< 1		1	15
Sterol esters			1	2
Phospholipids	1.5	10	20	47
Glycolipids		1.5	29	17
Sulfolipids				1
Others		0.54	7	15

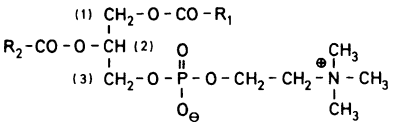
[a] Total lipids as %, while lipid fractions are expressed as percent of the total lipids.

Table 3.18. Occurrence of phosphatidyl derivates

Food	Lipid (g/kg)	P-containing lipids[a] (g/kg) PC	Phosphatidyl derivatives[b] (mg/kg)			
			PS	PE	PI	
Milk	37.8	0.35	120	10	100	2
Egg	113	35.1	27,000	¾	5810	¾
Meat (beef)	19	8.3	4290	690	1970	¾
Meat (chicken)	62	6.6	3320	850	1590	¾
Tuna fish	155	19.4	6410	1940	5030	¾
Potato	1.1	0.56	280	10	160	90
Rice	6.2	0.89	320	30	350	¾
Soybean	183	17.8	7980	¾	4660	2500

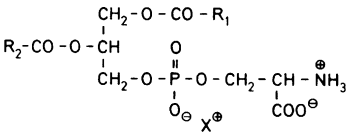
[a] Phosphatidyl derivatives and other P-containing lipids, e. g., plasmalogens, sphingomyelins.
[b] The abbreviations correspond to Formulas 3.26 – 3.29.

OH-group of choline, PC):



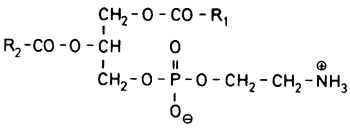
(3.26)

Phosphatidyl serine (phosphate group esterified with the HO-group of the amino acid serine, PS):



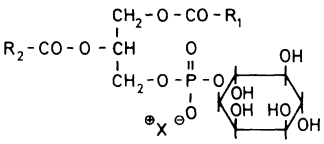
(3.27)

Phosphatidyl ethanolamine (phosphate group esterified with ethanolamine, PE):



(3.28)

Phosphatidyl inositol (phosphate group esterified with inositol, PI):



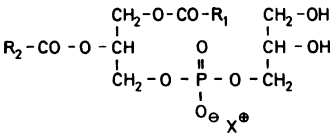
(3.29)

A mixture of phosphatidyl serine and phosphatidyl ethanolamine was once referred to as cephalin.

Examples of foods which contain phosphatidyl derivatives are shown in Table 3.18. The differences to the data in Table 3.17 are caused by the biological range of variations.

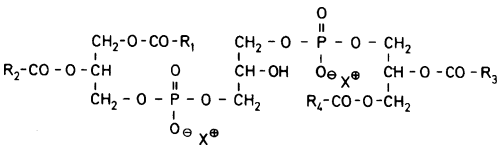
Only one acyl residue is cleaved by hydrolysis (cf. 3.7.1.2.1) with phospholipase A. This yields the corresponding lyso-compounds from lecithin or phosphatidyl ethanolamine. Some of these lyso-derivatives occur in nature, e. g., in cereals.

Phosphatidyl glycerol is invariably found in green plants, particularly in chloroplasts:



L-α-Phosphatidyl-D-glycerol (3.30)

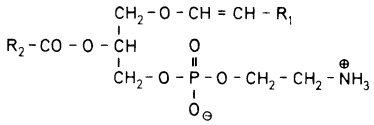
Cardiolipin, first identified in beef heart, is also a minor constituent of green plant lipids. Its chemical structure is diphosphatidyl glycerol:



Diphosphatidyl glycerol (cardiolipin) (3.31)

The plasmalogens occupy a special place in the class of phospho-glycerides. They are phosphatides in which position 1 of glycerol is linked to a straight-chain aldehyde with 16 or 18 carbons. The linkage is an enolether type with a dou-

ble bond in the cis-configuration. Plasmalogens of 1-O-(1-alkenyl)-2-O-acylglycerophospholipid type occur in small amounts in animal muscle tissue and also in milk fat. The enol-ether linkage, unlike the ether bonds of a 1-O-alkylglycerol (cf. 3.6.2), is readily hydrolyzed even by weak acids.



Plasmalogen (3.32)

Phospholipids are sensitive to autoxidation since they contain an abundance of linoleic acid. The other acid commonly present is palmitic acid.

Phospholipids are soluble in chloroform-methanol and poorly soluble in water-free acetone. The pK_s value of the phosphate group is between 1 and 2. Phosphatidyl choline and phosphatidyl ethanolamine are zwitter-ions at pH 7.

Phospholipids can be hydrolyzed stepwise by alcoholic KOH. Under mild conditions, only the fatty acids are cleaved, whereas, with strong alkalies, the base moiety is released. The bonds between phosphoric acid and glycerol or phosphoric acid and inositol are stable to alkalies, but are readily hydrolyzed by acids. Phosphatidyl derivatives, together with triacylglycerols and sterols, occur in the lipid fraction of lipoproteins (cf. 3.5.1).

Lecithin. Lecithin plays a significant role as a surface-active agent in the production of emulsions. "Raw lecithin", especially that of soya and that isolated from egg yolk, is available for use on a commercial scale. "Raw lecithins" are complex mixtures of lipids with phosphatidyl cholines, ethanolamines and inositols as main components (Table 3.19).

The major phospholipids of raw soya lecithin are given in Table 3.19. The manufacturer often separates lecithin into ethanol-soluble and ethanol-insoluble fractions.

Pure lecithin is a W/O emulsifier with a HLB-value (cf. 8.15.2.3) of about 3. The HLB-value rises to 8–11 by hydrolysis of lecithin to lysophosphatidyl choline; an o/w emulsifier is formed. Since the commercial lecithins are complex mixtures of lipids their HBL-values lie within a wide range. The ethanol-insoluble fraction (Table 3.19) is suitable for stabilization of W/O emulsions and the ethanol-soluble fraction for O/W emulsions. To increase the HLB-value, "hydroxylated lecithins" are produced by hydroxylation of the unsaturated acyl groups with hydrogen peroxide in the presence of lactic-, citric- and tartaric acid.

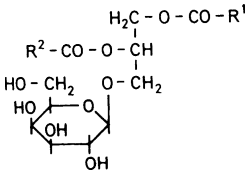
Table 3.19. Composition of glycerol phospholipids in soya "raw lecithin" and in the resulting fractions[a]

	Unfractionated	Ethanol soluble fraction	Ethanol insoluble fraction
Phosphatidyl ethanolamine	13–17	16.3	13.3
Phosphatidyl choline	20–27	49	6.6
Phosphatidyl inositol	9	1	15.2

[a] Values in weight%.

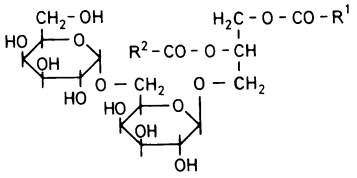
3.4.1.2 Glyceroglycolipids

These lipids consist of 1,2-diacylglycerols and a mono-, di- or, less frequently, tri- or tetrasaccharide bound in position 3 of glycerol. Galactose is predominant as the sugar component among plant glycerolipids. The glyceroglycolipids occurring in wheat influence the baking properties (cf. 15.2.5).



(3.33)

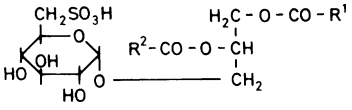
Monogalactosyl diacylglycerol (MGDG)
(1,2-diacyl-3-β-D-galactopyranosyl-L-glycerol)



(3.34)

Digalactosyl diacylglycerol (DGDG)

(1,2-diacyl-3-(α-D-galactopyranosyl-1,6-β-D-galactopyranosyl)-L-glycerol)
6-O-acyl-MGDG and 6-0-acyl-DGDG are minor components of plant lipids.
Sulfolipids are glyceroglycolipids which are highly soluble in water since they contain a sugar moiety esterified with sulfuric acid. The sugar moiety is 6-sulfochinovose. Sulfolipids occur in chloroplasts but are also detected in potato tubers:



(3.35)

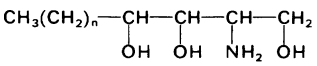
Sulfolipid(1,2-diacyl-(6-sulfo-α-D-chinovosyl-1,3)-L-glycerol)

3.4.1.3 Sphingolipids

Sphingolipids contain sphingosine, an amino alcohol with a long unsaturated hydrocarbon chain (D-*erythro*-1,3-dihydroxy-2-amino-trans-4-octadecene) instead of glycerol:

(3.36)

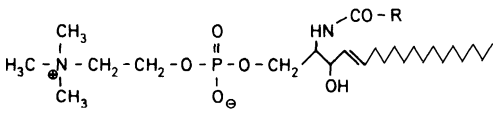
Sphingolipids which occur in plants, e. g., wheat, contain phytosphingosines:



n: 13, 14, 15, 16 (3.37)

The amino group in sphingolipids is linked to a fatty acid to form a carboxy amide, denoted as ceramide. The primary hydroxyl group is either esterified with phosphoric acid (sphingophospholipid: ceramide-phosphate-base) or bound glycosidically to a mono- di-, or oligosaccharide (sphingoglycolipid: ceramide-phosphate-sugar$_n$). In the third group of sphingolipids the ceramide moiety is linked by a phosphate residue to the carbohydrate building blocks. These compounds are also referred to as phytoglycolipids.

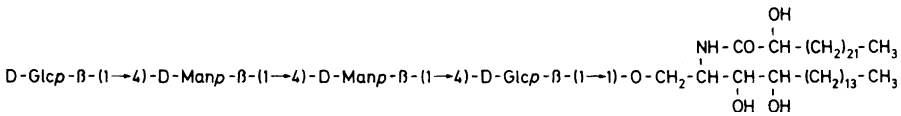
Sphingophospholipids. Sphingomyelin is one example of a sphingophospholipid. It is the most abundant sphingolipid and is found in myelin, the fatty substance of the sheath around nerve fibers. The structure of sphingomyelin is:



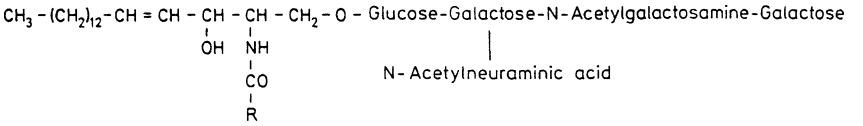
(3.38)

Sphingoglycolipids are found in tissue of animal origin, milk and in plants (especially cereals). Based on structural properties of the carbohydrate building blocks, one differentiates neutral and acid glycosphingolipids. The sulfatides and gangliosides also belong to this group.
Lactosylceramide in milk and the ceramide glycosides of wheat are examples of neutral glycosphingolipids that contain, next to glucose and mannose, also saturated (14:0–28:0) and monounsaturated (16:1–26:1) 2-hydroxy- or 2,3-dihydroxy fatty acids.
Formula 3.40 a depicts a sphingoglycolipid of wheat.
Gangliosides contain sialic acid (N-acetylneuraminic acid; cf. Formula 3.40 b). In the ganglioside fraction of milk monosialosyllactosyl-ceramide (cf. Formula 3.41) was identified.

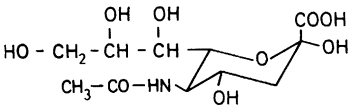
Phytosphingolipids. These lipids also have a complex structure. Total hydrolysis yields phytosphingosine, inositol, phosphoric acid and various monosaccharides (galactose, arabinose, mannose, glucosamine, glucuronic acid).



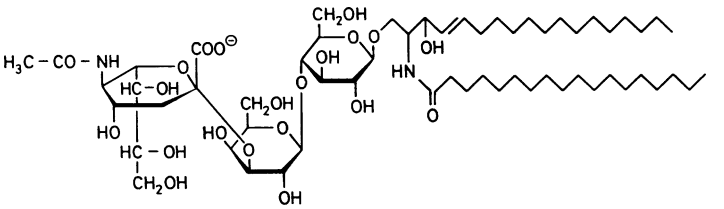
(3.39)



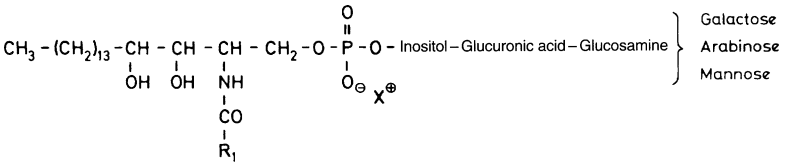
(3.40a)



(3.40b)



(3.41)



(3.42)

Phytosphingolipids are isolated from soya and peanuts (cf. Formula 3.42).

3.4.2 Analysis

3.4.2.1 Extraction, Removal of Nonlipids

A solvent mixture of chloroform/methanol (2 + 1 v/v) is suitable for a quantitative extraction of lipids. Addition of a small amount of BHA (cf. 3.7.3.2.2) is recommended for the stabilization of lipids against autoxidation.

Nonlipid impurities were earlier removed by shaking the extracts with a special salt solution under rather demanding conditions. An improved procedure, by which emulsion formation is avoided, is based on column chromatography with dextran gels.

3.4.2.2 Separation and Identification of Classes of Components

Isolated and purified lipids can be separated into classes by thin layer chromatography using developing solvents of different polarity. Figure 3.12 shows an example of the separation of neutral and polar lipids. Identification of polar lipids is based on using spraying reagents which react on the plates with the polar moiety of the lipid molecules. For example, phosphoric acid is identified by the molybdenum-blue reaction, monosaccharides by orcinol-$FeCl_3$, choline by bismuth iodide (*Dragendorff* reagent), ethanolamine and serine by the ninhydrin reaction, and sphingosine by a chlorine-benzidine reagent.

When sufficient material is available, it is advisable to perform a preliminary separation of lipids by column chromatography on magnesium sili-

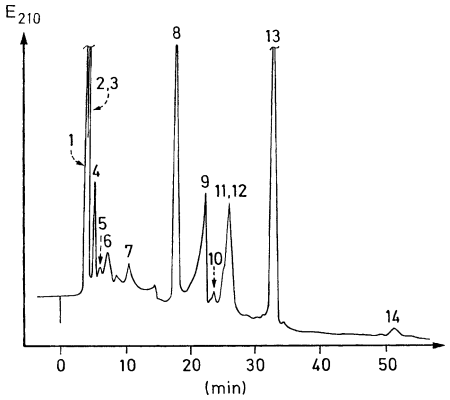


Fig. 3.11. HPLC-analysis of soy "raw lethicin" (according to *Sotirhos* et al. 1986) 1 Triacylglycerols, 2 free fatty acids, 3 phosphatidyl glycerol, 4 cerebrosides, 5 phytosphingosine, 6 diphosphatidyl glycerol, 7 digalactosyldiacyl glycerol, 8 phosphatidyl ethanolamine, 9 phosphatidyl inositol, 10 lysophosphatidyl ethanolamine, 11 phosphatidic acid, 12 phosphatidyl serine, 13 phosphatidyl choline, 14 lysophosphatidyl choline

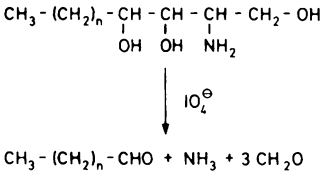
cate (florisil), silicic acid, hydrophobic dextran gel or a cellulose-based ion-exchanger, such as DEAE-cellulose.

Also the HPLC-analysis of phospho- and glycolipids is of growing importance. Figure 3.11 for example demonstrates the separation of soya "raw lecithin".

3.4.2.3 Analysis of Lipid Components

Fatty acid composition is determined after methanolysis of the lipid. For positional analysis of acyl residues (positions 1 or 2 in glycerol), phosphatidyl derivatives are selectively hydrolyzed with phospholipases (cf. 3.7.1.2.1) and the fatty acids liberated are analyzed by gas chromatography.

The sphingosine base can also be determined by gas chromatography after trimethylsilyl derivatization. The length of the carbon skeleton, of interest for phytosphingosine, can be determined by analyzing the aldehydes released after the chain has been cleaved by periodate:



(3.43)

The monosaccharides in glycolipids can also be determined by gas chromatography. The lipids are hydrolyzed with trifluoroacetic acid and then derivatized to an acetylated glyconic acid nitrile. By using this sugar derivative, the chromatogram is simplified because of the absence of sugar anomers (cf. 4.2.4.6).

3.5 Lipoproteins, Membranes

3.5.1 Lipoproteins

3.5.1.1 Definition

Lipoproteins are aggregates, consisting of proteins, polar lipids and triacylglycerols, which are water soluble and can be separated into protein and lipid moieties by an extraction procedure using suitable solvents. This indicates that only noncovalent bonds are involved in the formation of lipoproteins. The aggregates are primarily stabilized by hydrophobic interactions between the apolar side chains of hydrophobic regions of the protein and the acyl residues of the lipid. In addition, there is a contribution to stability by ionic forces between charged amino acid residues and charges carried by the phosphatides. Hydrogen bonds, important for stabilization of the secondary structure of protein, play a small role in binding lipids since phosphatidyl derivatives have only a few sites available for such linkages. Hydrogen bonds could exist to a greater extent between proteins and glycolipids; however, such lipids have not yet been found as lipoprotein components, but rather as building blocks of biological membranes. An exception may be their occurrence in wheat flour, where they are responsible for gluten stability of dough. Here, the lipoprotein complex consists of prolamine and glutelin attached to glycolipids by hydrogen bonds and hydrophobic forces.

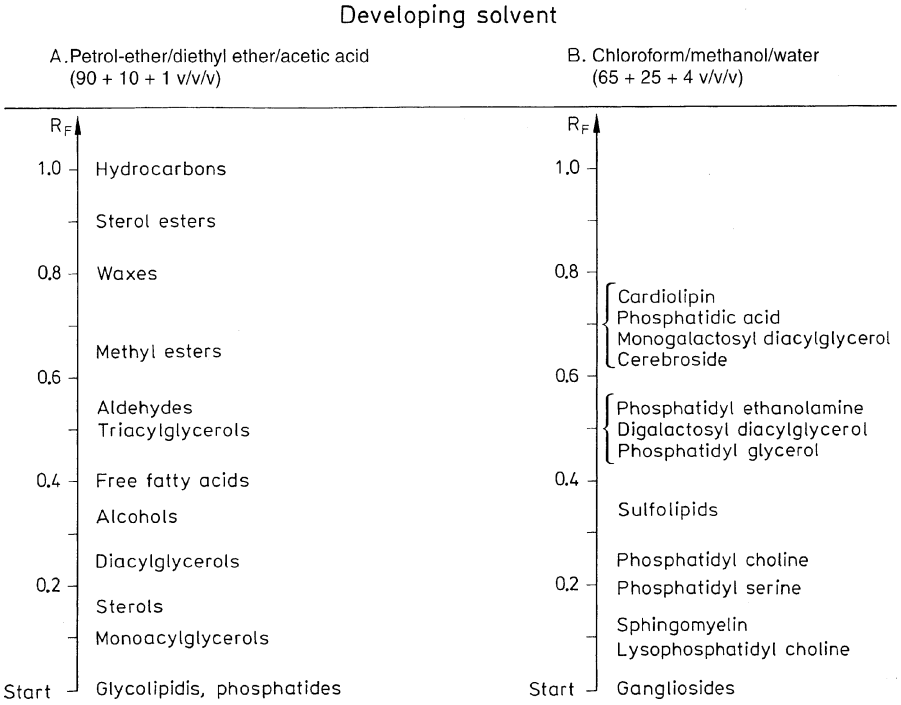


Fig. 3.12. Separation of lipid classes by thin layer chromatography using silicagel as an adsorbent. R_f values in two solvent systems

Thus, lipoproteins are held together only by non-covalent bonds.

3.5.1.2 Classification

Lipoproteins exist as globular particles in an aqueus medium. They are solubilized from biological sources by buffers with high ionic strength, by a change of pH or by detergents in the isolating medium. The latter, a more drastic approach, is usually used in the recovery of lipoproteins from membranes.

Lipoproteins are characterized by ultracentrifugation. Since lipids have a lower density (0.88–0.9 g/ml) than proteins (1.3–1.35 g/ml), the separation is possible because of differences in the ratios of lipid to protein within a lipoprotein complex. The lipoproteins of blood plasma have been thoroughly studied. They are separated by a stepwise centrifugation in solutions of NaCl into three fractions with different densities (Fig. 3.13). The "very low density lipoproteins" (VLDL; density <1.006 g/ml), the "low density lipoproteins" (LDL; 1.063 g/ml) and the "high density lipoproteins" (HDL; 1.21 g/ml) float, and the sediment contains the plasma proteins. The VLDL fraction can be separated further by electrophoresis into chylomicrons (the lightest lipoprotein, density <1.000 g/ml) and pre-β-lipoprotein.

Lipoproteins in the LDL fraction from an electrophoretic run have a mobility close to that of blood plasma β-globulin. Therefore, the LDL fraction is denoted as β-lipoprotein. An analogous designation of α-lipoprotein is assigned to the HDL fraction.

Chylomicrons, the diameters of which range from 1000–10,000 Å, are small droplets of triacylglycerol stabilized in the aqueous medium by a membrane-like structure composed of protein, phosphatides and cholesterol. The role of chylomicrons in blood is to transport triacylglycerols to various organs, but preferentially from the intestines to adipose tissue and the liver. The milk fat globules (cf. 10.1.2.3) have a structure

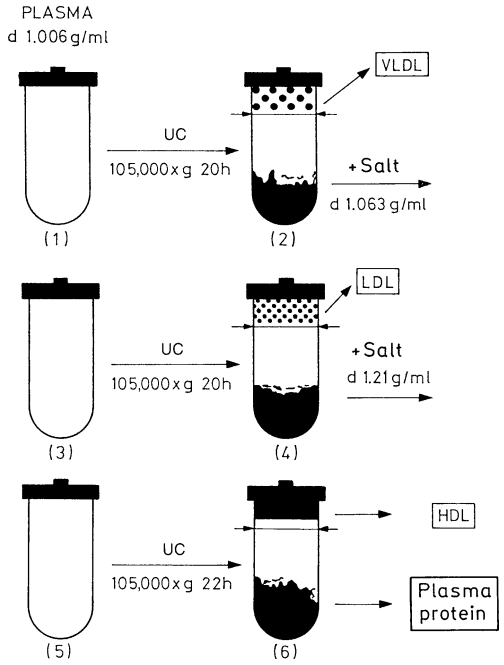


Fig. 3.13. Plasma protein fractionation by a preparative ultracentrifugation (UC) method (according to *Seidel*, 1971)

similar to that of chylomicrons. The composition of some lipoproteins is presented in Table 3.20.

Certain diseases related to fat metabolism (hyperlipidemias) can be clinically diagnosed by the content and composition of the plasma lipoprotein fractions.

Electron microscopy studies have revealed that the fat globules in milk have small particles attached to their membranes; these are detached by detergents and have been identified as LDL (cf. Table 3.20).

3.5.2 Involvement of Lipids in the Formation of Biological Membranes

Membranes that compartmentalize the cells and many subcellular particles are formed from two main building blocks: proteins and lipids (phospholipids and cholesterol). Differences in membrane structure and function are reflected by the compositional differences of membrane proteins and lipids (see examples in Table 3.18).

Studies of membrane structure are difficult since the methods for isolation and purification profoundly change the organization and functionality of the membrane.

Model membranes are readily formed. The major forces in such events are the hydrophobic interactions between the acyl tails of phospholipids, providing a bilayer arrangement. In addition, the amphipathic character of the lipid molecules makes membrane formation a spontaneous process. The acyl residues are sequestered and oriented in the nonpolar interior of the bilayer, whereas the polar hydrophilic head groups are oriented toward the outer aqueous phase.

Another arrangement in water that satisfies both the hydrophobic acyl tails and the hydrophilic polar groups is a globular micelle. Here, thehydrocarbon tails are sequestered inside, while the polar groups are on the surface of the sphere. There is no bilayer in this arrangement.

Table 3.20. Composition of typical lipoproteins

Source	Lipoprotein	Particle weight (kdal)	Protein (%)	Glycero-phospho-lipids (%)	Cholesterol		Triacyl-glycerols (%)
					free (%)	esterified (%)	
Human blood serum	Chylomicron	10^9–10^{10}	1–2	4	2.5–3	3–4	85–90
	Pre-β-lipoprotein	$5-100 \cdot 10^6$	8.3	19.2	7.4	11.1	54.2
	LDL (β-lipoprotein)	$2.3 \cdot 10^6$	22.7	27.9	8.5	28.8	10.5
	HDL (α-lipoprotein)	$1-4 \cdot 10^5$	58.1	24.7	2.9	9.2	5.9
Egg yolk (chicken)	β-Lipovitellin	$4 \cdot 10^5$	78	12	0.9	0.1	9
	LDL	$2-10 \cdot 10^6$	18	22	3.5	0.2	58
Bovine milk	LDL	$3.9 \cdot 10^6$	12.9	52	0	0	35.1

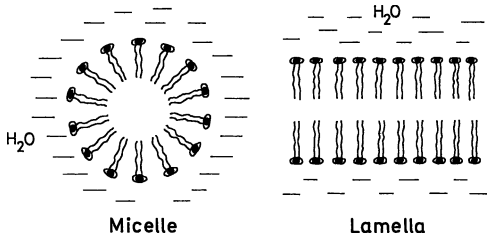


Fig. 3.14. Arrangement of polar acyl lipids in aqueous medium. ∞ Polar lipid tails; ≈ hydrophobic lipid tails

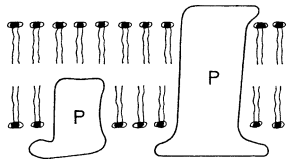


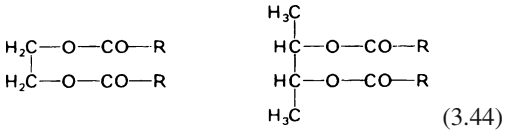
Fig. 3.15. Fluid mosaic model of a biological membrane. The protein (P) is not fixed but is mobile in the phospholipid phase

The favored structure for most phospho- and glycolipids in water is a bimolecular arrangement, rather than a micelle. Two model systems can exist for such bimolecular arrangements. The first is a lipid vesicle, known as a liposome, the core of which is an aqueous compartment surrounded by a lipid bilayer, and the second is a planar, bilayer membrane. The latter, together with the micellar model, is presented in Fig. 3.14. Globular proteins, often including enzymes, are found in animal cell membranes and are well embedded or inserted into the bimolecular layer. Some of these so-called integral membrane proteins protrude through both sides of the membrane (fluid mosaic model, Fig. 3.15). Although integral proteins interact extensively with the hydrophobic acyl tails of membrane lipids they are mobile within the lipid membrane.

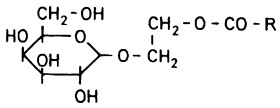
3.6 Diol Lipids, Higher Alcohols, Waxes and Cutin

3.6.1 Diol Lipids

The diol lipids which occur in both plant and animal tissues are minor lipid constituents. The diol content is about 1% of the content of glycerol. Exceptions are sea stars, sea urchins and mollusks, the lipids of which in summer contain 25–40% diol lipids. This proportion decreases sharply in winter and spring. Neutral and polar lipids derived from ethylene glycol, propane-(1,2 and 1,3)-diol and butane-(1,3; 1,4- and 2,3)-diol have been identified in the diol lipid fraction. Several of those isolated from corn oil have the following structures:



(3.44)



(3.45)

In a glycodiol lipid one hydroxyl group of ethylenediol is esterified with a fatty acid.
Diol lipids with structures analogous to phosphatidyl choline or plasmalogen have also been identified.

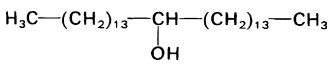
3.6.2 Higher Alcohols and Derivatives

3.6.2.1 Waxes

Higher alcohols occur either free or bound in plant and animal tissues. Free higher alcohols are abundant in fish oil and include:

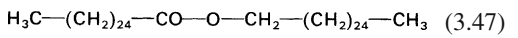
Cetyl alcohol	$C_{16}H_{33}OH$
Stearyl alcohol	$C_{18}H_{37}OH$
Oleyl alcohol	$C_{18}H_{35}OH$

Waxes are important derivatives of higher alcohols. They are higher alcohols esterified with long-chain fatty acids. Plant waxes are usually found on leaves or seeds. Thus, cabbage leaf wax consists of the primary alcohols C_{12} and C_{18}–C_{28} esterified with palmitic acid and other acids. The dominant components are stearyl and ceryl alcohol ($C_{26}H_{53}OH$). In addition to primary alcohols, esters of secondary alcohols, e. g., esters of nonacosane-15-ol, are present:



(3.46)

The role of waxes is to protect the surface of plant leaves, stems and seeds from dehydration and infections by microorganisms. Waxes are removed together with oils by solvent extraction of nondehulled seeds. Waxes are oil-soluble at elevated temperatures but crystallize at room temperature, causing undesired oil turbidity. Ceryl cerotate (ceryl alcohol esterified with cerotic acid, $C_{25}H_{51}COOH$)



(3.47)

is removed from seed hulls during extraction of sunflower oil. Waxes are removed by an oil refining winterization step during the production of clear edible oil.

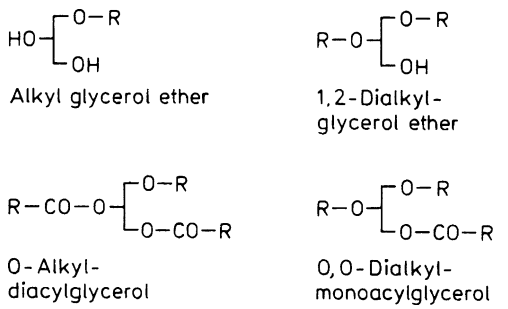
Waxes are also components of the mass used to cover fruit to protect it from drying out.

Waxes are present in fish oils, especially in sperm whale blubber and whale head oil, which contain a "reservoir" of spermaceti wax.

3.6.2.2 Alkoxy Lipids

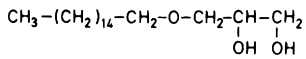
The higher alcohols, 16:0, 18:0 and 18:1 (9), form mono- and diethers with glycerol. Such alkoxylipids are widely distributed in small amounts in mammals and sea animals. Examples of confirmed structures are shown in Formula 3.48.

The elucidation of ether lipid structure is usually accomplished by cleavage by concentrated HI at elevated temperatures.

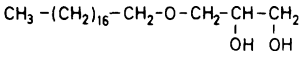


(3.48)

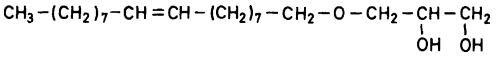
Common names of some deacylated alkoxy lipids (1-O-alkylglycerol) are the following:



Chimyl alcohol (3.49)



Batyl alcohol (3.50)



Selachyl alcohol (3.51)

3.6.3 Cutin

Plant epidermal cells are protected by a suberized or waxy cuticle. An additional layer of epicuticular waxes is deposited above the cuticle in many plants. The waxy cuticle consists of cutin. This is a complex, high molecular weight polyester which is readily solubilized in alkali. The structural units of the polymer are hydroxy fatty acids. The latter are similar in structure to the compounds given in 3.7.2.4.1. A segment of the postulated structure of cutin is presented in Fig. 3.16.

3.7 Changes in Acyl Lipids of Food

3.7.1 Enzymatic Hydrolysis

Hydrolases, which cleave acyl lipids, are present in food and microorganisms. The release of short-chain fatty acids ($<C_{14}$), e. g., in the hydrolysis of milk fat, has a direct effect on food aroma. Lipolysis is undesirable in fresh milk

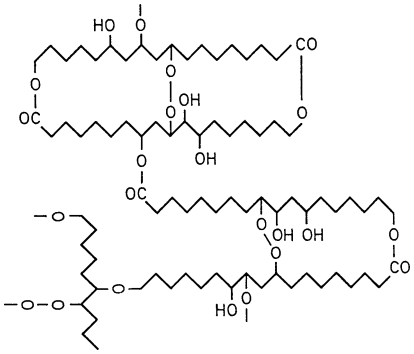


Fig. 3.16. A structural segment of cutin (according to *Hitchcock* and *Nichols*, 1971)

since the free C_4–C_{12} fatty acids (cf. Tables 3.3 to 3.5 for odor threshold values) are responsible for the rancid aroma defect. On the other hand, lipolysis occurring during the ripening of cheese is a desired and favorable process because the short-chain fatty acids are involved in the build-up of specific cheese aromas. Likewise, slight hydrolysis of milk fat is advantageous in the production of chocolate.

Linoleic and linoleiic acid released by hydrolysis and present in emulsified form affect the flavor of food even at low concentrations. They cause a bitter-burning sensation (cf. Table 3.9). In addition, they decompose by autoxidation (cf. 3.7.2.1) or enzymatic oxidation (cf. 3.7.2.2) into compounds with an intensive odor. In fruits and vegetables enzymatic oxidation in conjunction with lipolysis occur, as a rule, at a high reaction rate, especially when tissue is sliced or homogenized (an example for rapid lipolysis is shown in Table 3.21). Also, enzymatic hydrolysis of a small amount of the acyl lipids present can not be avoided during disintegration of oil seeds. Since the release of higher fatty acids promotes foaming, they are removed during oil refining (cf. 14.4.1).

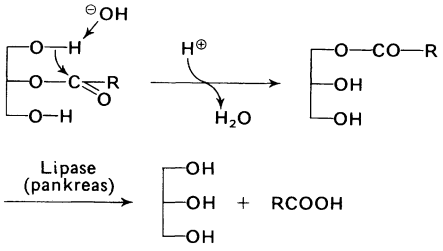
Enzymes with lipolytic activity belong to the carboxyl-ester hydrolase group of enzymes (cf. 2.2.6).

3.7.1.1 Triacylglycerol Hydrolases (Lipases)

Lipases (cf 2.2.6) hydrolyze only emulsified acyl lipids; they are active on a water/lipid interface. Lipases differ from esterase enzymes since the latter cleave only water-soluble esters, such as triacetylglycerol.

Lipase activity is detected, for example, in milk, oilseeds (soybean, peanut), cereals (oats, wheat), fruits and vegetables and in the digestive tract of mammals. Many microorganisms release lipase-type enzymes into their culture media.

As to their specificity, lipases are distinguished according to the criteria presented in Table 3.22. The lipase secreted by the swine pancreas has been the most studied. Its molecular weight is M_r = 48,000. The enzyme cleaves the following types of acyl glycerols with a decreasing rate of hydrolysis: triacyl- >diacyl- ≫monoacylglycerols. Table 3.22 shows that pancreatic lipase reacts with acyl residues at positions 1 and 3. The third acyl residue of a triacylglycerol is cleaved (cf. Reaction 3.52) only after acyl migration, which requires a longer incubation time.



(3.52)

The smaller the size of the oil droplet, the larger the oil/water interface and, therefore, the higher the lipase activity. This relationship should not

Table 3.21. Lipid hydrolysis occurring during potato tuber homogenization

	µmoles/g[a]	
	Acyl lipids	Free fatty acids
Potato	2.34	0.70
Homogenate[b]	2.04	1.40
Homogenate[b] kept for 10 min at 0 °C	1.72	1.75
Homogenate kept for 10 min at 25 °C	0.54	2.90

[a] Potato tissue fresh weight.
[b] Sliced potatoes were homogenized for 30 sec at 0 °C.

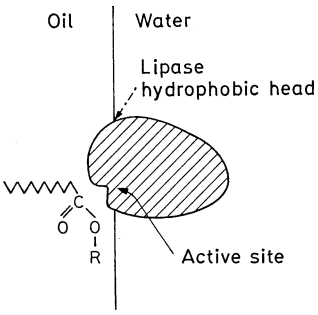


Fig. 3.17. A hypothetical model of pancreatic lipase fixation of an oil/water interphase (according to *Brockerhoff*, 1974)

Table 3.22. Examples of the specificity of lipases

Specificity	Lipase from
Substrate specific	
Monoacylglycerides	Rats (adipose tissue)
Mono- and diacyl-glycerides	*Penicillium camembertii*
Triacylglycerides	*Penicillium* sp.
Regiospecific	
1,3-Regioselective	Pancreas, milk, *Aspergillus niger*
sn-2-Regioselective	*Candida antarctia*
Non-regiospecific	
	Oats, castor, *Aspergillus flavus*
Acyl residue-specific	
Short chain fatty acids	*Penicillium roqueforti*
cis-9-Unsaturated fatty acids	*Geotrichum candidum*
Long chain fatty acids	*Botrytis cinerea*
Stereospecific[a]	
sn-1	*Pseudomonas neruginosa*
sn-3	Rabbit (digestive tract)

[a] Lipases differentiate between the sn-1 and sn-3 position in TGs.

be ignored when substrate emulsions are prepared for the assay of enzyme activities.

A model for pancreatic lipase has been suggested to account for the enzyme's activity on the oil/water interface (Fig. 3.17). The lipase's "hydrophobic head" is bound to the oil droplet by hydrophobic interactions, while the enzyme's active site aligns with and binds to the substrate molecule. The active site resembles that of serine proteinase. The splitting of the ester bond occurs with the involvement of Ser, His and Asp residues on the enzyme by a mechanism analogous to that of chymotrypsin (cf. 2.4.2.5). The dissimilarity between pancreatic lipase and serine proteinase is in the active site: lipase has a leucine residue within this site in order to establish hydrophobic contact with the lipid substrate and to align it with the activity center.

Lipase-catalyzed reactions are accelerated by Ca^{2+} ions since the liberated fatty acids are precipitated as insoluble Ca-salts.

The properties of milk lipase closely resemble those of pancreatic lipase.

Lipases of microbiological origin are often very heat stable. As can be seen from the example of a lipase of *Pseudomonas fluorescence* (Table 3.23), such lipases are not inactivated by pasteurization, ultra high temperature treatment, as well as drying procedures, e. g., the production of dry milk. These lipases can be the cause of decrease in quality of such products during storage.

A lipase of microbial origin has been detected which hydrolyzes fatty acids only when they have a cis-double bond in position 9 (Table 3.22). It is used to elucidate triacylglyceride structure. The use of lipases in food processing was outlined under 2.7.2.2.14.

Lipase activities in foods can be measured very sensitively with fluorochromic substrates, e. g., 4-methyl umbelliferyl fatty acid esters. Of course it is not possible to predict the storage stability of a food item with regard to lipolysis based only on such measurements. The substrate specificity of the lipases, which can vary widely as shown in Table 3.22, is of essential importance for the aroma quality. Therefore, individual fatty acids can increase in different amounts even at the same lipase activity measured against a standard substrate. Since the odor and taste threshold values of the fatty acids differ greatly (cf. Tables 3.3–3.5), the effects of the lipases on the aroma are very variable. It is not directly possible to predict the point of time when rancid aroma notes will be present from the determination of the lipase activity. More precise information about the changes to be expected is obtained through storage experiments during which the fatty acids are quantitatively determined by gas chromatographic analysis. Table 3.24 shows the change in the concentrations of free fatty acids in sweet cream butter together with the resulting rancid aroma notes.

Table 3.23. Heat inactivation of a lipase of *Pseudomonas fluorescence* dissolved in skim milk

Temperature °C	D-value[a] (min)
100	23.5
120	7.3
140	2.0
160	0.7

[a] Time for 90% decrease in enzyme activity (cf. 2.5.4.1).

Table 3.24. Free fatty acids in butter (sweet cream) samples of different quality

Fatty acid	Butter A (mg/kg)	B	C	D	E
4:0	0	5	38	78	119
6:0	0	4	28	25	46
8:0	8	22	51	51	86
10:0	38	58	104	136	229
12:0	78	59	142	137	231
14:0	193	152	283	170	477
Aroma[a]	2.3	2.8	3.0	4.6	5.4

[a] Classification: 2 not rancid, 3 slightly rancid, 4 rancid, 5 very rancid.

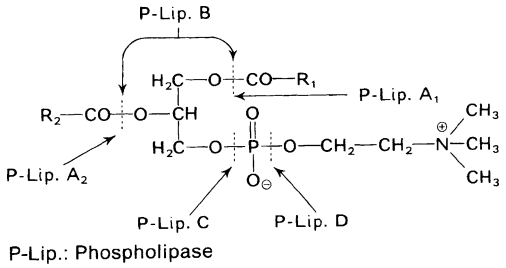
3.7.1.2 Polar-Lipid Hydrolases

These enzymes are denoted as phospholipases, lysophospholipases or glycolipid hydrolases, depending on the substrate.

3.7.1.2.1 Phospholipases

Phospholipase A_1. The enzyme is present together with phospholipase A_2 in many mammals and bacteria. It cleaves specifically the sn-1 ester bonds of diacylphosphatides (Formula 3.53).

Phospholipase A_2. Enzymes with sn-2 specificity isolated form snake and bee venoms. They are very stable, are activated by Ca^{2+}-ions and are amongst the smallest enzyme molecules (molecular weight about 14,000).



(3.53)

Phospholipase B. The existence of phospholipase B, which hydrolyzes in a single-step reaction both acyl groups in diacylphosphatides, is controversial. Other than the phospholipases A_1, A_2, C and D, the B-type could not be isolated in its pure form. A phospholipase B has been enriched from germinating barley. However, the B-specificity appears to be only a secondary activity because the enzyme hydrolyzes the acyl residue of lysolecithin considerably faster than the acyl residues of lecithin.

Phospholipase C. It hydrolyzes lecithin to a 1,2-diacylglyceride and phosphoryl choline. The enzyme is found in snake venom and in bacteria.

Phospholipase D. This enzyme cleaves the choline group in the presence of water or an alcohol, such as methanol, ethanol or glycerol, yielding free or esterified phosphatidic acid. For example:

$$\text{Phosphatidylcholine} + \text{ROH} \longrightarrow \text{Phosphatidyl-OR} + \text{Choline}$$
$$\text{R}: \text{H}, \text{CH}_3, \text{CH}_2, \text{CH}_2(\text{OH}) - \text{CH}(\text{OH}) - \text{CH}_2 \tag{3.54}$$

Phospholipase D cannot cleave phosphatidyl inositol. The enzyme is present in cereals, such as rye and wheat, and in legumes. It was isolated and purified from peanuts.

Lysophospholipases. The enzymes, hydrolyzing only lysophosphatides, are abundant in animal tissue and bacteria. There are lysophospholipases that split preferentially 1-acylphosphatides while others prefer 2-acylphosphatides, and a third group doesn't differentiate at all between the two lysophosphatide types.

3.7.1.2.2 Glycolipid Hydrolases

Enzymes that cleave the acyl residues of mono- and digalactosyl-diacylglycerides are localized in green plants. A substrate specificity study for such a hydrolase from potato (Table 3.25) shows that plants also contain enzymes that are able to hydrolyze polar lipids in general. The potato enzyme preferentially cleaves the acyl residue from monoacylglycerols and lysolecithin, whereas triacylglycerols, such as triolein, are not affected.

Table 3.25. Purified potato acyl hydrolase: substrate specificity

Substrate	Relative activity (%)	Substrate	Relative activity (%)
Monolein	100	Lecithin	13
Diolein	21	Monogalactosyl-diacylglycerol	31
Triolein	0.2	Digalactosyl-diacylglycerol	17
Methyloleate	28		
Lysolecithin	72		

3.7.2 Peroxidation of Unsaturated Acyl Lipids

Acyl lipid constituents, such as oleic, linoleic and linolenic acids, have one or more allyl groups within the fatty acid molecule (cf. Table 3.7) and thus are readily oxidized to hydroperoxides. The latter, after subsequent degradation reaction, yield a great number of other compounds. Therefore, under the usual conditions of food storage, unsaturated acyl lipids cannot be considered as stable food constituents.

Autoxidation should be distinguished from *lipoxygenase catalysis* in the process denoted as *lipid peroxidation.* Both oxidations provide hydroperoxides, but the latter occurs only in the presence of the enzyme.

Lipid peroxidation provides numerous volatile and nonvolatile compounds. Since some of the volatiles are exceptionally odorous compounds, lipid peroxidation is detected even in food with unsaturated acyl lipids present as minor constituents, or in food in which only a small portion of lipid was subjected to oxidation.

Induced changes in food aroma are frequently assessed by consumers as objectionable, for example, as rancid, fishy, metallic or cardboardlike, or as an undefined old or stale flavor. On the other hand, the fact that some volatile compounds, at a level below their off-flavor threshold values, contribute to the pleasant aroma of many fruits and vegetables and to rounding-off the aroma of many fator oil-containing foods should not be neglected.

3.7.2.1 Autoxidation

Autoxidation is quite complex and involves a great number of interrelated reactions of intermediates. Hence, autoxidation of food is usually imitated by the study of a model system in which, for example, changes of one unsaturated fatty acid or one of its intermediary oxidation products are recorded in the presence of oxygen under controlled experimental conditions.

Model system studies have revealed that the rate of autoxidation is affected by fatty acid composition, degree of unsaturation, the presence and activity of pro- and antioxidants, partial pressure of oxygen, the nature of the surface being exposed to oxygen and the storage conditions (temperature, light exposure, moisture content, etc.) of fat/oil-containing food. The position of the unsaturated fatty acid in the triacylglyceride molecule also influences the rate of autoxidation. TGs with an unsaturated fatty acid in the 1- or 3-position oxidize faster than TGs with an unsaturated acyl residue in the more protected 2-position.

The oxygen uptake of an unsaturated fatty acid as a function of time is shown in Fig. 3.18. Studying this figure helps in the understanding of the elementary steps involved in autoxidation. The extreme case 1 demonstrates what has invariably been found in food: the initial oxidation products are detectable only after a certain elapsed storage time. When this *induction period*, which is typical for a given autoxidation process, has expired, a steep rise occurs in the reaction rate. The proox-

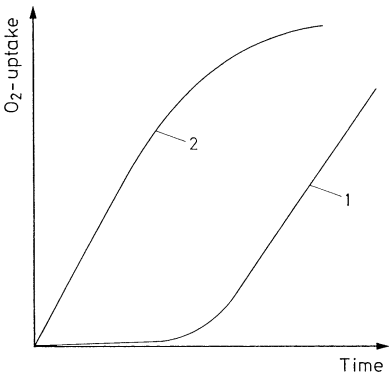


Fig. 3.18. Autoxidation of unsaturated acyl lipids. Prooxidant concentration: 1 low, 2 high

Table 3.26. Induction period and relative rate of oxidation for fatty acids at 25 °C

Fatty acid	Number of allyl groups	Induction period (h)	Oxidation rate (relative)
18:0	0		1
18:1 (9)	1	82	100
18:2 (9, 12)	2	19	1,200
18:3 (9, 12, 15)	3	1.34	2500

idant concentration is high in some foods. In these cases, illustrated in Fig. 3.18-2, the induction period may be nonexistent.

3.7.2.1.1 Fundamental Steps of Autoxidation

The length of the induction period and the rate of oxidation depend, among other things, on the fatty acid composition of the lipid (Table 3.26); the more allyl groups present, the shorter the induction period and the higher the oxidation rate.
Both phenomena, the induction period and the rise in reaction rate in the series, oleic, linoleic and linolenic acid can be explained as follows: Oxidation proceeds by a sequential free radical chain-reaction mechanism. Relatively stable radicals that can abstract H-atoms from the activated methylene groups in an olefinic compound are formed. On the basis of this assumption and, in addition, on the fact that the oxidation rate is exponential, *Farmer* et al. (1942) and *Bolland* (1949) proposed an autoxidation mechanism for olefinic compounds and, thus, also for unsaturated fatty acids. This mechanism has several fundamental steps. As shown in Fig. 3.19, the oxidation process is essentially a radical-induced chain reaction divided into initiation (start), propagation, branching and termination steps. Autoxidation is initiated by free radicals of frequently unknown origin.
Measured and calculated reaction rate constants for the different steps of the radical chain reaction show that due to the stability of the peroxy free radicals ($ROO^{\bullet}$), the whole process is limited by the conversion of these free radicals into monohydroperoxide molecules (ROOH). This reaction is achieved by abstraction of an H-atom from a fatty acid molecule [reaction step 2 (RS-2 in Fig. 3.19)]. The H-abstraction is the slowest and, hence, the rate limiting step in radical ($R^{\bullet}$) formation. Peroxidation of unsaturated fatty acids is accelerated autocatalytically by radicals generated from the degradation of hydroperoxides by a monomolecular reaction mechanism (RS-4 in Fig. 3.19). This reaction is promoted by heavy metal ions or heme(in)-containing molecules (cf. 3.7.2.1.7). Also, degradation of hydro-peroxides is considered to be a starting point for the formation of volatile reaction products (cf. 3.7.2.1.9).
After a while, the hydroperoxide concentration reaches a level at which it begins to generate free radicals by a bimolecular degradation mechanism (RS-5 in Fig. 3.19). Reaction RS-5 is exothermic, unlike the endothermic monomolecular decomposition of hydroperoxides (RS-4 in Fig. 3.19) which needs approx. 150 kJ/mol. However, in most foods, RS-5 is of no relevance since fat (oil) oxidation makes a food unpalatable well before reaching the necessary hydroperoxide level for the RS-5 reaction step to occur. RS-4 and RS-5 (Fig. 3.19) are the branching reactions of the free radical chain.

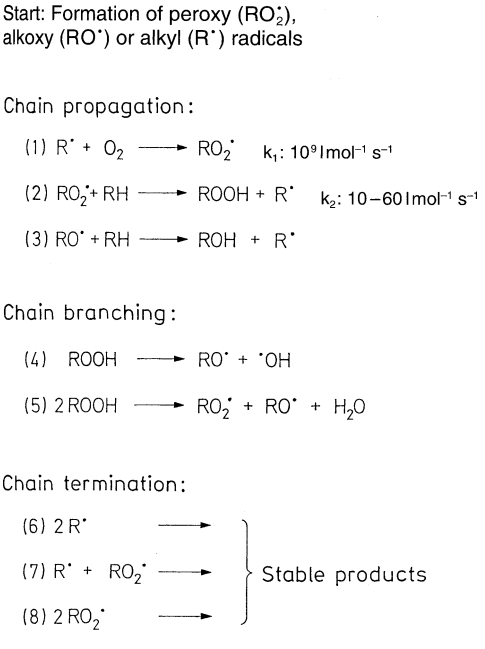


Fig. 3.19. Basic steps in the autoxidation of olefins

At room temperature, a radical may inititiate the formation of 100 hydroperoxide molecules before chain termination occurs. In the presence of air (oxygen partial pressure >130 mbar), all alkyl radicals are transformed into peroxy radicals through the rapid radical chain reaction 1 (RS-1, Fig. 3.19). Therefore, chain termination occurs through collision of two peroxy radicals (RS-8, Fig. 3.19).

Termination reactions RS-6 and RS-7 in Fig. 3.19 play a role when, for example, the oxygen level is low, e. g. in the inner portion of a fatty food.

The hypothesis presented in Fig. 3.19 is valid only for the initiation phase of autoxidation. The process becomes less and less clear with increasing reaction time since, in addition to hydroperoxides, secondary products appear that partially autoxidize into tertiary products. The stage at which the process starts to become difficult to survey depends on the stability of the primary products. It is instructive here to compare the difference in the structures of monohydroperoxides derived from linoleic and linolenic acids.

3.7.2.1.2 Monohydroperoxides

The peroxy radical formed in RS-1 (Fig. 3.19) is slow reacting and therefore it selectively abstracts the most weakly bound H-atom from a fat molecule. It differs in this property from, for example, the substantially more reactive hydroxy ($HO^{\bullet}$) and alkoxy($RO^{\bullet}$) radicals (cf. 3.7.2.1.8). RS-2 in Fig. 3.19 has a high reaction rate only when the energy for H-abstraction is clearly lower than the energy released in binding H to O during formation of hydroperoxide groups (about 376 kJ mol^{-1}).

Table 3.27 lists the energy inputs needed for H-abstraction from the carbon chain segments or groups occurring in fatty acids. The peroxy radical abstracts hydrogen more readily from a methylene group of a 1,4-pentadiene system than from a single allyl group. In the former case, the 1,4-diene radical that is generated is more effectively stabilized by resonance, i. e. electron delocalization over 5 C-atoms. Such considerations explain the difference in rates of autoxidation for unsaturated fatty acids and show why, at room temperature, the unsaturated fatty acids are attacked very selectively by peroxy radicals while the saturated acids are stable.

Table 3.27. Energy requirement for a H-atom abstraction

	$D_{R\text{-}H}$, (kJ/mole)
H | CH_2-	422
H | CH_3-CH-	410
H | $-CH-CH=CH-$	322
H | $-CH=CH-CH-CH-$	272

The general reaction steps shown in Fig. 3.19 are valid for all unsaturated fatty acids. In the case of oleic acid, H-atom abstraction occurs on the methylene group adjacent to the double bond, i. e. positions 8 and 11 (Fig. 3.20). This would give rise to four hydroperoxides. In reality, they have all been isolated and identified as autoxidation products of oleic acid. The configuration of the newly formed double bond of the hydroperoxides is affected by temperature. This configuration has 33% of cis and 67% of the more stable trans-configuration at room temperature.

Oxidation of the methylene group in position 11 of linoleic acid is activated especially by the two neighboring double bonds. Hence, this is the initial site for abstraction of an H-atom (Fig. 3.21). The pentadienyl radical generated is stabilized by formation of two hydroperoxides at positions 9 and 13, each retaining a conjugated diene system. These hydroperoxides have an UV maximum absorption at 235 nm and can be separated by high performance liquid chromatography as methyl esters, either directly or after reduction to hydroxydienes (Fig. 3.22).

The monoallylic groups in linoleic acid (positions 8 and 14 in the molecule), in addition to the bis-allylic group (position 11), also react to a small extent, giving rise to four hydroperoxides (8-, 10-, 12- and 14-OOH), each isomer having two isolated double bonds. The proportion of these minor monohydroperoxides is about 4% of the total (Table 3.28).

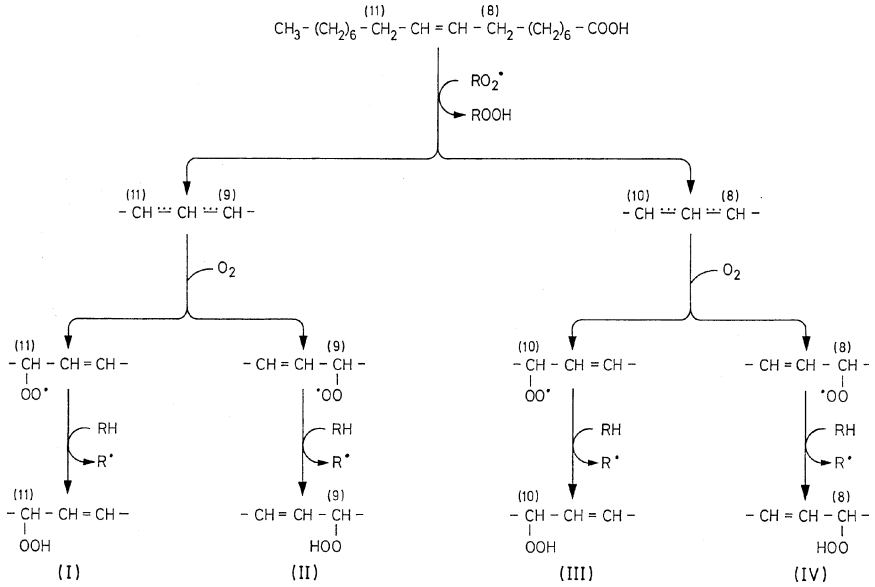


Fig. 3.20. Autoxidation of oleic acid. Primary reaction products: I 11-Hydroperoxyoctadec-9-enoic acid; II 9-hydroperoxyoctadec-10-enoic acid, III 10-hydroperoxyoctadec-8-enoic acid, IV 8-hydroperoxyoctadec-9-enoic acid

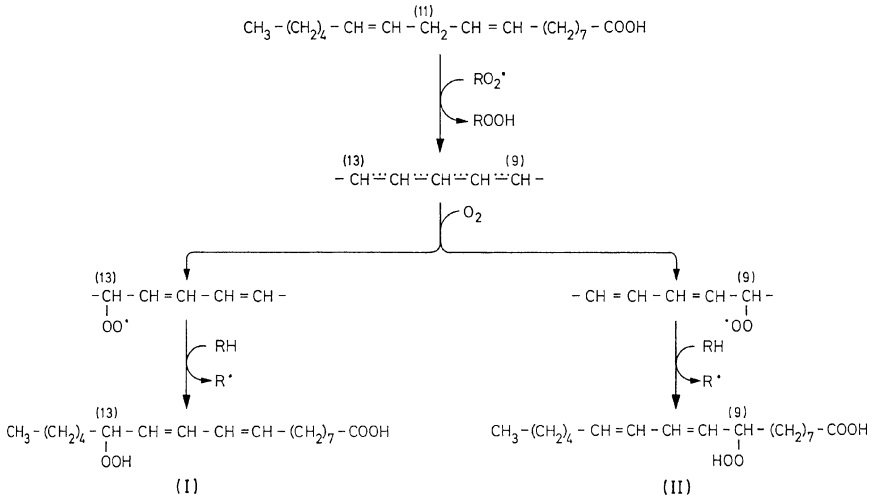


Fig. 3.21. Autoxidation of linoleic acid. Primary reaction products: I 13-Hydroperoxyoctadeca-9,11-dienoic acid, II 9-hydroperoxyoctadeca-10,12-dienoic acid

Autoxidation of linolenic acid yields four monohydroperoxides (Table 3.28). Formation of the monohydroperoxides is easily achieved by H-abstraction from the bis-allylic groups in positions 11 and 14. The resultant two pentadiene radicals then stabilize analogously to linoleic acid oxidation (Fig. 3.21); each radical corresponds to two monohydroperoxides. However, the four isomers are not formed in equimolar amounts; the 9- and 16-isomers predominate (Table 3.28). The configuration of the conjugated double bonds again depends on the reaction

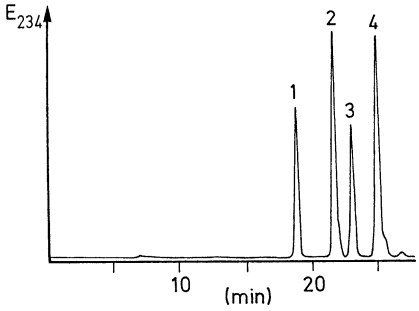
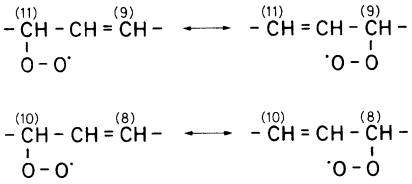


Fig. 3.22. Autoxidation of linoleic acid methyl ester. Analysis of primary products (after reduction of the hydroperoxy group) by HPLC (according to *Chan* and *Levett*, 1977). 1 13-Hydroxy-cis-9, trans-11-octadecadienoic acid methyl ester, 2 13-hydroxytrans-9,trans-11-octadecadienoic acid methyl ester, 3 9-hydroxy-trans-10, cis-12-octadecadienoic acid methyl ester, 4 9-hydroxy-trans-10, trans-12-octadecadienoic acid methyl ester

Table 3.28. Monohydroperoxides formed by autoxidation ($^3O_2$) and photooxidation ($^1O_2$) of unsaturated fatty acids

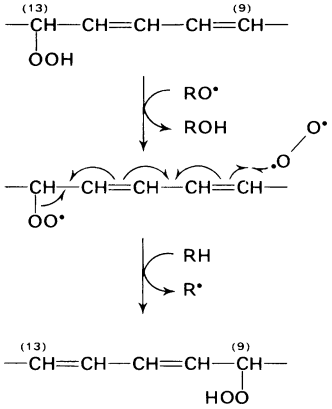
Fatty acid	Monohydroperoxide Position of		Proportion (%)	
	HOO-group	double bond	$^3O_2$	$^1O_2$
Oleic acid	8	9	27	
	9	10	23	48
	10	8	23	52
	11	9	27	
Linoleic acid	8	9,12	1.5	
	9	10,12	46.5	32
	10	8,12	0.5	17
	12	9,13	0.5	17
	13	9,11	49.5	34
	14	9,12	1.5	
Linolenic acid	9	10,12,15	31	23
	10	8,12,15		13
	12	9,13,15	11	12
	13	9,11,15	12	14
	15	9,12,16		13
	16	9,12,14	46	25

conditions. Cis-hydroperoxides are the main products at temperatures $<40\,^{\circ}C$. Competition exists between conversion of the peroxy radical to monohydroperoxide and reactions involving β-fragmentation and cyclization. Allyl peroxy radicals can undergo β-fragmentation which results, after a new oxygen molecule is attached, in a peroxy radical positional isomer, e. g. rearrangement of an oleic acid peroxy radical:



(3.55)

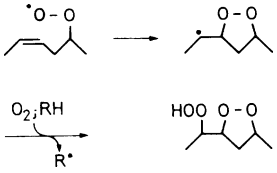
However, hydroperoxides can also be isomerized by such a reaction pathway. When they interact with free radicals (H-abstraction from –OOH group) or with heavy metal ions (cf. Reaction 3.64), they are again transformed into peroxy radicals. Thus, the 13-hydroperoxide of linoleic acid isomerizes into the 9-isomer and vice versa:



(3.56)

3.7.2.1.3 Hydroperoxide-Epidioxides

Peroxy radicals which contain isolated β,γ double bonds are prone to cyclization reactions in competition with reactions leading to monohydroperoxides. A hydroperoxide-epidioxide results through attachment of a second oxygen molecule and abstraction of a hydrogen atom:



(3.57)

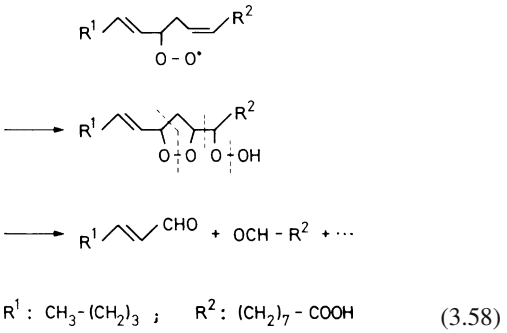
Peroxy radicals with isolated β,γ double bonds are formed as intermediary products after autoxidation and photooxidation (reaction with singlet O_2) of unsaturated fatty acids having two or more double bonds.

For this reason the 10- and 12-peroxy radicals obtained from linoleic acid readily form hydroperoxy-epidioxides. While such radicals are only minor products in autoxidation, in photooxidation they are generated as intermediary products in yields similar to the 9- and 13-peroxy radicals, which do not cyclize. Ring formation by 10- and 12-peroxy radicals decreases formation of the corresponding monohydroperoxides (Table 3.28; reaction with $^1O_2$).

Among the peroxy radicals of linolenic acid which are formed by autoxidation, the isolated β,γ double bond system exists only for the 12- and 13-isomers, and not for the 9- and 16-isomers. Also, the tendency of the 12- and 13-peroxy radicals of linolenic acid to form hydroperoxy-epidioxides results in the formation of less monohydroperoxide of the corresponding isomers as opposed to the 9- and 16-isomers (Table 3.28).

Peroxy radicals interact rapidly with antioxidants which may be present to give monohydroperoxides (cf. 3.7.3.1). Thus, it is not only the chain reaction which is inhibited by antioxidants, but also β-fragmentation and peroxy radical cyclization.

Fragmentation occurs when a hydroperoxideepidioxide is heated, resulting in formation of aldehydes and aldehydic acids. For example,



(3.58)

hydroperoxide-epidioxide fragments derived from the 12-peroxy radical of linoleic acid are formed as shown in Reaction 3.58.

Peroxy radicals formed from fatty acids with three or more double bonds can form bicycloendoperoxides with an epidioxide radical as intermediate. This is illustrated in Reaction 3.74.

3.7.2.1.4 Initiation of a Radical Chain Reaction

Since autoxidation of unsaturated acyl lipids frequently results in deterioration of food quality, an effort is made to at least decrease the rate of this deterioration process. However, pertinent measures are only possible when more knowledge is acquired about the reactions involved during the induction period of autoxidation and how they trigger the start of autoxidation.

In recent decades model system studies have revealed that two fundamentally different groups of reactions are involved in initiating autoxidation.

The first group is confined to the initiating reactions which overcome the energy barrier required for the reaction of molecular oxygen with an unsaturated fatty acid. The most important is photosensitized oxidation (photooxidation) which provides the "first" hydroperoxides. These hydroperoxides are then converted further into radicals in the second group of reactions. Heavy metal ions and heme(in) proteins are involved in this second reaction group. Some enzymes which generate the superoxide radical anion can be placed in between these two delineated reaction groups since at least H_2O_2 is necessary as reactant for the formation of radicals.

The following topics will be discussed here:

- Photooxidation
- Effect of heavy metal ions
- Heme(in) catalysis
- Activated oxygen from enzymatic reactions.

3.7.2.1.5 Photooxidation

In order to understand photooxidation and to differentiate it from autoxidation, the electronic configuration of the molecular orbital energy levels for oxygen should be known. As presented in Fig. 3.23, the allowed energy levels correspond to $^3\Sigma^-$ g, $^1\Delta$ g and $^1\Sigma^+$ g.

The notation for the molecular orbital of O_2 is $(\sigma\ 2s)^2\ (\sigma^*\ 2s)^2\ (\sigma\ 2p)^2\ (\pi\ 2p)^4\ (\pi^*\ 2p)^2$.

In the ground state, oxygen is a triplet ($^3O_2$). As seen from the above notation, the term $(\pi^*\ 2p)^2$

accounts for two unpaired electrons in the oxygen molecule. These are the two antibonding π orbitals available: π^*2p_y and π^*2p_z. The two electrons occupy these orbitals alone. The net angular momentum of the unpaired electrons has three components, hence the term "triplet". When the electrons are paired, the angular momentum can not be split into components and this represents a singlet state. In the triplet state, oxygen reacts preferentially with radicals, i. e. molecules having one unpaired electron. In contrast, direct reactions of tripletstate oxygen with molecules which have all electrons paired, as in the case of fatty acids, are prevented by spin barriers. For this reason the activation energy of the reaction

$$RH + {}^3O_2 \longrightarrow ROOH \qquad (3.59)$$

is so high (146–273 kJ/mole) that it does not occur without some assistance.

Oxygen goes from the ground state to the short-lived 1-singlet-state ($^1O_2$) by the uptake of 92 kJ/mole of energy (Fig. 3.23). The previously unpaired single electrons are now paired on the π^*2p_y antibonding orbital. The reactivity of this molecule resembles ethylenic or general olefinic π electron pair reactions, but it is more electrophilic. Hence, in the reaction with oleic acid, the 1-singlet-state oxygen attacks the 9−10 double bond, generating two monohydroperoxides, the 9- and 10-isomers (cf. Table 3.28). The second singlet-state of oxygen ($^1\Sigma^+$ g) has a much shorter life than the 1-singlet-state and plays no role in the oxidation of fats or oils.

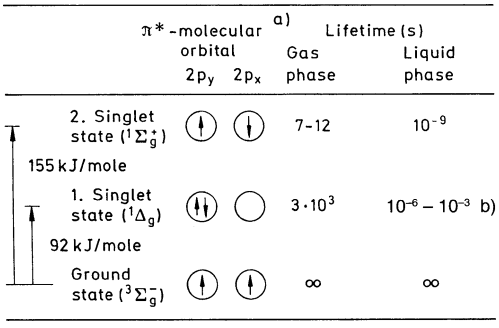


Fig. 3.23. Configuration of electrons in an oxygen molecule

[a] Electrons in $2p_x$ and $2p_y$ orbitals

[b] Dependent on solvent, e. g. 2 µs in water, 20 µs in D_2O and 7 µs in methanol

For a long time it has been recognized that the stability of stored fat (oil) drops in the presence of light. Light triggers lipid autoxidation. Low amounts of some compounds participate as sensitizers.

According to *Schenk* and *Koch* (1960), there are two types of sensitizers. Type I sensitizers are those which, once activated by light (sen*), react directly with substrate, generating substrate radicals. These then trigger the autoxidation process. Type II sensitizers are those which activate the ground state of oxygen to the $^1O_2$ singlet state.

Type I and II photooxidation compete with each other. Which reaction will prevail depends on the structure of the sensitizer but also on the concentration and the structure of the substrate available for oxidation.

Table 3.28 shows that the composition of hydroperoxide isomers derived from an unsaturated acid by autoxidation ($^3O_2$) differs from that obtained in the reaction with $^1O_2$. The isomers can be separated by analysis of hydroperoxides using high performance liquid chromatography and, thus, one can distinguish Type I from Type II photooxidation. Such studies have revealed that sensitizers, such as chlorophylls a and b, pheophytins a and b and riboflavin, present in food, promote the Type II oxidation of oleic and linoleic acids.

As already stated, the Type II sensitizer, once activated, does not react with the substrate but with ground state triplet oxygen, transforming it with an input of energy into 1-singlet-state oxygen:

$$\begin{array}{lcl} & \xrightarrow{h\nu} & \\ Sen & \longrightarrow & Sen^* \\ Sen^* + O_2 & \longrightarrow & Sen + {}^1O_2 \end{array} \qquad (3.60)$$

The singlet $^1O_2$ formed now reacts directly with the unsaturated fatty acid by a mechanism of "cyclo-addition":

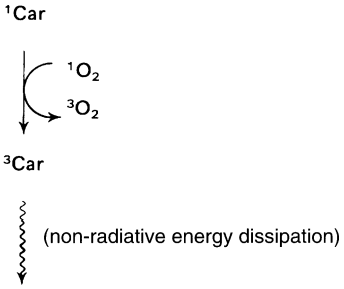
(3.61)

The fact that the number of hydroperoxides formed are double the number of isolated double bonds present in the fatty acid molecule is in

agreement with the above reaction mechanism. The reaction is illustrated in Fig. 3.24 for the oxidation of linoleic acid. In addition to the two hydroperoxides with a conjugated diene system already mentioned (Fig. 3.21), two hydroperoxides are obtained with isolated double bonds.

The mechanism reveals that double bonds of unsaturated fatty acids containing more than one double bond behave as isolated C=C units rather than 1,4-diene systems. Consequently, the difference in the reaction rates of linoleic acid and oleic acid with singlet oxygen is relatively small. In model reactions linoleic acid was photooxidized only 2 to 3 times faster. This is in contrast to autoxidation where linoleic acid is oxidized at least 12 times faster (cf. 3.7.2.1.1). However, furan fatty acids (cf. 3.2.1.3) react much faster with $^1O_2$ than linoleic or linolenic acid. An endo peroxide is the main reaction product. In addition, diacetyl, 3-methyl-2,4-nonandione (MND) and 2,3-octandione are formed. MND contributes to the aroma of tea (cf. 21.2.5.8) and, with diacetyl, to the aroma changes in stored soybean oil (cf. 14.3.2.2.5). The formation of MND is explained by the secondary reactions shown in Fig. 3.25. First, the allyl group of C-9 to C-11 is oxidized to an 11-hydroperoxide. The following β-cleavage of hydroperoxide results in the formation of a carbonyl group at C-11 and a hydroxyl radical which combines with C-13 after homolysis of the furan ring. The furan fatty acid thus is split into MND and a fragment of unknown structure. The odor threshold of MND in water is fairly low (Table 3.32), even in comparison with that of octandione.

Formation of 1-singlet oxygen ($^1O_2$) is inhibited by carotenoids (car):



(3.62)

The quenching effect of carotenoids (transition of $^1O_2$ to $^3O_2$) is very fast ($k = 3 \times 10^{10}\,1\,mole^{-1}s^{-1}$). They also prevent energy transfer from excited-state chlorophyll to $^3O_2$. Therefore, carotenoids are particularly suitable for protecting fat (oil)-containing food from Type II photooxidation.

3.7.2.1.6 Heavy Metal Ions

These ions are involved in the second group of initiation reactions, namely, in the decomposition of initially-formed hydroperoxides into radicals

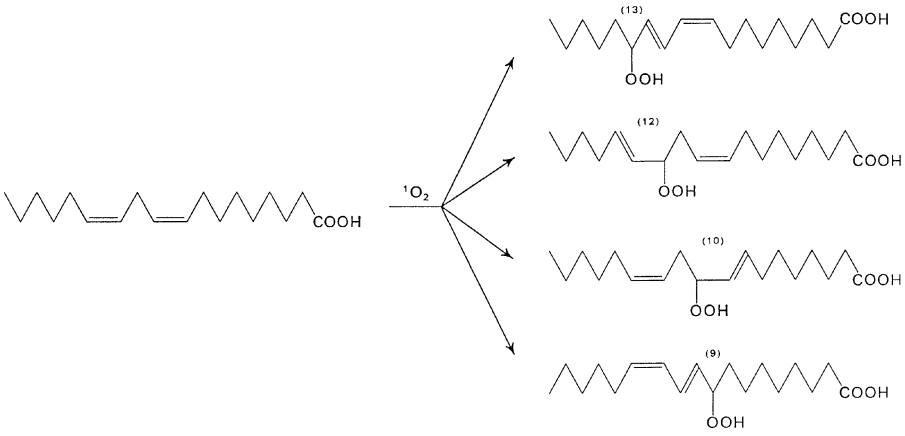


Fig. 3.24. Hydroperoxides derived from linoleic acid by type-2 photooxidation

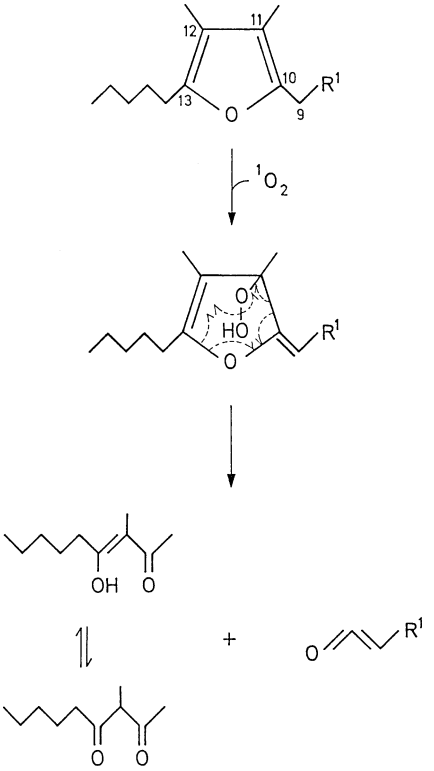


Fig. 3.25. Side reaction of a branched furan fatty acid with singlet oxygen (R^1: $(CH_2)_7COOH$)

which then propel the radical chain reaction of the autooxidation process. Fats, oils and foods always contain traces of heavy metals, the complete removal of which in a refining step would be uneconomical. The metal ions, primarily Fe, Cu and Co, may originate from:

- Raw food. Traces of heavy metal ions are present in many enzymes and other metal-bound proteins. For example, during the crushing and solvent extraction of oilseeds, metal bonds dissociate and the free ions bind to fatty acids.
- From processing and handling equipment. Traces of heavy metals are solubilized during the processing of fat (oil). Such traces are inactive physiologically but active as prooxidants.
- From packaging material. Traces of heavy metals from metal foils or cans or from wrapping paper can contaminate food and diffuse into the fat or oil phase.

The concentration of heavy metal ions that results in fat (oil) shelf-life instability is dependent on the nature of the metal ion and the fatty acid composition of the fat (oil). Edible oils of the linoleic acid type, such as sunflower and corn germ oil, should contain less than 0.03 ppm Fe and 0.01 ppm Cu to maintain their stability. The concentration limit is 0.2 ppm for Cu and 2 ppm for Fe in fat with a high content of oleic and/or stearic acids, e. g. butter.

Heavy metal ions trigger the autoxidation of unsaturated acyl lipids only when they contain hydroperoxides. That is, the presence of a hydroperoxide group is a prerequisite for metal ion activity, which leads to decomposition of the hydroperoxide group into a free radical:

$$Me^{n\oplus} + ROOH \rightarrow Me^{(n+1)\oplus} + RO^{\cdot} + OH^{\ominus} \quad (3.63)$$

$$Re^{(n+1)\oplus} + ROOH \rightarrow RO_2^{\cdot} + H^{\oplus} + Me^{n\oplus} \quad (3.64)$$

Reaction rate constants for the decomposition of linoleic acid hydroperoxide are given in Table 3.29. As seen with iron, the lower oxidation state (Fe^{2+}) provides a ten-fold faster decomposition rate than the higher state (Fe^{3+}). Correspondingly, Reaction 3.63 proceeds much faster than Reaction 3.64 in which the reduced state of the metal ion is regenerated. The start of autoxidation then is triggered by radicals from generated hydroperoxides.

The decomposition rates for hydroperoxides emulsified in water depend on pH (Table 3.29). The optimal activity for Fe and Cu ions is in the pH range of 5.5–6.0. The presence of ascorbic acid, even in traces, accelerates the decomposition. Apparently, it sustains the reduced state of the metal ions.

The direct oxidation of an unsaturated fatty acid to an acyl radical by a heavy metal ion

$$RH + Me^{(n+1)\oplus} \rightarrow R^{\cdot} + H^{\oplus} + Me^{n\oplus} \quad (3.65)$$

proceeds, but at an exceptionally slow rate. It seems to be without significance for the initiation of autoxidation.

The autoxidation of acyl lipids is also influenced by the moisture content of food. The reaction rate is high for both dehydrated and water-containing food, but is minimal at a water activity (a_w) of 0.3 (Fig. 0.4). The following hypotheses are discussed to explain these differences: The high reaction rate in dehydrated food is due to metal

Table 3.29. Linoleic acid hydroperoxides[a]: decomposition by heavy metal or heme compounds at 23 °C. Relative reaction rates k_{rel} are given at two pH's[a]

Heavy metal ion[b]	k_{rel}		Heme compound[b]	k_{rel}	
	pH 7	pH 5.5		pH 7	pH 5.5
Fe^{3+}	1	10^2	Hematin	4.10^3	4.10^4
Fe^{2+}	14	10^3	Methemoglobin	5.10^3	$7.6.10^3$
Cu^{2+}	0.2	1.5	Cytochrome C	$2.6.10^3$	$3.9.10^3$
Co^{3+}	6.10^2	1	Oxyhemoglobin	$1.2.10^3$	
Mn^{2+}	0	0	Myoglobin	$1.1.10^3$	
			Catalase	1	
			Peroxidase	1	

[a] Linoleic acid hydroperoxide is emulsified in a buffer.
[b] Reaction rate constant is related to reaction rate in presence of Fe^{3+} at pH 7 ($k_{rel} = 1$).

ions with depleted hydration shells. In addition, ESR spectroscopic studies show that food drying promotes the formation of free radicals which might initiate lipid peroxidation. As the water content starts to increase, the rate of autoxidation decreases. It is assumed that this decrease in rate is due to hydration of ions and also of radicals. Above an a_w of 0.3, free water is present in food in addition to bound water. Free water appears to enhance the mobility of prooxidants, thus accounting for the renewed increase in autoxidation rate that is invariably observed at high moisture levels in food.

3.7.2.1.7 Heme(in) Catalysis

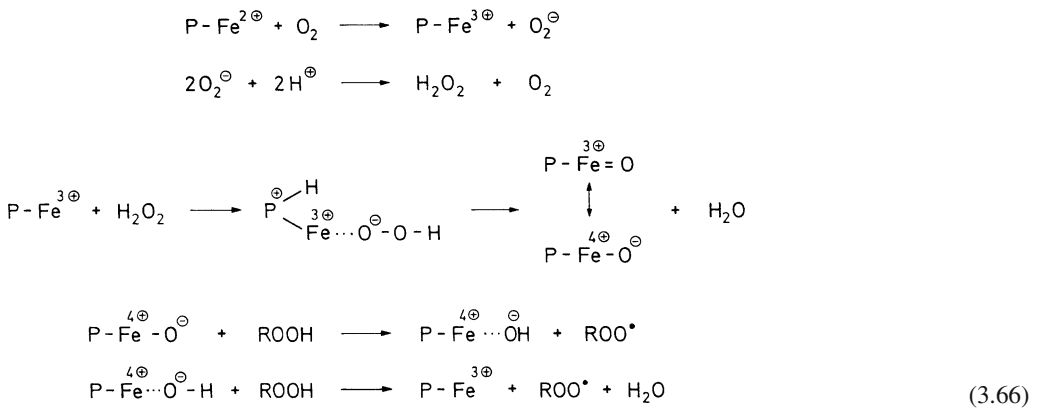
Heme (Fe^{2+}) and hemin (Fe^{3+}) proteins are widely distributed in food. Lipid peroxidation in animal tissue is accelerated by hemoglobin, myoglobin and cytochrome C. These reactions are often responsible for rancidity or aroma defects occurring during storage of fish, poultry and cooked meat. In plant food the most important heme(in) proteins are peroxidase and catalase. Cytochrome P_{450} is a particularly powerful catalyst for lipid peroxidation, although it is not yet clear to what extent the compound affects food shelf life "in situ".

During heme catalysis, a Fe^{2+} protoporphyrin complex (P–Fe^{2+}), like in myoglobin, will be oxidized by air to P–Fe^{3+} as indicated in Formula 3.66. The formed superoxide radical anion O_2^-, whose properties are discussed below, will further react yielding H_2O_2. Hydrogen peroxide will then oxidize P–Fe^{3+} to the oxene species P–Fe=O. The reaction with H_2O_2 is accelerated by acid/base catalysis, facilitating the loss of the water molecule; the hemin protein and one carboxylic group of the protoporphyrin system acts as proton acceptor and proton donor respectively.

Oxene is the active form of the hemin catalyst. It oxidizes two fatty acid hydroperoxide molecules to peroxy radicals that will then initiate lipid peroxidation.

In comparison with iron ions, some heme(in) compounds degrade the hydroperoxides more rapidly by several orders of magnitude (cf. Table 3.29). Therefore they are more effective as initiators of lipid peroxidation. Their activity is also negligibly influenced by a decrease in the pH-value.

However, the activity of a heme(in) protein towards hydroperoxides is influenced by its steric accessibility to fatty acid hydroperoxides. Hydroperoxide binding to the Fe-porphyrin moiety of native catalase and peroxidase molecules is obviously not without interferences. The prosthetic group is free to promote hydroperoxide decomposition only after heat denaturation of the enzymes. Indeed, a model experiment with peroxidase showed that the peroxidation of linoleic acid increased by a factor of 10 when the enzyme was heated for 1 minute to 140 °C. As expected, the enzymatic activity of peroxidase decreased and was only 14%. Similar results were obtained in reaction systems containing catalase.

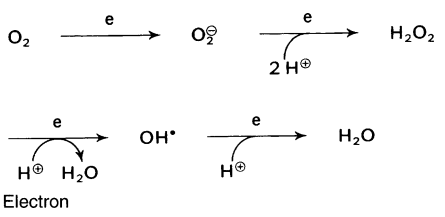


(3.66)

Suppression of peroxidase and catalase activity is of importance for the shelf life of heat-processed food. As long as the protein moiety has not been denatured, it is the lipoxygenase enzyme which is the most active for lipid peroxidation (cf. 3.7.2.2). After lipoxygenase activity is destroyed by heat denaturation, its role is replaced by the heme(in) proteins. As already suggested, an assay of heme(in) protein enzyme activity does not necessarily reflect its prooxidant activity.

3.7.2.1.8 Activated Oxygen

In enzymatic reactions oxygen can form three intermediates, which differ greatly in their activities and which are all ultimately reduced to water:



(3.67)

Oxygen takes up one electron to form the superoxide radical anion ($O_2^{\ominus}$). This anion radical is a reducing agent with chemical properties dependent on pH, according to the equilibrium:

$$O_2^{\ominus} + H^{\oplus} \rightleftharpoons HO_2^{\bullet} (pK_S : 4.8) \qquad (3.68)$$

Based on its pK_s value under physiological conditions, this activated oxygen species occurs as an anion with its radical character suppressed. It acts as a nucleophilic reagent (e. g. it promotes phospholipid hydrolysis within the membranes) under such conditions, but is not directly able to abstract an H-atom and to initiate lipid peroxidation. The free radical activity of the superoxide anion appears only in acidic media, wherein the perhydroxy radical form ($HO_2^{\bullet}$) prevails. Some reactions of ($HO_2^{\bullet}$) are presented in Table 3.30.
$O_2^{\ominus}$ is comparatively inactive (Table 3.30). As shown in Reaction 3.69, it dismutates at a rate that is dependent on the pH, e.g., pH 7: $k = 5.105\,l\,mol^{-1}\,s^{-1}$, pH 11: $k = 102\,l mol^{-1}\,s^{-1}$.

$$2O_2^{\ominus} + 2H^{\oplus} \longrightarrow H_2O_2 + O_2 \qquad (3.69)$$

An enzyme with superoxide dismutase activity which significantly accelerates ($k = 2 \times 10^9\,l mol^{-1}\,s^{-1}$) Reaction 3.69 occurs in numerous animal and plant tissues.
The superoxide radical anion, O_2^-, is generated especially by flavin enzymes, such as xanthine oxidase (cf. 2.3.3.2). The involvement of this enzyme in the development of milk oxidation flavor has been questioned for a long time.
The superoxide radical anion reacts at an exceptionally high rate ($k = 1.9 \times 10^{10}\,l mol^{-1}\,s^{-1}$) with nitrogen oxide (NO), the monomer being present as the free radical, to give peroxy nitrite ($ONOO^{\ominus}$). NO is formed in animal and plant foods from arginine by nitrogen oxide synthase (cf. 9.8.1). It is relatively stable with a half life of 400 s (H_2O). Peroxy nitrite is a versatile oxidant; it oxidizes unsaturated fatty acids, ascorbic acid, tocopherols, uric acid and amino acids, among

Table 3.30. Rate constants of reactions of reactive oxygen species with food constituents

Constituent	$^1O_2$	HO$^\bullet$	$O_2^{\ominus}$	HOO$^\bullet$
Lipid		k ($l \times mol^{-1} \times s^{-1}$)		
Oleic acid	5.3×10^4			
Linoleic acid	7.3×10^4			No reaction
Linolenic acid	1.0×10^5			1.2×10^3
Arachidonic acid				1.7×10^3
Cholestererol	2.5×10^8			3.1×10^3
Amino acids				
Histidine	4.6×10^7	4.8×10^9	<1.0	<95
Tryptophane	1.3×10^7	1.3×10^{10}	<24	
Cysteine	5.0×10^7	1.9×10^{10}	<15	<600
Cystine		2.1×10^9	$<4.0 \times 10^{-1}$	
Methionine	1.3×10^7	7.4×10^9	$<3.3 \times 10^{-1}$	<49
Sugar				
Glucose	1.4×10^4	1.5×10^9		
Fructose		1.6×10^9		
Sucrose	2.5×10^4	2.3×10^9		
Maltose		2.3×10^9		
Vitamins				
β-Carotene	5.0×10^9			
Riboflavin	6.0×10^7	1.2×10^{10}		
Ascorbic acid	1.1×10^7	8.2×10^9		1.6×10^4
Vitamin D	2.3×10^7			
α-Tocopherol	13.2×10^7		No reaction	2.0×10^5

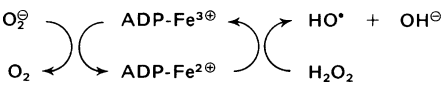
other substances. It easily decomposes with the formation of radicals, which can start lipid peroxidation.

Hydrogen peroxide, H_2O_2, is the second intermediate of oxygen reduction. In the absence of heavy metal ions, energy-rich radiation including UV light and elevated temperatures, H_2O_2 is a rather indolent and sluggish reaction agent. On the other hand, the hydroxy radical (HO$^\bullet$) derived from it is exceptionally active. During the abstraction of an H-atom,

$$R\text{—}H + HO^\bullet \longrightarrow R^\bullet + H_2O \tag{3.70}$$

the energy input in the HO-bond formed is 497 kJ/mol, thus exceeding the dissociation energy for abstraction of hydrogen from each C–H bond by at least 75 kJ/mol (cf. Table 3.27). Therefore, the HO$^\bullet$ radical reacts non-selectively with all organic constituents of food at an almost diffusion-controlled rate. Consequently, it can directly initiate lipid peroxidation. However, in a complex system such as food, the following question is always pertinent: "Has the HO$^\bullet$ radical actually reached the unsaturated acyl lipid, or was it trapped prior to lipid oxidation by some other food ingredient?".

The reaction of the superoxide radical anion with hydrogen peroxide should be emphasized in relation to initiation of autoxidation. This is the so-called *Fenton* reaction in particular of an Fe-complex:



(3.71)

The Fe-complex (e. g. with ADP) occurs in food of plant and animal origin. The Fe^{2+} obtained by reduction with O_2^- can then reduce the H_2O_2 present and generate free HO$^\bullet$ radicals.

3.7.2.1.9 Secondary Products

The primary products of autoxidation, the monohydroperoxides, are odorless and tasteless (such as linoleic acid hydroperoxides; cf. 3.7.2.4.1). Food quality is not affected until volatile compounds are formed. The latter are usually powerfully odorous compounds and, even in the very small amounts in which they occur, affect the odor and flavor of food.

From the numerous volatile secondary products of lipid peroxidation the following compounds will be discussed in detail

- odor-active carbonyl compounds
- malonic dialdehyde
- alkanes, alkenes

Odor-Active Monocarbonyl Compounds. Model expriments showed that the volatile fractions formed during the autoxidation of oleic, linoleic and linolenic acid contain mainly aldehydes and ketones (Table 3.31). Linoleic acid, a component of all lipids sensitive to autoxidation, is a precursor of hexanal that is predominant in the volatile fraction. Therefore this substance, since it can easily be determined by headspace analysis, is used as an indicator for the characterization of off-flavors resulting from lipid peroxidation.

A comparison of the sensory properties (Table 3.32) shows that some carbonyl compounds, belonging to side components of the volatile fractions, may intensively contribute to an off-flavor due to their low threshold values. Food items containing linoleic acid, especially (E)-2-nonenal, trans-4,5-epoxy-(E)-2-decenal and 1-octen-3-one, are very aroma active.

The rapid deterioration of food containing linolenic acid should not be ascribed solely to the preferential oxidation of this acid but also to the low odor threshold values of the carbonyl compounds formed, such as (Z)-3-hexenal, (E,Z)-2,6-nonadienal and (Z)-1,5-octadien-3-one (Table 3.32). Aldehydes with exceptionally strong aromas can be released in food by the autoxidation of some fatty acids, even if they are present in low amounts. An example is octadeca-(Z,Z)-11, 15-dienoic acid (the precursor for

Table 3.31. Volatile compounds formed by autoxidation of unsaturated fatty acids (μg/g)[a]

Oleic acid		Linoleic acid		Linolenic acid	
Heptanal	50	Pentane[b]	+[c]	Propanal[b]	
Octanal	320	Pentanal	55	1-Penten-3-one	30
Nonanal	370	Hexanal	5,100	(E)-2-Butenal	10
Decanal	80	Heptanal	50	(E)-2-Pentenal	35
(E)-2-Decenal	70	(E)-2-Heptenal	450	(Z)-2-Pentenal	45
(E)-2-Undecenal	85	Octanal	45	(E)-2-Hexenal	10
		1-Octen-3-one	2	(E)-3-Hexenal	15
		1-Octen-3-hydroperoxide	+[c]	(Z)-3-Hexenal	90
		(Z)-2-Octenal	990	(E)-2-Heptenal	5
		(E)-2-Octenal	420	(E,Z)-2,4-Heptadienal	320
		(Z)-3-Nonenal	30	(E,E)-2,4-Heptadienal	70
		(E)-3-Nonenal	30	(Z,Z)-2,5-Octadienal	20
		(Z)-2-Nonenal	+[c]	3,5-Octadien-2-one	30
		(E)-2-Nonenal	30	(Z)-1,5-Octadien-3-one	+[c]
		(Z)-2-Decenal	20	(Z)-1,5-Octadien-3-hydroperoxide	+[c]
		(E,E)-2,4-Nonadienal	30	(E,Z)-2,6-Nonadienal	10
		(E,Z)-2,4-Decadienal	250	2,4,7-Decatrienal	85
		(E,E)-2,4-Decadienal	150		
		trans-4,5-Epoxy-(E)-2-decenal	+[c]		

[a] Each fatty acid in amount of 1 g was autoxidized at 20 °C by an uptake of 0.5 mole oxygen/mole fatty acid.
[b] Major compound of autoxidation.
[c] Detected, but not quantified.

Table 3.32. Sensory properties of aroma components resulting from lipid peroxidation

Compound	Flavor quality	Odor threshold (μg/kg) in oil		in water
		nasal	retronasal	nasal
Aldehydes				
2:0	fruity, pungent	0.22	7.1	–
3:0	fruity, pungent	9.4	68	–
5:0	pungent, like bitter almonds	240	150	18
6:0	tallowy, green leafy	320	75	12
7:0	oily, fatty	3200	50	5
8:0	oily, fatty, soapy	55	515	0.7
9:0	tallowy, soapy-fruity	13,500	260	1.0
10:0	orange peel like	300	75	5
5:1 (E-2)	pungent, apple	2300	600	–
6:1 (E-2)	apple	420	250	316
6:1 (Z-3)	green leafy	1.7	1.2	0.03
7:1 (E-2)	fatty, bitter almond	14,000	400	51
7:1 (Z-4)	cream, putty	2	1	0.8
8:1 (Z-2)	walnut	–	50	–
8:1 (E-2)	fatty, nutty	7000	125	4
9:1 (Z-2)	fatty, green leafy	4.5	0.6	0.02
9:1 (E-2)	tallowy, cucumber	900	65	0.25
9:1 (Z-3)	cucumber	250	35	–
10:1 (E-2)	tallowy, orange	33,800	150	–
7:2 (E,Z-2,4)	frying odor, tallowy	4000	50	–
7:2 (E,E-2,4)	fatty, oily	10,000	30	–
9:2 (E,E-2,4)	fatty, oily	2500	460	–
9:2 (E,Z-2,6)	like cucumber	4	1.5	–
9:2 (Z,Z-3,6)	fatty, green	–	–	0.05[a]
9:3 (E,E,Z-2,4,6)	Oat flakes	–	–	0.026
10:2 (E,Z-2,4)	frying odor	10	–	–
10:2 (E,E-2,4)	frying odor	180	40	0.2
10:3 (E,Z,Z-2,4,7)	cut beans	–	24	–
trans-4,5-Epoxy-(E)-2-decenal	metallic	1.3	3	–
Ketones				
1-Penten-3-one	hot, fishy	0.73	3	–
1-Octen-3-one	like mushrooms, fishy	10	0.3	0.05
1-Nonen-3-one	like mushrooms, earthy	–	–	8×10^{-6}
(Z)-1,5-Octadien-3-one	like geraniums, metallic	0.45	0.03	1.2×10^{-3}
(E,E)-3,5-Octadien-2-one	fatty, fruity	300	–	–
(E,Z)-3,5-Octadien-2-one	fatty, fruity	200	–	–
3-Methyl-2,4-nonanedione	like straw, fruity, like butter	23	1.5	0.01
Miscellaneous compounds				
1-Octen-3-hydroperoxide	metallic	240	–	–
2-Pentylfuran	like butter, like green beans	2000	–	–

[a] retronasal.

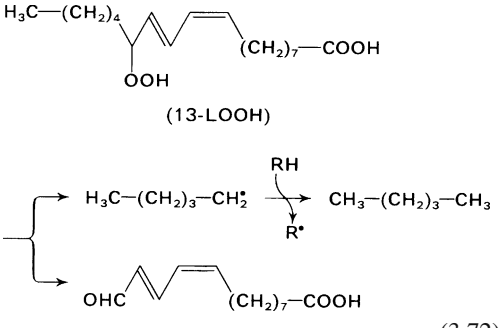
(Z)-4-heptenal), which occurs in beef and mutton and often in butter (odor threshold in Table 3.32). Also, the processing of oil and fat can provide an altered fatty acid profile. These can then provide new precursors for a new set of carbonyls. For example, (E)-6-nonenal, the precursor of which is octadeca-(Z,E)-9,15-dienoic acid, is a product of the partial hydrogenation of linolenic acid. This aldehyde can be formed during storage of partially hardened soya and linseed oils. The aldehyde, together with other compounds, is responsible for an off-flavor denoted as "hardened flavor".

Several reaction mechanisms have been suggested to explain the formation of volatile carbonyl compounds. The most probable mechanism is the β-scission of monohydroperoxides with formation of an intermediary short-lived alkoxy radical (Fig. 3.26). Such β-scission is catalyzed by heavy metal ions or heme(in) compounds (cf. 3.7.2.1.7).

There are two possibilities for β-scission of each hydroperoxide fatty acid (Fig. 3.26). Option "B", i. e. the cleavage of the C–C bond located further away from the double bond position, is the energetically preferred one since it leads to resonance-stabilized "oxoene" or "oxo-diene" compounds. Applying this β-scission mechanism ("B") to both major monohydroperoxide isomers of linoleic acid gives the products shown in Formula 3.72 and 3.73.

From the volatile autoxidation products which contain the methyl end of the linoleic acid molecule, the formation of 2,4-decadienal and pentane can be explained by reaction 3.72.

The formation of hexanal among the main volatile compounds derived from linoleic acid (cf. Table 3.31) is still an open question. The preferential formation of hexanal in aqueous



(3.72)

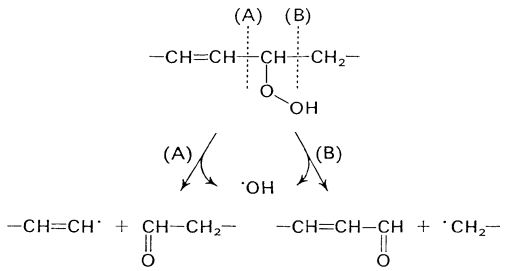
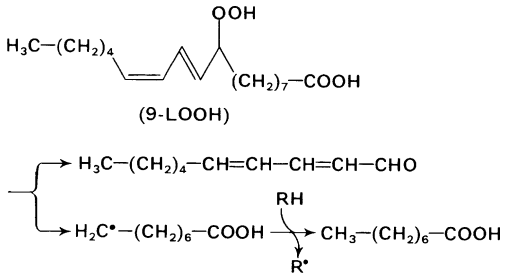


Fig. 3.26. β-Scission of monohydroperoxides (according to *Badings*, 1970)



(3.73)

systems can be explained with an ionic mechanism. As shown in Fig. 3.27, the heterolytic cleavage is initiated by the protonation of the hydroperoxide group. After elimination of a water molecule, the oxo-cation formed is subjected to an insertion reaction exclusively on the C–C linkage adjacent to the double bond. The carbonium ion then splits into an oxo-acid and hexanal. The fact that linoleic acid 9-hydroperoxide gives rise to 2-nonenal is in agreement with this outline.

However, in the water-free fat or oil phase of food, the homolytic cleavage of hydroperoxides presented above is the predominant reaction mechanism. Since option "A" of the cleavage reaction is excluded (Fig. 3.26), some other reactions should be assumed to occur to account for formation of hexanal and other aldehydes from linoleic acid. The further oxidation reactions of monohydroperoxides and carbonyl compounds are among the possibilities.

The above assumption is supported by the finding that 2-alkenals and 2,4-alkadienals are oxidized substantially faster than the unsaturated

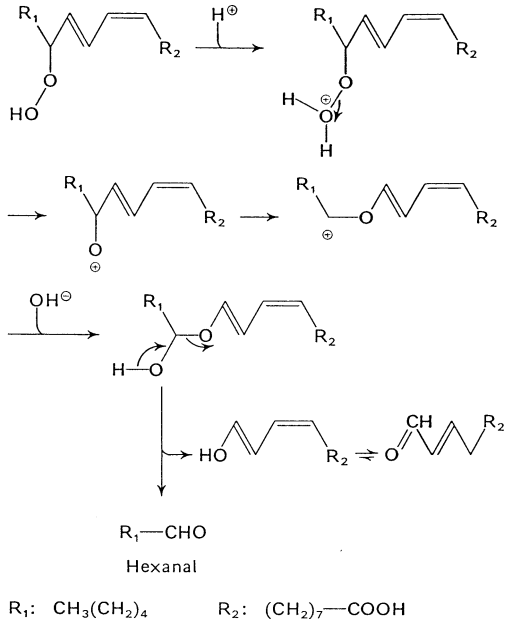


Fig. 3.27. Proton-catalyzed cleavage of linoleic acid 13-hydroperoxide (according to *Ohloff*, 1973)

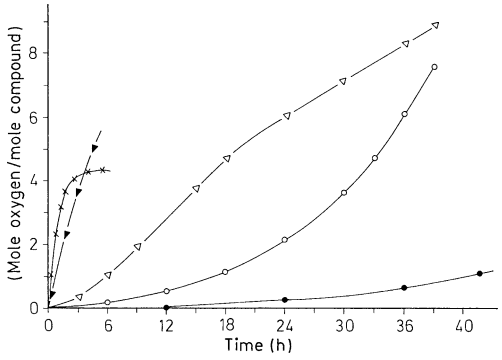


Fig. 3.28. Reaction rate of an autoxidation process (according to *Lillard* and *Day*, 1964).
–▽–▽– Linolenic acid methyl ester, –○–○– linoleic acid methyl ester, ×–×– 2-nonenal, ▼–▼–▼ 2,4-heptadienal, –●–●– nonanal

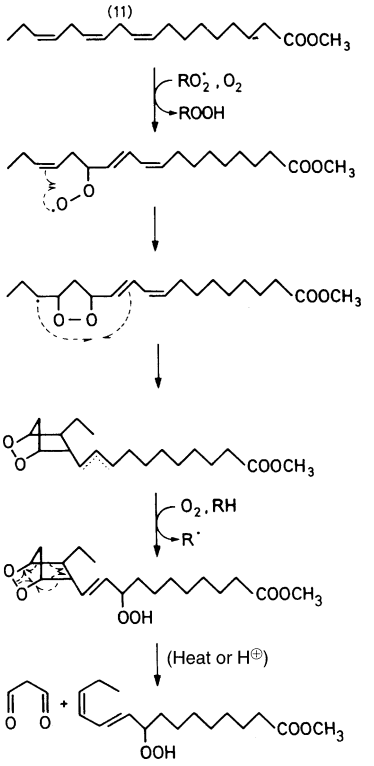
fatty acids (Fig. 3.28). In addition, the autoxidation of 2,4-decadienal yields hexanal and other volatiles which coincide with those obtained from linoleic acid. Since saturated aldehydes oxidize slowly, as demonstrated by nonanal (Fig. 3.28), they will enrich the oxidation products and become predominant.

Also the delayed appearance of hexanal during the storage of linoleic acid containing fats and oils compared to pentane and 2,4-decadienal, supports the hypothesis that hexanal is not directly formed by a β-scission of the 13-hydroperoxide. It is mainly produced in a tertiary reaction, e. g., during the autoxidation of 2,4-decadienal.

Other studies to elucidate the multitude of aldehydes which arise suggest that the decomposition of minor hydroperoxides formed by autoxidation of linoleic acid (cf. Table 3.28) contribute to the profile of aldehydes. This suggestion is supported by pentanal, which originates from the 14-hydroperoxide.

The occurrence of 2,4-heptadienal (from the 12-hydroperoxide isomer) and of 2,4,7-decatrienal (from the 9-hydroperoxide isomer) as oxidation products is, thereby, readily explained by accepting the fragmentation mechanism outlined above (option "B" in Fig. 3.26) for the autoxidation of α-linolenic acid. The formation of other volatile carbonyls can then follow by autoxidation of these two aldehydes or from the further oxidation of labile monohydroperoxides.

Malonic Aldehyde. This dialdehyde is preferentially formed by autoxidation of fatty acids with three or more double bonds. The compound is odorless. In food it may be bound to proteins by a double condensation, crosslinking the proteins (cf. 3.7.2.4.3). Malonic aldehyde is formed from α-linolenic acid by a modified reaction pathway, as outlined under the formation of hydroperoxide-epidioxide (cf. 3.7.2.1.3). However, a bicyclic compound is formed here as an intermediary product that readily fragments to malonic aldehyde:



(3.74)

Alkanes, Alkenes. The main constituents of the volatile hydrocarbon fraction are ethane and pentane. Since these hydrocarbons are readily quantitated by gas chromatography using head-space analysis, they can serve as suitable indicators for *in vivo* detection of lipid peroxidation. Pentane is probably formed from the 13-hydroperoxide of linoleic acid by the β-scission mechanism (cf. reaction 3.72). The corresponding pathway for 16-hydroperoxide of linolenic acid should then yield ethane.

3.7.2.2 Lipoxygenase: Occurrence and Properties

A lipoxygenase (linoleic acid oxygen oxidoreductase, EC 1.13.11.12) enzyme occurs in many plants and also in erythrocytes and leucocytes. It catalyzes the oxidation of some unsaturated fatty acids to their corresponding monohydroperoxides. These hydroperoxides have the same structure as those obtained by autoxidation. Unlike autoxidation, reactions catalyzed by lipoxygenase are characterized by all the features of enzyme catalysis: substrate specificity, peroxidation selectivity, occurrence of a pH optimum, susceptibility to heat treatment and a high reaction rate in the range of 0–20 °C. Also, the activation energy for linoleic acid peroxidation is rather low: 17 kJ/mol (as compared to the activation energy of a noncatalyzed reaction, see 3.7.2.1.5). Lipoxygenase oxidizes only fatty acids which contain a 1-cis,4-cis-pentadiene system. Therefore, the preferred substrates are linoleic and linolenic acids for the plant enzyme, and arachidonic acid for the animal enzyme; oleic acid is not oxidized.

Lipoxygenase is a metal-bound protein with an Fe-atom in its active center. The enzyme

Table 3.33. Regio- and stereospecificity of lipoxygenases (LOX)

Origin (isoenzyme)		Hydroperoxide from 18:2 (9,12)[a]					PH
		13S	13R	9S	9R	8R	
Soybean, seed	(LOX I)	94	2	2	2		10.5
	(LOX II)	77	3	18	2		7
Pea, seed	(LOX I)	23	16	32	29		6.8
	(LOX II)	87	2	6	5		6.8
Corn, germ		3.5	3.5	89	4		6.5
Tomato, fruit		13	2	84	<1		5.5
Potato, tuber		1.6	2.4	96	0		6.8
Barley, seed				92	3		7.0
Wheat, germ		10	5	83	2		6.8
Gaeumannomyces graminis						100	7.4
Marchantia polymorpha		89	2				9.0

[a] Composition of the hydroperoxide fraction in %.

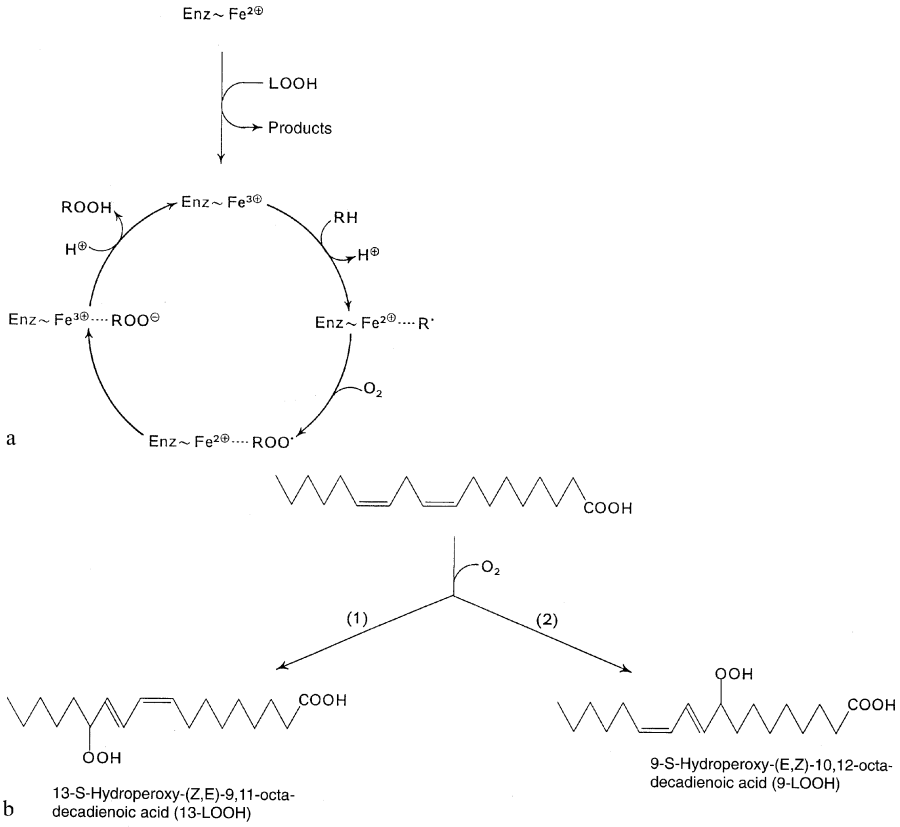


Fig. 3.29. Lipoxygenase catalysis

a Proposed mechanism of reaction (according to *Veldink*, 1977); RH: linoleic acid; LOOH: linoleic acid hydroperoxide

b Regio- and stereospecificity for linoleic acid oxidation. (1) Lipoxygenase from soybean (LOX 1; cf. Table 3.33); (2) lipoxygenase from tomato (cf. Table 3.33)

is activated by its product and during activation, Fe^{2+} is oxidized to Fe^{3+}. The catalyzed oxidation pathway is assumed to have the following reaction steps (cf. Fig. 3.29a): abstraction of a methylene H-atom from the substrate's 1,4-pentadiene system and oxidation of the H-atom to a proton. The pentadienyl radical bound to the enzyme is then rearranged into a conjugated diene system, followed by the uptake of oxygen. The peroxy radical formed is then reduced by the enzyme and, after attachment of a proton, the hydroperoxide formed is released.

In the rate-limiting step of catalysis, the isoenzyme LOX 1 from soybeans abstracts the pro-(S)-hydrogen from the n-8 methylene group[a] of linoleic acid. Molecular oxygen is then introduced into the fatty acid present as a pentadienyl radical from the opposite side at n-6 with the formation of the 13S-hydroperoxide (Fig. 3.29b). Another group of LOX, to which the enzyme from tomatoes belongs, abstracts the pro-(R)-hydrogen. This results in the formation of a 9S-hydroperoxide (Fig. 3.29b) if the oxygen coming from the opposite side docks onto C-9.

Lipoxygenases from plants mostly exhibit 9- or 13-regiospecificity. A LOX with C-8 specificity has been found in a mushroom (Table 3.33).

[a] "n": the C atoms are counted from the methyl end of the fatty acid.

Non-specific LOX occur in legumes, e. g., LOX 1 in peas (Table 3.33) and LOX III in soybeans (pH optimum: 6.5). These enzymes oxidize linoleic acid to mixtures of 9- and 13-hydroperoxides, which approach racemic proportions. In addition, oxo fatty acids and volatile compounds are formed, i. e., the product spectrum resembles that formed by the autoxidation of linoleic acid. Moreover, they also react with esterified substrate fatty acids. In contrast to specific LOX, they do not require prior release of fatty acids by a lipase enzyme for activity in food.

The non-specific lipoxygenases can cooxidize carotenoids and chlorophyll and thus can degrade these pigments to colorless products. This property is utilized in flour "bleaching" (cf. 15.4.1.4.3). The involvement of LOX in cooxidation reactions can be explained by the possibility that the peroxy radicals are not as rapidly and fully converted to their hydroperoxides as in the case of specifically reacting enzymes. Thus, a fraction of the free peroxy radicals are released by the enzyme. It can abstract an H-atom either from the unsaturated fatty acid present (pathway 2a in Fig. 3.30) or from a polyene (pathway 2b in Fig. 3.30).

The non-specific lipoxygenases present in legumes produces a wide spectrum of volatile aldehydes from lipid substrates. These aldehydes, identical to those of a noncatalyzed autoxidation, can be further reduced to their alcohols, depending on the status of NADH-$NAD^{\oplus}$.

3.7.2.3 Enzymatic Degradation of Hydroperoxides

Animals and plants degrade fatty acid hydroperoxides differently. In animal tissue, the enzyme glutathione peroxidase (cf. 7.3.2.8) catalyzes a reduction of the fatty acid hydroperoxides to the corresponding hydroxy acids, while in plants and mushrooms, hydroperoxide lyase (HPL), hydroperoxide isomerase, allene oxide synthase (AOS) and allene oxide cyclase (AOC) are active. The HPL reaction is highly interesting with regard to food chemistry since the hydroperoxides, which are formed by lipoxygenase catalysis of linoleic and linolenic acid, are precursors of odorants. Those are important for fruits, vegetables and mushrooms, like the green-grassy or cucumberlike smelling aldehydes hexanal, (Z)-3-hexenal ("leafy aldehyde"), (Z,Z)-3,6-nonadienal and the mushroomlike (R)-l-octen-3-ol (Table 3.34). The suggested mechanism is a β-cleavage of the hydroperoxide (Fig. 3.31).

The difference in volatile products in plants (aldehydes) and mushrooms (allyl alcohols) is due to the different substrate and reaction specificity of HPL. In the first case, in hydroperoxides with conjugated diene systems (Fig. 3.31a), the bond between the C-atom bearing the HOO-group and the C-atom of the diene system is cleaved. In the second case (Fig. 3.31b), cleavage of hydroperoxides with isolated double bonds occurs in the opposite direction between the C-atom

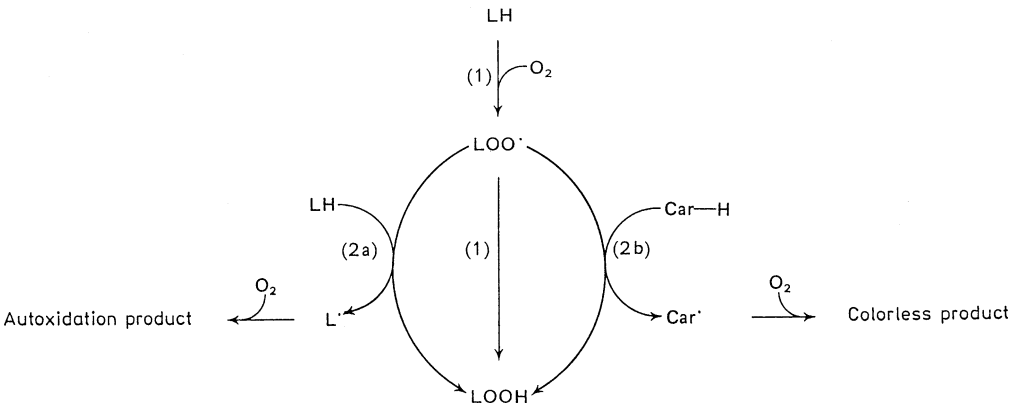
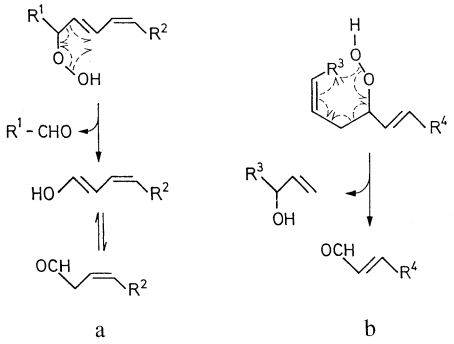


Fig. 3.30. Reactions of non-specific lipoxygenase (according to *Weber* and *Grosch*, 1976). (1) Main catalysis pathway; (2a) and (2b) cooxidation pathways. LH: linoleic acid; Car-H: carotenoid; LOOH: linoleic acid hydroperoxide



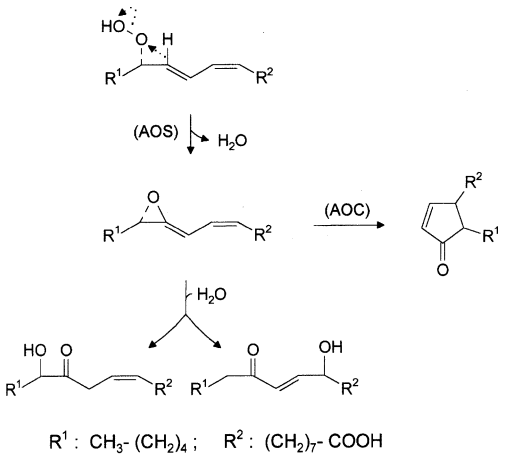
	R^1	R^2
13 - LOOH	$CH_3(CH_2)_4$	$(CH_2)_7COOH$
13 - LnOOH	$CH_3CH_2CH=CHCH_2$	$(CH_2)_7COOH$
9 - LOOH	$HOOC(CH_2)_7$	$(CH_2)_4CH_3$
9 - LnOOH	$HOOC(CH_2)_7$	$CH_2CH=CHCH_2CH_3$
	R^3	R^4
10 - LOOH	$CH_3(CH_2)_4$	$(CH_2)_6COOH$
10 - LnOOH	$CH_3CH_2CH=CHCH_2$	$(CH_2)_6COOH$

Fig. 3.31. Mechanism of the cleavage of hydroperoxides by lyases (according to *Wurzenberger and Grosch*, 1986)
a in plants, **b** in mushrooms

with the OOH-group and the C-atom with the adjacent methylene group. The (Z)-3-aldehydes in plants formed by the splitting reaction can transform themselves into the respective (E)-2-aldehydes. Isomerases that catalyze this reaction were identified in cucumbers, apples and tea chloroplasts.

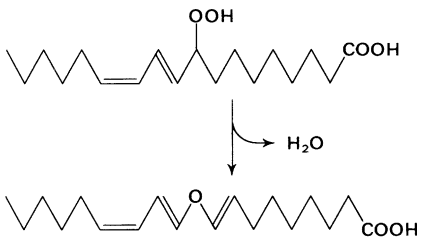
The widespread presence of the C6- and C9-aldehydes in fruits and vegetables as well as the C8-alcohols in mushrooms (Table 3.34) permits the conclusion to be drawn that enzymatic-oxidative cleavage of linoleic and linolenic acid with the enzymes lipoxygenase, hydroperoxide-lyase and, if necessary, an aldehyde-isomerase generally contributes to the formation of aroma in these food items. This process is intensified when oxygen can permeate the cells freely by destruction of tissue (during the chopping of fruits and vegetables).

Allene oxide synthase (AOS) is a cytochrome P_{450} enzyme which was first found in flaxseed. It catalyzes the degradation of hydroperoxides to very instable allene oxides ($t_{1/2}$ at 0 °C: 33 s). On hydrolysis, the allene oxide originating from the 13-hydroperoxide of linoleic acid gives rise to α- or γ-ketol fatty acids depending on whether the OH-ion attacks at C-13 or C-9 (Formula 3.75). The allene oxide can also react with other nucleophiles, e. g., ROH, RSH, as well as the anion of linoleic acid. Allene oxide cyclase (AOC) competes with these non-enzymatic reactions, cyclizing the allene oxide to 15,16-dihydro-12oxophyto-dienoic acid (Formula 3.75). AOS and AOC convert the 13-hydroperoxide of linolenic acid to 12-oxophytadienoic acid.



(3.75)

Linoleic acid 9-hydroperoxide formed by lipoxygenase in potato is changed enzymatically by elimination of water into a fatty acid with a dienyl-ether structure:



(1′-trans, 3′-cis-Nonadienyloxa)-trans-8-nonenoic acid (3.76)

In addition to lipoxygenase, lipoperoxidase activity has been observed in oats. The 9-hydroperoxide formed initially is reduced to 9-hydroxy-trans-10,cis-12-octadecadienoic

Table 3.34. Occurrence and properties of various hydroperoxide-lyases

Occurrence	Substrate	Products of the catalyses
Apple, tomato, cucumber, tea leaf (chloroplasts), soy beans, grape	13(S)-hydroperoxy-9-cis,11-trans-octadecadienoic acid (13-LOOH)	hexanal + 12-oxo-9-cis-dodecenoic acid
Apple, tomato, cucumber, tea leaf (chloroplasts), soy beans, grape	13(S)-hydroperoxy-9-cis,11-trans, 15-cis-octadecatrienoic acid (13-LnOOH)	(Z)-3-hexenal + 12-oxo-9-cis-dodecenoic acid
Cucumber, pear	9(S)-hydroperoxy-10-trans, 12-cis-octadecadienoic acid (9-LOOH)	(Z)-3-nonenal + 9-oxo-nonanoicacid
Cucumber, pear	9(S)-hydroperoxy-8-trans, 12-cis, 15-cis-octadecatrienoic acid (9-LnOOH)	(Z,Z)-3,6-nonadienal + 9-oxononanoic acid
Champignon	10(S)-hydroperoxy-10-trans, 12-cis-octadecadienoic acid (10-LOOH)	1-octen-3(R)-ol + 10-oxo-8-trans-dcenoic acid
Champignon	10(S)-hydroperoxy-8-trans,12-cis-15-cis-octadecatrienoic acid (10-LnOOH)	(Z)-1,5-octadien-3(R)-ol+ 10-oxo-8-trans-decenoicacid

acid. Since hydroxy but not hydroperoxy acids taste bitter, this reaction should contribute to the bitter taste generated during the storage of oats (cf. 15.2.2.3).

3.7.2.4 Hydroperoxide–Protein Interactions

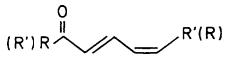
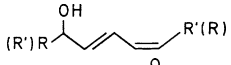
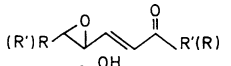
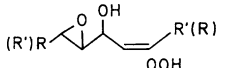
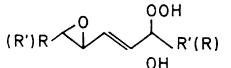
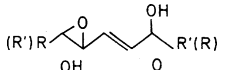
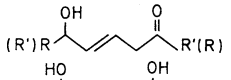
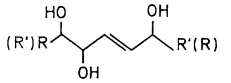
3.7.2.4.1 Products Formed from Hydroperoxides

Hydroperoxides formed enzymatically in food are usually degraded further. This degradation can also be of a nonenzymatic nature. In nonspecific reactions involving heavy metal ions, heme(in) compounds or proteins, hydroperoxides are transformed into oxo, expoxy, mono-, di- and trihydroxy carboxylic acids (Table 3.35). Unlike hydroperoxides, i. e. the primary products of autoxidation, some of these derivatives are characterized as having a bitter taste (Table 3.35). Such compounds are detected in legumes and cereals. They may play a role in other foods rich in unsaturated fatty acids and proteins, such as fish and fish products.

In order to clarify the formation of the compounds presented in Table 3.35, the reaction sequences given in Fig. 3.32 have been assumed to occur. The start of the reaction is from the alkoxydiene radical generated from the 9- or 13-hydroperoxide by the catalytic action of heavy metal ions or heme(in) compounds (cf. 3.7.2.1.7). The alkoxydiene radical may disproportionate into a hydroxydiene and an oxodiene fatty acid. Frequently this reaction is only of secondary importance since the alkoxydiene radical rearranges immediately to an epoxyallylic radical which is susceptible to a variety of radical combination reactions. Under aerobic conditions the epoxyallylic radical combines preferentially with molecular oxygen. The epoxyhydroperoxides formed are, in turn, subject to homolysis via an oxyradical. A disproportionation reaction leads to epoxyoxo and epoxyhydroxy compounds. Under anaerobic conditions the epoxyallylic radical combines with other radicals, e. g. hydroxy radicals (Fig. 3.32) or thiyl radicals (Fig. 3.33).

Of the epoxides produced, the allylic epoxides are known to be particularly susceptible to hydrolysis in the presence of protons. As shown in Fig. 3.32 trihydroxy fatty acids may result from the hydrolysis of an allylic epoxyhydroxy compound.

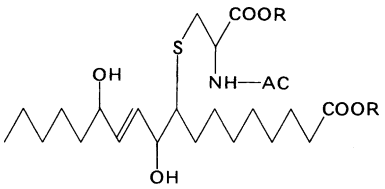
Table 3.35. Products obtained by non-enzymic degradation of linoleic acid hydroperoxides

Product[a]	Hydroperoxide interaction with				
	Fe^{3+} cysteine	Hemo-globin	Soya homogenate	Pea homogenate	Wheat flour
	+	+	+	+	
	+	+	+	+	+
	+		+		
	+	+	+		
	+		+		
		+		+	+
	+				
	+		+	+	+

[a] As a rule a mixture of two isomers are formed with R: $CH_3(CH_2)_4$ and R′: $(CH_2)_7COOH$.

3.7.2.4.2 Lipid–Protein Complexes

Studies related to the interaction of hydroperoxides with proteins have shown that, in the absence of oxygen, linoleic acid 13-hydroperoxide reacts with N-acetylcysteine, yielding an adduct of which one isomer is shown:



(3.77)

However, in the presence of oxygen, covalently bound amino acid-fatty acid adduct formation is significantly suppressed; instead, the oxidized fatty acids listed in Table 3.35 are formed.

The difference in reaction products is explained in the reaction scheme shown in Fig. 3.33 which gives an insight into the different reaction pathways. The thiyl radical, derived from cysteine by abstraction of an H-atom, is added to the epoxyallyllic radical only in the absence of oxygen (pathway 2 in Fig. 3.33). In the presence of oxygen, oxidation of cysteine to cysteine oxide and of fatty acids to their more oxidized forms (Fig. 3.32) occur with a higher reaction rate than in the previous reaction.

Table 3.36. Taste of oxidized fatty acids

Compound	Threshold value for bitter taste (mmol/1)
13-Hydroperoxy-cis-9,trans-11-octadecadienoic acid	not bitter[a]
9-Hydroperoxy-trans-10,cis-12-octadecadienoic acid	not bitter[a]
13-Hydroxy-cis-9, trans-11-octadecadienoic acid	7.6–8.5[a]
9-Hydroxy-trans-10,cis-12-octadecadienoic acid	6.5–8.0[a]
9,12,13-Trihydroxy-trans-10-octadecenoic acid	0.6–0.9[b]
9, 10, 13-Trihydroxy-trans-11-octadecenoic acid	

[a] A burning taste sensation.

[b] A blend of the two trihydroxy fatty acids was assessed.

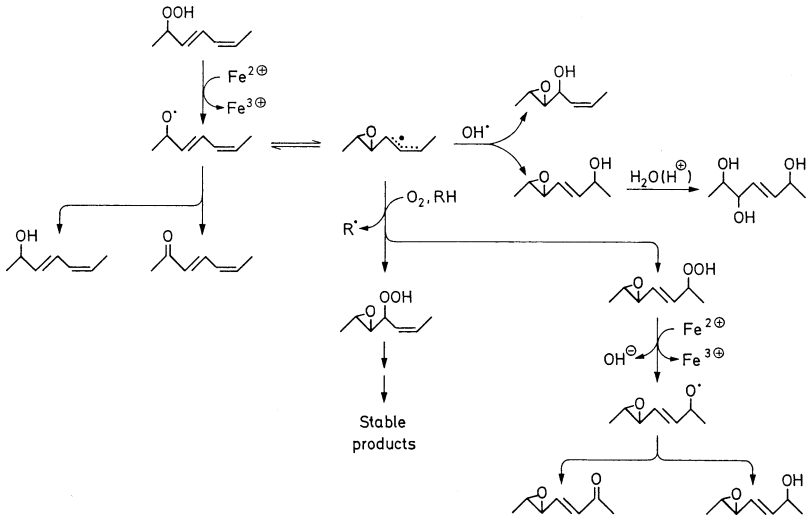


Fig. 3.32. Degradation of linoleic acid hydroperoxides to hydroxy-, epoxy- and oxo-fatty acids. The postulated reaction sequence explains the formation of identified products. Only segments of the structures are presented (according to *Gardner*, 1985)

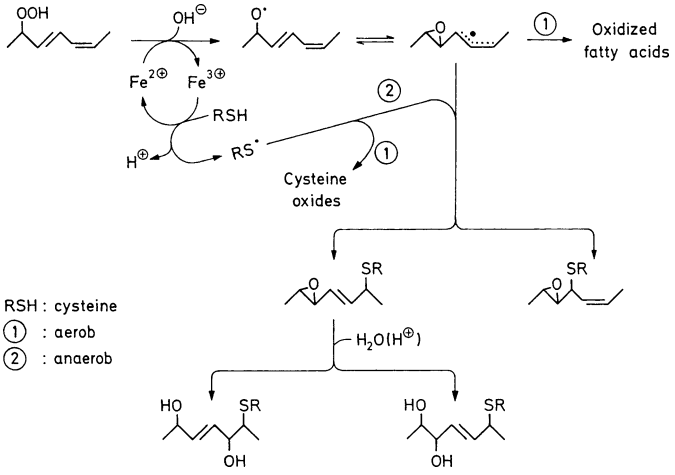


Fig. 3.33. Interaction of linoleic acid hydroperoxides with cysteine. A hypothesis to explain the reaction products obtained. Only segments of the structures are presented (according to *Gardner*, 1985)

As a consequence, a large portion of the oxidized lipid from protein-containing food stored in air does not have lipid–protein covalent bonds and, hence, is readily extracted with a lipid solvent such as chloroform/methanol (2:1).

3.7.2.4.3 Protein Changes

Some properties of proteins are changed when they react with hydroperoxides or their degradation products. This is reflected by changes in food

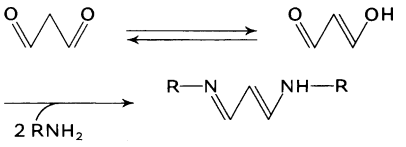
texture, decreases in protein solubility (formation of cross-linked proteins), color (browning) and changes in nutritive value (loss of essential amino acids).

The radicals generated from hydroperoxides (cf. Fig. 3.33) can abstract H-atoms from protein (PH), preferentially from the amino acids Trp, Lys, Tyr, Arg, His and cysteine, in which the phenolic HO-, S- or N-containing groups react:

$$RO^{\bullet} + PH \longrightarrow P^{\bullet} + ROH \quad (3.78)$$

$$2\,P^{\bullet} \longrightarrow P\text{–}P \quad (3.79)$$

In Reaction 3.79. protein radicals combine with each other, resulting in the formation of a protein network. Malonaldehyde is generated (cf. 3.7.2.1.9) under certain conditions during lipid peroxidation. As a bifunctional reagent, malonaldehyde can crosslink proteins through a *Schiff* base reaction with the ε-NH_2 groups of two lysine residues:



(3.80)

The *Schiff* base adduct is a conjugated fluorochrome that has distinct spectral properties (λ_{max} excitation ~350 nm; λ_{max} emission ~450 nm). Hence, it can be used for detecting lipid peroxidation and the reactions derived from it with the protein present.

Reactions resulting in the formation of a protein network like that oulined above also have practical implications, e. g., they are responsible for the decrease in solubility of fish protein during frozen storage.

Also, the monocarbonyl compounds derived from autoxidation of unsaturated fatty acids readily condense with protein-free NH_2 groups, forming *Schiff* bases that can provide brown polymers by repeated aldol condensations (Fig. 3.34). The brown polymers are often N-free since the amino compound can be readily eliminated by hydrolysis. When hydrolysis occurs in the early stages of aldol condensations (after the first or second condensation; cf. Fig. 3.34) and the released aldehyde, which has a powerful odor, does not reenter the reaction, the condensation process results not only in discoloration (browning) but also in a change in aroma.

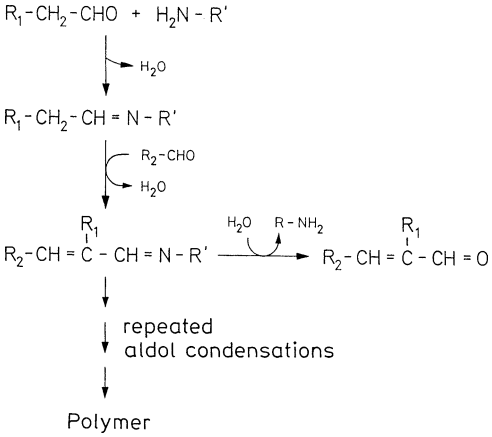


Fig. 3.34. Reaction of volatile aldehydes with protein amino groups

3.7.2.4.4 Decomposition of Amino Acids

Studies of model systems have revealed that protein cleavage and degradation of side chains, rather than formation of protein networks, are the preferred reactions when the water content of protein/lipid mixtures decreases. Several examples of the extent of losses of amino acids in a protein in the presence of an oxidized lipid are presented in Table 3.37. The strong dependence of this loss on the nature of the protein and reaction conditions is obvious. Degradation products obtained in model systems of pure amino acids and oxidized lipids are described in Table 3.38.

3.7.3 Inhibition of Lipid Peroxidation

Autoxidation of unsaturated acyl-lipids can be retarded by:

- Exclusion of oxygen. Possibilities are packaging under a vacuum or addition of glucose oxidase (cf. 2.7.2.1.1).

Table 3.37. Amino acid losses occurring in protein reaction with peroxidized lipids

Reaction system		Reaction conditions		Amino acids lost
protein	lipid	time	T (°C)	(% loss)
Cytochrome C	Linolenic acid	5 h	37	His(59),Ser(55), Pro(53),Val(49), Arg(42),Met(38), Cys(35)[a]
Trypsin	Linoleic acid	40 min	37	Met(83),His(12)[a]
Lysozyme	Linoleic acid	8 days	37	Trp(56),His(42), Lys(17),Met(14), Arg(9)
Casein	Linoleic acid ethyl ester	4 days	60	Lys(50), Met(47), Ile(30),Phe(30), Arg(29),Asp(29), Gly(29),His(28), Thr(27),Ala(27), Tyr(27)[a,b]
Ovalbumin	Linoleic acid ethyl ester	24 h	55	Met(17),Ser(10), Lys(9),Ala(8), Leu(8)[a,b]

[a] Trp analysis was not performed.
[b] Cystine analysis was not performed.

Table 3.38. Amino acid products formed in reaction with peroxidized lipid

Reaction system		Compounds formed from amino acids
amino acid	lipid	
His	Methyl linoleate	Imidazolelactic acid, Imidazoleacetic acid
Cys	Ethyl arachidonate	Cystine, H_2S, cysteic acid, alanine, cystine-disulfoxide
Met	Methyl linoleate	Methionine-sulfoxide
Lys	Methyl linoleate	Diaminopentane, aspartic acid, glycine, alanine, α-aminoadipic acid, pipecolinic acid, 1,10-diamino-1,10-dicarboxydecane

- Storage at low temperature in the dark. The autoxidation rate is thereby decreased substantially. However, in fruits and vegetables which contain the lipoxygenase enzyme, these precautions are not applicable. Food deterioration is prevented only after in activation of the enzyme by a blanching process (cf. 2.6.4).
- Addition of antioxidants to food.

3.7.3.1 Antioxidant Activity

The peroxy and oxy free radicals formed during the propagation and branching steps of the autoxidation radical chain (cf. Fig. 3.19) are scavenged by antioxidants (AH; cf. Fig. 3.35). Antioxidants containing a phenolic group play the major role in food. In reactions 1 and 2 in Fig. 3.35, they form radicals which are stabilized by an aromatic resonance system. In contrast to the acyl peroxy and oxy free radicals, they are not able to abstract a H-atom from an unsaturated fatty acid and therefore cannot initiate lipid peroxidation. The end-products formed in reactions 3 and 4 in Fig. 3.35 are relatively stable and in consequence the autoxidation radical chains are shortened.
The reaction scheme (Fig. 3.35) shows that one antioxidant molecule combines with two radicals. Therefore, the maximum achievable stoichiometric factor is $n = 2$. In practice, the value of n is between 1 and 2 for the antioxidants used. Antioxidants, in addition to their main role as radical scavengers, can also partially reduce hydroperexides to hydroxy compounds.

3.7.3.2 Antioxidants in Food

3.7.3.2.1 Natural Antioxidants

The unsaturated lipids in living tissue are relatively stable. Plants and animals have the necessary complement of antioxidants and of enzymes, for instance, glutathione peroxidase and superoxide dismutase, to effectively prevent lipid oxidation.
During the isolation of oil from plants (cf. 3.8.3), tocopherols are also isolated. A sufficient level is retained in oil even after refining, thus, toco-

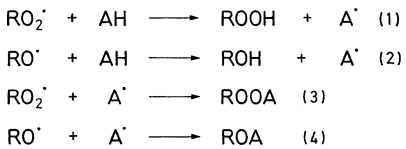
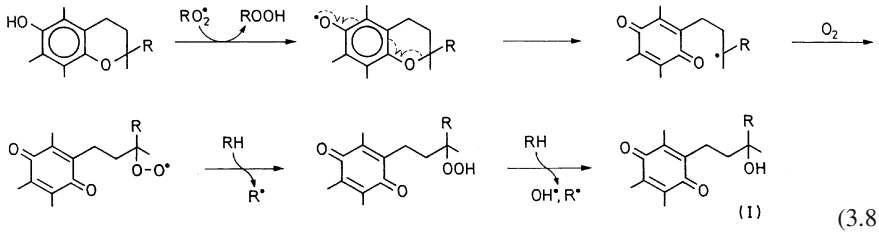


Fig. 3.35. Activity of an antioxidant as a radical scavenger. AH: Antioxidant



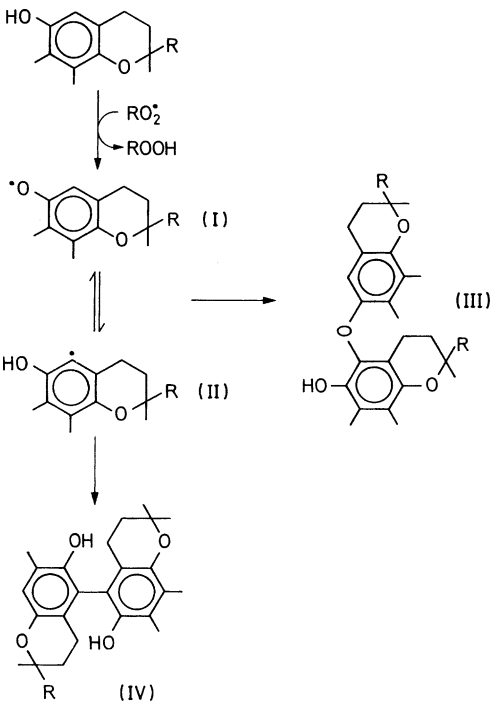
(3.81)

pherols secure the stability of the oil end-product. Soya oil, due to its relatively high level of furan fatty acids and linolenic acid (cf. 14.3.2.2.5), is an exception. The tocopherol content of animal fat is influenced by animal feed.

The antioxidant activity of tocopherols increases from α → δ. It is the reverse of the vitamin E activity (cf. 6.2.3) and of the rate of reaction with peroxy radicals. Table 3.39 demonstrates that α-tocopherol reacts with peroxy radicals faster than the other tocopherols and the synthetic antioxidants DBHA and BHT.

The higher efficiency of γ-tocopherol in comparison to α-tocopherol is based on the higher stability of γ-tocopherol and on different reaction products formed during the antioxidative reaction.

After opening of the chroman ring system, α-tocopherol is converted into an alkyl radical which in turn oxidizes to a hydroxy-alkylquinone (I in Formula 3.81). α-Tocopherol is a faster scavenger for peroxy radicals formed during autoxidation than γ-tocopherol (Table 3.39), but α-tocopherol then generates an alkyl radical which, in contrast to the slow reacting chromanoxyl radical, can start autoxidation of unsaturated fatty acids. Therefore, the peroxidation rate of an unsaturated fatty acid increases with higher α-tocopherol concentrations after going through a minimum. This prooxidative effect is smaller in the case of γ-tocopherol because in contrast to α-tocopherol, no opening of the chroman ring takes place but formation of diphenylether and biphenyl dimers occurs. The supposed explanation for these reaction products is: The peroxy radical of a fatty acid abstracts a hydrogen atom from γ-tocopherol (Formula 3.82). A chromanoxyl radical (I) is formed, that can transform into a chromanyl radical (II). Recombination of (I) and (II) results in the diphenylether dimer (III) and recombination of two radicals (II) into the biphenyl dimer (IV). Unlike p-quinone from the reaction of α-tocopherol, the dimer structures (III) and (IV) possess one or two phenolic OH-groups that are also antioxidatively active.

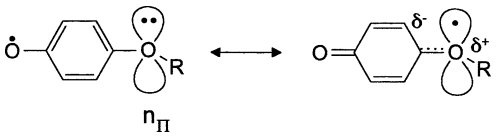


(3.82)

Table 3.39. Rate constants of tocopherols and BHT for reaction 2 in Fig. 3.35 at 30°C

Antioxidant	$k(1 \cdot mol^{-1} \cdot s^{-1}) \cdot 10^{-5}$
α-Tocopherol	23.5
β-Tocopherol	16.6
γ-Tocopherol	15.9
δ-Tocopherol	6.5
2,6-Di-*tert*-butyl-4-hydroxyanisole (DBHA)	1.1
2,6-Di-tert-butyl-p-cresol (BHT)	0.1

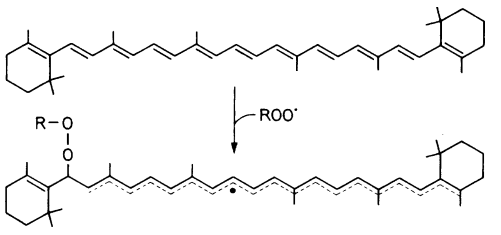
In the reaction with peroxyl radicals, the higher rate of tocopherols compared with DBHA (cf. Table 3.39) is based on the fact that the chromanoxyl radical formed on H-abstraction is more stable than the phenoxyl radical. Both types of radical are stabilized by the following resonance:



(3.83)

This resonance effect is the highest when the orbital of the 2p electron pair of the ether oxygen and the half occupied molecule orbital of the radical oxygen are aligned parallel to each other, i. e., vertical to the plane of the aromatic ring. Any deviation lowers the stability, slowing down H-abstraction. Due to incorporation into a six-membered ring present in the *half-chair* conformation, the ether oxygen is so strongly fixed in the chromanoxyl radical that the deviation is only 17°. The methoxy group in the DBHA phenoxyl radical is freely rotatable so that the orbital of the 2p electron pair is oriented to the plane of the aromatic ring; thus the deviation is ∼90°. BHT reacts even slower than DBHA (Table 3.39) because there is no ether oxygen.

Ascorbic acid (cf. 6.3.9) is active as an antioxidant in aqueous media, but only at higher concentrations (∼10^{-3} mol/l). A prooxidant activity is observed at lower levels (10^{-5} mol/l), especially in the presence of heavy metal ions. The effect of tocopherols is enhanced by the addition of fat soluble ascorbyl palmitate or ascorbic acid in combination with an emulsifier (e. g. lecithin) since the formed tocopherol radical from reaction 2 in Fig. 3.35 is rapidly reduced to α-tocopherol by vitamin C.



(3.84)

Carotinoids also can act as scavengers for alkyl radicals. Radicals stabilized by resonance are formed (Formula 3.84), unable to initiate lipid peroxidation. β-Carotenes are most active at a concentration of $5 \cdot 10^{-5}$ mol/l, while at higher concentrations the prooxidative effect is predominant. Also the partial pressure of oxygen is critical, it should be below 150 mm Hg.

Phenolic compounds (cf. 18.1.2.5) which are widely distributed in plant tissues, act as natural antioxidants. The protective effect of several herbs, spices (e. g. sage or rosemary) and tea extracts against fat (oil) oxidation is based on the presence of such natural antioxidants (cf. 21.2.5.1 and 22.1.1.4). The antioxidative effect of phenols depends on the pH. It is low in an acidic medium (pH 4) and high in an alkaline medium (pH 8) when phenolation occurs.

In the protection of linoleic acid micelles, the antioxidative activity of quercetin is approximately as high as that of α-tocopherol (Table 3.40). The activity of the two synthetic dihydroxyflavones at 70% and 63% is also high. Therefore, it is not only the number of OH-groups in the molecule, but the presence of OH-groups in the ortho position that is important. But this characteristic feature is not enough to explain the high activity of quercetin compared with that of catechin, which is four times less active (Table 3.40) although the OH patterns correspond. Obviously the carbonyl group, which is absent in catechin, increases the stability of the phenoxyl radical by electron attraction which

Table 3.40. Relative antioxidative activity (RAA) of flavonoids, cumarins and hydroxycinnamic acids[a,b]

Compound	RAA × 100
α-Tocopherol	100
Quercetin (cf. Formula 18.32)	90
Cyanidin	90
Catechin (cf. Formula 18.20)	22
6,7-Dihydroxyflavonc	70
7,8-Dihydroxyflavone	63
7,8-Dihydroxycumarin	3.3
Ferulic acid	< 0.1
Caffeic acid	< 0.1

[a] Test system: linoleic acid micelles stabilized with Na dodecyl sulfate (pH 7.4, T: 50 °C).
[b] RAA with reference to the activity of α-tocopherol.

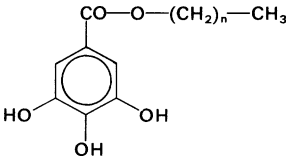
includes the 2,3-double bond. The phenoxyl radical arises from the trapping reaction of peroxyl radicals with quercetin. Another factor is the lipophilicity. Catechin is more hydrophobic than caffeic acid, which is present as an anion at pH 7.4 and, consequently, cannot penetrate the linoleic acid micelle. For this reason, although caffeic acid has OH-groups in ortho position, it is not an antioxidant under the conditions given in Table 3.40.

Polyphenols in wood, such as lignin, undergo thermal cracking, resulting in volatile phenols, during the generation of smoke by burning wood or, even more so, sawdust. These phenols deposit on the food surface during smoking and then penetrate into the food, thus acting as antioxidants.

It was also demonstrated that vanillin, in food items where its aroma is desired, plays an important role as an antioxidant. Finally, some of the *Maillard* reaction products, such as reductones (cf. 4.2.4.4), should be considered as naturally active antioxidants.

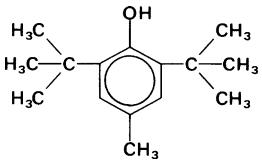
3.7.3.2.2 Synthetic Antioxidants

In order to be used as an antioxidant, a synthetic compound has to meet the following requirements: it should not be toxic; it has to be highly active at low concentrations (0.01–0.02%); it has to concentrate on the surface of the fat or oil phase. Therefore, strongly lipophilic antioxidants are particularly suitable (with low HLB values, e. g. BHA, BHT or tocopherols, dodecylgallate) for o/w emulsions. On the other hand, the more polar antioxidants, such as TBHQ and propyl gallate, are very active in fats and oils since they are enriched at the surface of fat and come in contact with air. Antioxidants should be stable under the usual food processing conditions. This stability is denoted as the "carry through" effect. Some of the synthetic antioxidants used worldwide are:



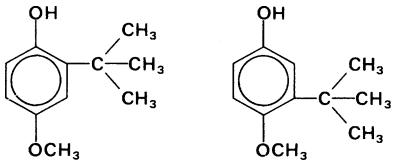
(3.85)

Propyl (n = 2); octyl (n = 7) and dodecyl (n = 11) gallate



(3.86)

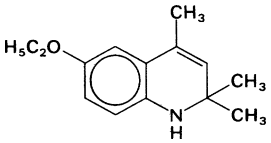
2,6-Di-tert-butyl-p-hydroxytoluene (BHT)



(3.87)

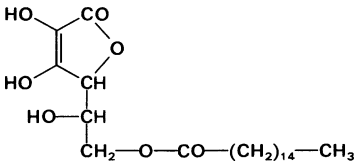
tert-Butyl-4-hydroxyanisole (BHA)

Commercial BHA is a mixture of two isomers, 2- and 3-tert-butyl-4-hydroxyanisole



(3.88)

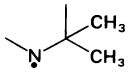
6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin)



(3.89)

Ascorbyl palmitate

ESR spectroscopy has demonstrated that a large portion of ethoxyquin is present in oil as a free radical



(3.90)

and stabilization by dimerization of the radical occurs. The radical, and not the dimer, is the active antioxidant.

tert-Butylhydroquinone (TBHQ) is a particularly powerful antioxidant used, for example, for stabilization of soya oil. The "carry through" proper-

ties are of importance in the use of BHA, TBHQ and BHT in food processing. All three antioxidants are steam distillable at higher temperatures. Utilization of antioxidants is often regulated by governments through controls on the use of food additives. In North America incorporation of antioxidants is permitted at a maximum level of 0.01% for any one antioxidant, and a maximum of 0.02% for any combination. The regulations related to permitted levels often vary from country to country.

The efficiency of an antioxidant can be evaluated by a comparative assay, making use of an "antioxidative factor" (AF):

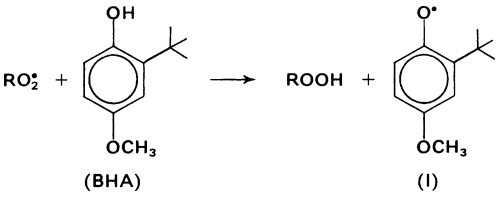
$$AF = I_A/I_0 \tag{3.91}$$

where I_A = oxidation induction period for a fat or oil (cf. 3.7.2.1.1) in the presence of an antioxidant and I_0 = oxidation induction period of a fat or oil without an antioxidant.

Hence, the efficiency of an antioxidant increases with an increase in the AF value. As illustrated by the data in Table 3.41, BHA in comparison with BHT shows a higher efficiency in a lard sample. This result is understandable since in BHT both tertiary butyl substituents sterically hinder the reaction with radicals to a certain extent (reaction 1 in Fig. 3.35). The effect on antioxidants depends not only on the origin of fat or oil but, also, on the processing steps used in the isolation and refining procedures. Hence, data in Table 3.41 serve only as an illustration.

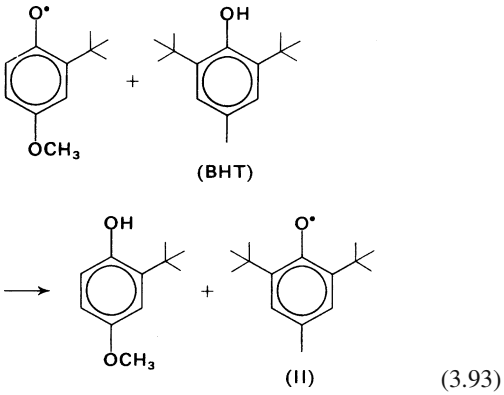
BHA and BHT together at a given total concentration are more effective in extending shelf-life of a fat or oil than either antioxidant alone at the same level of use (Table 3.41).

To explain this, it is suggested that BHA, by participating in reaction 1 (Fig. 3.35), provides a phenoxy radical (I):



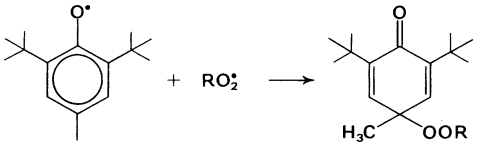
(3.92)

which is then regenerated into the original molecule by rapid interaction with BHT:



(3.93)

On the other hand, the phenoxy radical (II) derived from BHT can react further with an additional peroxy radical:



(3.94)

Propyl gallate (PG) increases the efficiency of BHA, but not that of BHT. Ascorbyl palmitate, which is by itself a rather weak antioxidant, substantially sustains the antioxidative activity of γ,d,l-tocopherol.

Table 3.41. Antioxidative factor (AF) values of some antioxidants (0.02%) in refined lard

Antioxidant	AF	Antioxidant	AF
d-α-Tocopherol	5	Octyl gallate	6
dl-γ-Tocopherol	12	Ascorbyl palmitate	4
BHA	9.5	BHA and	
BHT	6	BHT[a]	12

[a] Each compound is added in amount of 0.01%.

3.7.3.2.3 Synergists

Substances which enhance the activity of antioxidants are called synergists. The main examples are lecithin, amino acids, citric, phosphoric, citraconic and fumaric acids, i. e. compounds which

complex heavy metal ions (chelating agents, sequesterants or scavengers of trace metals). Thus, initiation of heavy metal-catalyzed lipid autoxidation can be prevented (cf. 3.7.2.1.6). Results compiled in Table 3.42 demonstrate the synergistic activities of citric and phosphoric acids in combination with lauryl gallate. Whereas citric acid enhances the antioxidant effectiveness in the presence of all three metal ions, phosphoric acid is able to do so with copper and nickel, but not with iron. Also, use of citric acid is more advantageous since phosphoric acid promotes polymerization of fat or oil during deep frying.

The synergistic effect of phospholipids is different. Addition of dipalmitoylphosphatidylethanolamine (0.1–0.2 weight %) to lard enhances the antioxidative activity of α-tocopherol, BHA, BHT and propyl gallate, while phosphatidylcholine shows no activity.

The reaction of ascorbic acid with tocopherol radicals as described in 3.7.3.2 is a synergistic effect.

3.7.3.2.4 Prooxidative Effect

The activity of antioxidants reverses under certain conditions: they become prooxidants. One way in which α-tocopherol can become peroxidatively active is shown in Formula 3.81. Another way is through the formation of the chromanoxyl radical in concentrations high enough to overcome the inertness mentioned in 3.7.3.1 and abstract H-atoms from unsaturated acyl lipids to a definite extent, starting lipid peroxidation. This activity reversion, which is also undesirable from a nutritional and physiological point of view, is prevented by co-antioxidants, e.g., vitamin C (cf. 3.7.3.2.1), which can reduce the chromanoxyl radical to α-tocopherol.

In the presence of heavy metal ions, e.g., $Fe^{3\oplus}$, ascorbic acid becomes a peroxidant. It reduces $Fe^{3\oplus}$ to $Fe^{2\oplus}$, which can produce superoxide radical anions or hydroxyl radicals with oxygen or H_2O_2 (*Fenton* reaction, cf. 3.7.2.1.8)

Prooxidative effects have also been observed with carotenoids and flavonoids at higher concentrations.

Table 3.42. Synergistic action of citric (C) and phosphoric acids (P) in combination with lauryl gallate (LG) on oxidation of fats and oils

Added to fat/oil	AF value after addition of				
	0.01% C	0.01% P	0.01% LG	0.01%LG + 0.01% C	0.01% LG + 0.01% P
0.2 ppm Cu	0.3	0.2	0.9	4.7	4.1
2 ppm Fe	0.6	0.5	0.1	5.7	0.2
2 ppm Ni	0.5	0.6	3.0	7.0	4.4

3.7.4 Fat or Oil Heating (Deep Frying)

Deep frying is one of the methods of food preparation used both in the home and in industry. Meat, fish, doughnuts, potato chips or french fries are dipped into fat (oil) heated to about 180 °C. After several minutes of frying, the food is sufficiently tender to be served.

The frying fat or oil changes substantially in its chemical and physical properties after prolonged use. Data for a partially hydrogenated soybean oil

Table 3.43. Characteristics of partially hydrogenated soybean oil before and after simulated deep fat frying[a]

Characteristics	Fresh oil	Heated oil
Iodine number	108.9	101.3
Saponification number	191.4	195.9
Free fatty acids[b]	0.03	0.59
Hydroxyl number	2.25	9.34
DG	1.18	2.73
Composition of fatty acids (weight %)		
14:0	0.06	0.06
16:0	9.90	9.82
18:0	4.53	4.45
18:1 (9)	45.3	42.9
18:2 (9, 12)	37.0	29.6
18:3 (9, 12, 15)	2.39	1.67
20:0	0.35	0.35
22:0	0.38	0.38
Other	0.50	0.67

[a] The oil was heated for 80 h (8 h/day) at 195 °C. Batches of moist cotton balls containing 75% by weight of water were fried at 30-min intervals (17 frying operations/day) in order to simulate the deep frying process.

[b] Weight % calculated as oleic acid.

compiled in Table 3.43 indicate that heating of oil causes reactions involving double bonds. This will result in a decrease in iodine number. As can be deduced from changes in the composition of fatty acids (Table 3.43), in the case of soybean oil, linoleic and linolenic acid are the most affected. Peroxides formed at elevated temperatures fragment immediately with formation of hydroxy compounds thus increasing the hydroxyl number (Table 3.43). Therefore, determination of peroxide values to evaluate the quality of fat or oil in deep frying is not appropriate.

Unsaturated TG polymerize during heating thus increasing the viscosity of the fat. Di- and trimeric TG are formed. The increase of these components can be monitored by means of gel permeation chromatography (GPC) (Fig. 3.36).

Before or after methanolysis of the oil sample, GPC is a valuable first tool to analyze the great number of reaction products formed during deep frying. Monomeric methyl esters are further fractionated via the urea adducts, while the cyclic fatty acids enrich themselves in the supernatant. Dimeric methyl esters can be preseparated by RP-HPLC and further analyzed by GC/MS after silylation of the OH-groups. A great number of volatile and nonvolatile products are obtained during deep frying of oil or fat. The types of reactions involved in and responsible for changes in oil and fat structures are compiled in Table 3.44. Some of the reactions presented will be outlined in more detail.

Table 3.44. A review of reactions occurring in heat treated fats and oils

Fat/oil heating	Reaction	Products
1. Deep frying without food	Autoxidation Isomerization Polymerization	Volatile acids aldehydes esters alcohols Epoxides Branched chain fatty acids Dimeric fatty acids Mono- and bicyclic compounds Aromatic compounds Compounds with trans double bonds Hydrogen, CO_2
2. Deep frying with food added	As under 1. and in addition hydrolysis	As under 1. and in addition free fatty acids, mono- and diacylglycerols and glycerol

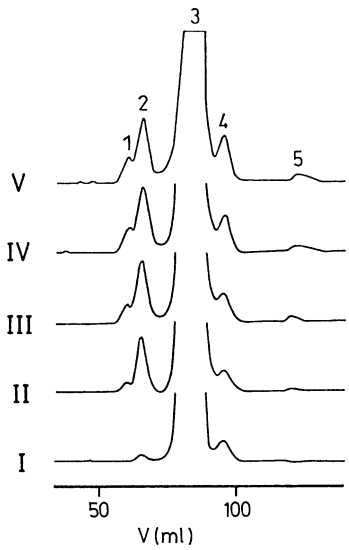


Fig. 3.36. Gel permeation chromatography of heated soybean oil (according to *Rojo* and *Perkins*, 1987). Oil samples (composition and heating conditions see Table 3.41) were analyzed immediately (I), as well as after 8 h (II), 24 h (III), 48 h (IV) and 80 h (V), *1* Trimeric TG, *2* Dimeric TG, *3* TG, *4* DG, *5* free fatty acids

3.7.4.1 Autoxidation of Saturated Acyl Lipids

The selectivity of autoxidation decreases above 60 °C since the hydroperoxides formed are subjected to homolysis giving hydroxy and alkoxy radicals (Reaction RS-4 in Fig. 3.19) which, due to their high reactivity, can abstract H-atoms even from saturated fatty acids.

Numerous compounds result from these reactions. For example, Table 3.45 lists a series of aldehydes and methyl ketones derived preferentially from tristearin. Both classes of compounds are also formed by thermal degradation of free fatty acids. These acids are formed by triglyceride hydrolysis or by the oxidation of aldehydes.

Table 3.45. Volatile compounds formed from heat-treated tristearin[a]

Class of compound	Portion	C-number	Major compounds
Alcohols	2.7	4–14	n-Octanol n-Nonanol n-Decanol
γ-Lactones	4.1	4–14	γ-Butyrolactone γ-Pentalactone γ-Heptalactone
Alkanes	8.8	4–17	n-Heptadecane n-Nonane n-Decane
Acids	9.7	2–12	Caproic acid Valeric acid Butyric acid
Aldehydes	36.1	3–17	n-Hexanal n-Heptanal n-Octanal
Methyl ketones	38.4	3–17	2-Nonanone 2-Heptanone 2-Decanone

[a] Tristearin is heated in air at 192 °C.

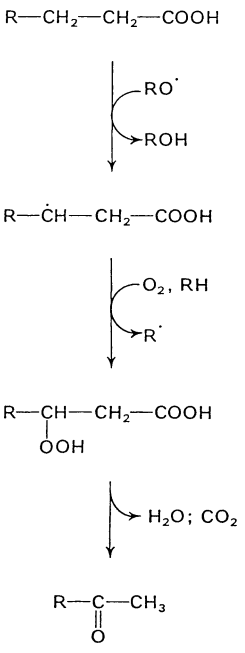
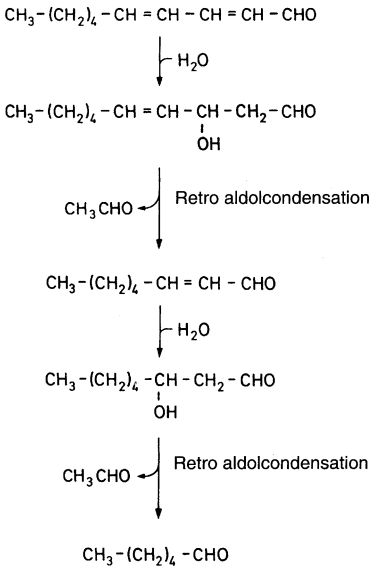


Fig. 3.37. Autoxidation of saturated fatty acids. Postulated reaction steps involved in formation of methyl ketones

Methyl ketones are obtained by thermally induced β-oxidation followed by a decarboxylation reaction (Fig. 3.37). Aldehydes are obtained from the fragmentation of hydroperoxides by a β-scission mechanism (Fig. 3.38) occurring nonselectively at elevated temperatures (compare the difference with 3.7.2.1.9).

Unsaturated aldehydes with a double bond conjugated to the carbonyl group are easily degraded during the deep frying process (Formula 3.95). Addition of water results in the formation of a 3-hydroxyaldehyde that is split by retro aldol condensation catalyzed by heat. Examples of this mechanism are the degradation of (E,Z)-2,6-nonadienal to (Z)-4-heptenal and acetaldehyde, as well as the cleavage of 2,4-decadienal into 2-octenal and acetaldehyde.



(3.95)

Some volatiles are important odorous compounds. In particular, (E,Z)- and (E,E)-2,4-decadienal are responsible for the pleasant deep-fried flavor (cf. 5.2.7). Since these aldehydes are formed by thermal degradation of linoleic acid, fats or oils containing this acid provide a better aroma during deep frying than hydrogenated fats. However, if a fat is heated for a prolonged period of time, the volatile compounds produce an off-flavor.

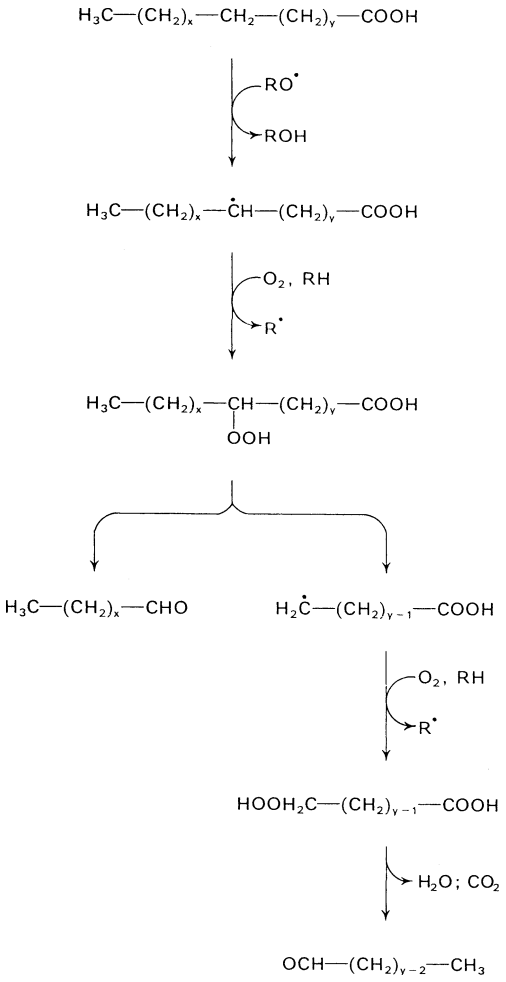
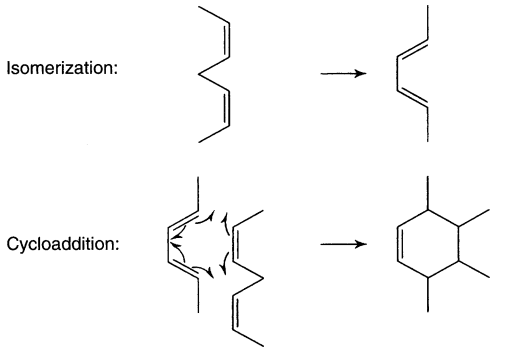
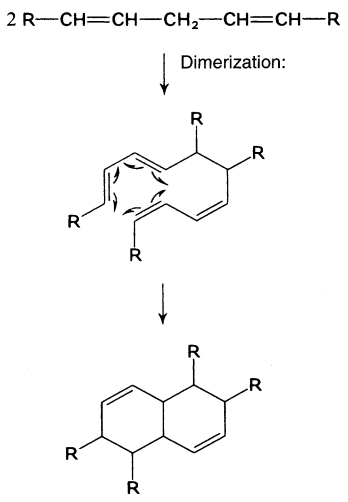


Fig. 3.38. Autoxidation of saturated fatty acids. Hypothetical reactions involved in formation of volatile aldehydes



(3.96)

The side chains of the resultant tetra-substituted cyclohexene derivatives are shortened by oxidation to oxo, hydroxy or carboxyl groups. In addition, the cyclohexene ring is readily dehydrogenated to an aromatic ring, hence compounds related to benzoic acid can be formed. The fatty acid or triacylglycerol radicals formed by H-abstraction in the absence of oxygen can dimerize and then form a ring structure:

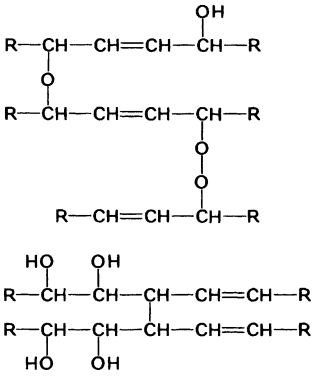


(3.97)

3.7.4.2 Polymerization

Under deep frying conditions, the isolenic fatty acids are isomerized into conjugated fatty acids which in turn interact by a 1,4-cycloaddition, yielding so-called *Diels–Alder* adducts (cf. Reaction 3.96).

On the other hand, polymers with ether and peroxide linkages are formed in the presence of oxygen. They also may contain hydroxy, oxo or epoxy groups. The following structures, among others, have been identified:



(3.98)

Such compounds are undesirable in deep-fried oil or fat since they permanently diminish the flavoring characteristics of the oil or fat and, because of their HO-groups, behave like surface-active agents, i. e. they foam.

Disregarding the odor or taste deficiencies developed in a fat or oil heated for a prolonged period of time, the oil is considered spoiled when its petroleum ether-insoluble oxidized fatty acids reach a level $\geq$1% (or $\geq$0.7% at the decreased smokepoint temperature of $\leq$170 °C). The fats or oils differ in their heat stability (Table 3.46). The stability is increased by hydrogenation of the double bonds.

Table 3.46. Relative stability of various fats and oils on deep-frying (RSDF)

Oil/fat	RSDF	Oil/fat	RSDF
Sunflower	1.0	Coconut	2.4
Rapeseed	1.0	Edible beef tallow	2.4
Soya	1.0	Soya oil,	
Peanut	1.2	hydrogenated	2.3
Palm	1.5	Peanut oil,	
Lard	2.0	hydrogenated	4.4
Butter fat	2.3		

3.7.5 Radiolysis

Alkyl and acyloxy radicals are formed during radiolysis of acyl lipids. These will further react to form volatile compounds. The formation of alkanes and alkenes, that lack one or two C-atoms, from the original acyl residue are of interest for the detection of irradiation (Fig. 3.39).

The proposed indicators for the irradiation of meat are the hydrocarbons 14:1, 15:0, 16:1, 16:2, 17:0 and 17:1 which are formed during radiolysis of palmitic, oleic and stearic acid. It was demonstrated that their concentrations in fat increased depending on the radiation dose, e. g., in chicken meat (Fig. 3.40).

Alkyl cyclobutanones are another group of compounds which can be used as indicators of irradiation. They are produced from triacylglycerides (cf. Formula 3.99), but are not formed on heating, e. g., meat, or by microorganisms. On the irradiation of chicken meat with a dose of 1 kGy, 0.72 µ g of 2-dodecyl cyclobutanone per g of lipid were detected. The indicator is stable because this value fell by only 15% in 18 days.

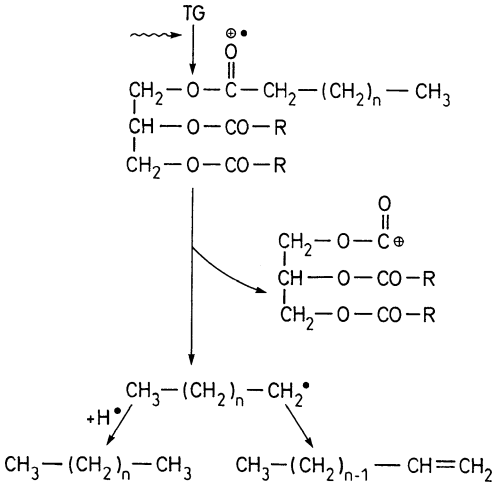
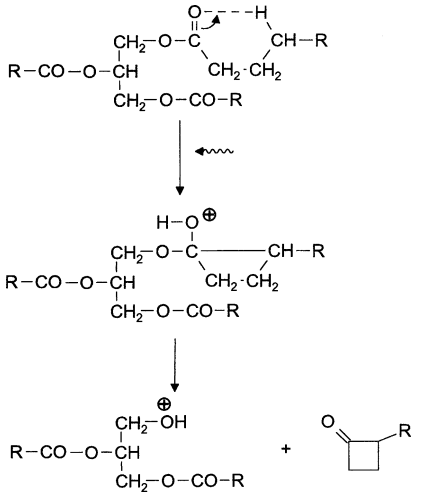


Fig. 3.39. Formation of alkanes and 1-alkenes during radiolysis of saturated triacyl glycerols



(3.99)

The dose used to decontaminate meat and spices is in the range 3–30 kGy. Therefore, the chemical reactions mentioned here are to be expected. Considerably lower doses are used for the treatment of fruit and vegetables as shown in the following examples: disinfection of fruit and nuts (0.15–0.5 kGy), inhibition of the germination of potatoes, onions and garlic (0.2–0.15 kGy), delayed ripening of bananas, mangoes and papayas (0.12–0.75 kGy). Consequently, the chemical detection of the irradiation of these foods is quite difficult.

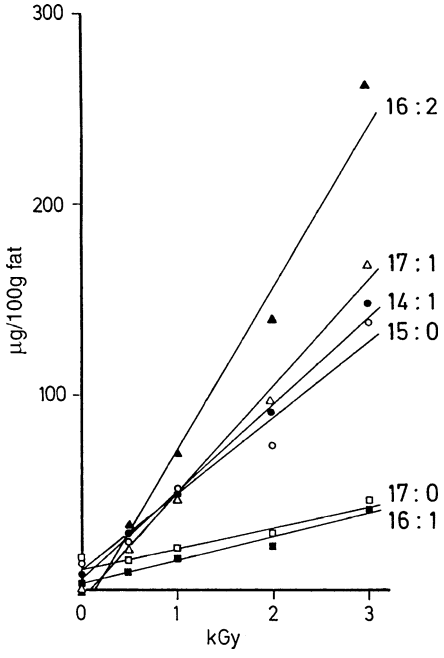


Fig. 3.40. Increase in the concentration of hydrocarbons relative to the radiation dose during irradiation of chicken meat (according to *Nawar* et al., 1990)

3.7.6 Microbial Degradation of Acyl Lipids to Methyl Ketones

Fatty acids of short and medium chain lengths present in milk fat, coconut and palm oils are degraded to methyl ketones by some fungi. A number of *Penicillium* and *Aspergillus* species, as well as several *Ascomycetes, Phycomycetes* and *fungi imperfecti* are able to do this.

The microorganisms first hydrolyze the triglycerides enzymatically (cf. 3.7.1) and then they degrade the free acids by a β-oxidation pathway (Fig. 3.41). The fatty acids $<C_{14}$ are transformed to methyl ketones, the C-skeletons of which have one C-atom less than those of the fatty acids. Apparently, the thiohydrolase activity of these fungi is higher than the β-ketothiolase activity. Hence, ester hydrolysis occurs instead of thioclastic cleavage of the thioester of a β-keto acid (see a textbook of biochemistry). The β-keto acid released is rapidly decarboxylated enzymatically; a portion of the methyl ketones is reduced to the corresponding secondary alcohols.

The odor threshold values for methyl ketones are substantially higher than those for aldehydes (cf. Tables 3.32 and 3.47). Nevertheless, they act as aroma constituents, particularly in flavors of mold-ripened cheese (cf. 10.2.8.3). However, methyl ketones in coconut or palm oil or in milk fat provide an undesirable, unpleasant odor denoted as"perfume rancidity".

3.8 Unsaponifiable Constituents*

Disregarding a few exceptions, fats and oils contain an average of 0.2–1.5% unsaponifiable compounds (Table 3.48). They are isolated from

* The free higher alcohols described under 3.6.2 and the deacylated alkoxy-lipids belong to this class.

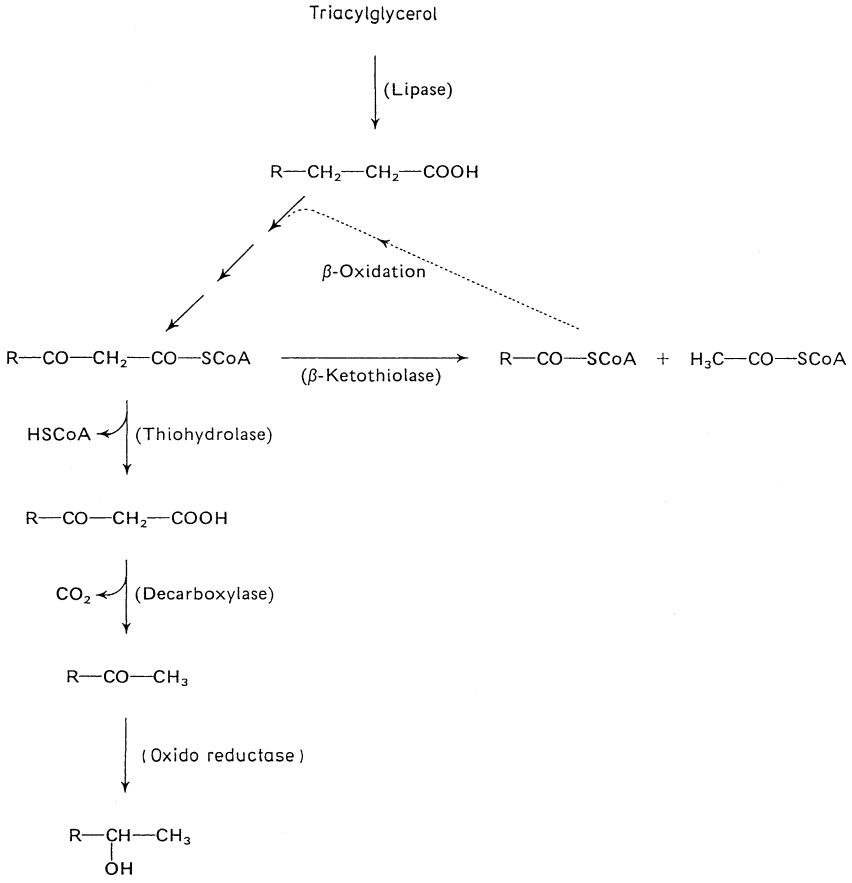


Fig. 3.41. Fungal degradation of triacylglycerols to methyl ketones (according to *Kinsella* and *Hwang*, 1976)

Table 3.47. Sensory properties of methyl ketones

Compound	Odor description	Odor threshold (ppb; in water)
2-Pentanone	Fruity, like bananas	2300
2-Hexanone		930
2-Heptanone	Fragrant, herbaceous	650
2-Octanone	Flowery, refreshing	190
2-Nonanone	Flowery, fatty	190

Table 3.48. Content of unsaponifiables in various fats and oils

Fat/oil	Unsaponifiable (weight-%)	Fat/oil	Unsaponifiable (weight-%)
Soya	0.6–1.2	Shea	3.6–10.0
Sunflower	0.3–1.2	Lard	0.1–0.2
Cocoa	0.2–0.3	Shark (refined)	15–17
Peanut	0.2–4.4		
Olive	0.4–1.1	Herring (refined)	0.7–1.0
Palm	0.3–0.9		
Rapeseed	0.7–1.1		

a soap solution (alkali salts of fatty acids) by extraction with an organic solvent.

The unsaponifiable matter contains hydrocarbons, steroids, tocopherols and carotenoids. In addition, contaminants or fat or oil additives, such as mineral oil, plasticizers or pesticide residues, can be found.

Each class of compounds in the unsaponifiable matter is represented by a number of components,

the structures and properties of which have been thoroughly elucidated in the past decade or two, thus reflecting the advance in the analytical chemistry of fats and oils.
Studies aimed at elucidating the constituents, and their structures, of unsaponifiable matter are motivated by a desire to find compounds which can serve as a reliable indicator for the identity of a fat or an oil.

3.8.1 Hydrocarbons

All edible oils contain hydrocarbons with an even or an odd C-number (C_{11} to C_{35}). Olive, rice and fish oils are particularly rich in this class of compounds. The main hydrocarbon constituent of olive oil (1–7 g/kg) and rice oil (3.3 g/kg) is a linear triterpene (C_{30}), squalene:

(3.100)

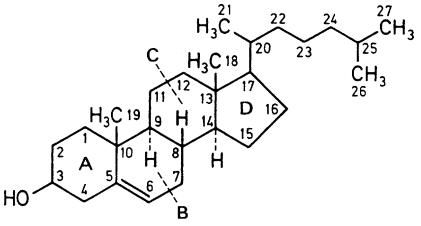
This compound is used as an analytical indicator for olive oil (cf. Table 14.24).
Squalene is present in a substantially higher concentration in fish liver oil. For example, shark liver oil has up to 30% squalene, and 7% pristane (2,6,10,14-tetramethylpentadecane) and some phytane (3,7,11,15-tetramethylhexadecane).

3.8.2 Steroids

The unsaponifiable part of edible fats contains a series of cyclic triterpenes which have structures related to that of steroids. Quantitatively, the 3β-hydroxysteroids are the sterols which are to the fore. Especially diverse is the sterol spectrum of plant fats which contain not only desmethyl, but also 4-methyl and 4,4-dimethyl sterols.

3.8.2.1 Structure, Nomenclature

The steroid skeleton is made up of four condensed rings; A, B, C and D. The first three are in the chair conformation, whereas ring D is usually planar. While rings B and C, and C and D are fused in a trans-conformation, rings A and B can be fused in a trans- or in a cis-conformation.



(3.101)

Conformational isomers introduced by fusing rings A and B in cholest-5-ene-3-β-ol (cholesterol; cf. Formula 3.101) are not possible since the C-5 position has a double bond.
By convention, the steric arrangement of substituents and H-atoms is related to the angular methyl group attached at C-10. When the plane containing the four rings is assumed to be the plane of this page, the substituent at C-10, by definition, is above the plane; all substituents below the plane are denoted by dashed or dotted lines. They are said to be α-oriented and have a trans-conformation. Substituents above the plane are termed β-oriented and are shown by solid line bonds and, in relation to the angular C-10 methyl group, are of cis-conformation.
In cholesterol (cf. Formula 3.101) the HO-group, the angular methyl group at C-13, the side chain on C-17 and the H-atom on C-8 are β-oriented (cis), whereas the H-atoms at C-9, C-14 and C-17 are α-oriented (trans). These steroids that are not methylated at position C-4 are also denoted as desmethyl steroids.

3.8.2.2 Steroids of Animal Food

3.8.2.2.1 Cholesterol

Cholesterol (cf. Formula 3.101) is obtained biosynthetically from squalene (see a textbook of biochemistry). It is the main steroid of mammals and occurs in lipids in free form or esterified with saturated and unsaturated fatty acids. The content of cholesterol in some foods is illustrated by the data in Table 3.49.
Autoxidation of cholesterol, which is accelerated manyfold by 18:2 and 18:3 fatty acid

Table 3.49. Cholesterol content of some food

Food	Amount (mg/100 g)
Calf brain	2000
Egg yolk[a]	1010
Pork kidney	410
Pork liver	340
Butter	215–330
Pork meat, lean	70
Beef, lean	60
Fish (Halibut; *Hypoglossus vulgaris*)	50

[a] Egg white is devoid of cholesterol.

peroxy radicals, proceeds through the intermediary 3β-hydroxycholest-5-en-7α- and 7β-hydroperoxides, of which the 7β-epimer is more stable because of its quasi-equatorial conformation and, hence, is formed predominantly. Unlike autoxidation, the photosensitized oxidation (reaction with a singlet oxygen) of cholesterol yields 3β-hydroxycholest-6-en-5α-hydroperoxide.

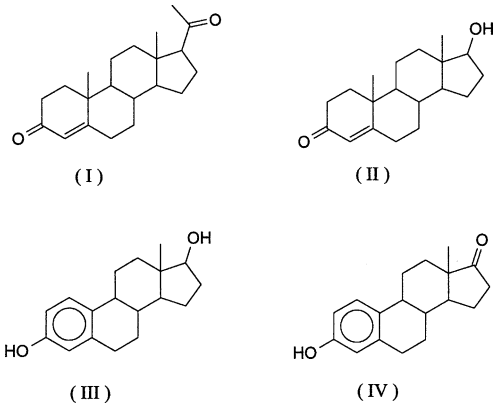
Among the many derivatives obtained by the further degradation of the hydroperoxides, cholest-5-en-3β,7α-diol, cholest-5en-3β,7β-diol, 3β-hydroxycholest-5-en-7-one, 5,6β-epoxy-5β-cholestan-3β-ol and 5α-cholestan-3β,5,6β-triol have been identified as major products. These so-called "oxycholesterols" have been detected as side components in some food items (dried egg yolk, whole milk powder, butter oil and heated meat). It is difficult to quantify these oxidation products because significant losses can occur in the clean-up of the analyte, e. g., in the case of polar cholestantriol. In addition, artifacts are easily formed. For this reason, quantitative values found in the literature are frequently only approximations.

In the animal organism, cholesterol is the starting point for the synthesis of other steroids, such as sex hormones and bile acids. In fact, GC-MS analyses and radio immunoassays show that among the sex hormones, progesterone (I in Formula 3.102) appears most often in animal food. It is enriched in the fat phase, leading to relatively high concentrations in butter (Table 3.50). Traces of this steroid also occur in plant foods. Testosterone (II in Formula 3.102), 3,17-estradiol (III) and 17-estrone (IV) are other sex hormones which have been identified as natural trace components of meat, milk and their products.

Table 3.50. Progesterone in foods

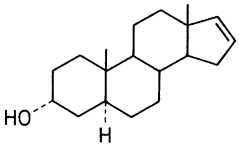
Food	Progesterone (μg/kg)
Beef, male[a]	0.01–5
Beef, female[a]	0.5–40
Pork (muscle)	1.1–1.8
Chicken	0.24
Turkey	8.18
Chicken egg	12.5–43.6
Skim milk (0.1% fat)	1.3–4.6
Whole milk (3.5% fat)	9.5–12.5
Cream (32% fat)	42–73
Butter (82% fat)	133–300
Cheese (Gouda, 29% fat)	44
Potatoes	5.1
Wheat	0.6–2.9
Corn germ oil	0.3
Safflower oil	0.7

[a] edible parts.



(3.102)

Products of cholesterol metabolism include C_{19}-sterols which produce the specific smell of boar in boar meat. Five aroma components (Table 3.51) were identified; 5α-androst-16-en-3α-ol (Formula 3.103) has also been detected in truffels (cf. 17.1.2.6.1).



(3.103)

Table 3.51. Odor-active C_{19}-steroids

Compound	Odor threshold (mg/kg ; oil)
5α-Androst-16-en-3-one	0.6
5α-Androst-16-en-3α-ol	0.9
5α-Androst-16-en-3β-ol	1.2
4,16-Androstadien-3-one	7.8
5,16-Androstadien-3β-ol	8.9

3.8.2.2.2 Vitamin D

Cholecalciferol (vitamin D_3) is formed by photolysis of 7-dehydrocholesterol, a precursor in cholesterol biosynthesis. As shown in Fig. 3.42, UV radiation opens the B-ring. The precalciferol formed is then isomerized to vitamin D_3 by a rearrangement of the double bond which is influenced by temperature. Side-products, such as lumi- and tachisterol, have no vitamin D activity. Cholecalciferol is converted into the active hormone, 1,25-dihydroxy-cholecalciferol, by hydroxylation reactions in liver and kidney.
7-Dehydrocholesterol, the largest part of which is supplied by food intake and which accumulates in human skin, is transformed by UV light into vitamin D_3. The occurrence and the physiological significance of the D vitamins are covered in Sect. 6.2.2.
Ergosterol (ergosta-5,7,22-trien-3β-ol), which occurs in yeast, moulds and algae, is provitamin D_2. It can serve as an indicator for fungal contamination. A tolerance limit of 15 mg/kg solid has been proposed for tomato products.

3.8.2.3 Plant Steroids (Phytosterols)

The sterols and stanols (hydrogenation products of sterols) occurring in plants are known as phytosterols. The best known representatives are the desmethylsterols shown in 3.8.2.3.1.
The phytosterols are of interest from a nutritional and physiological point of view because they lower the concentration of cholesterol and LDL in the blood plasma (cf. 3.5.1.2). The absorption of cholesterol is inhibited, a significant effect being reached with an intake of 1 g/day of phytosterol. Since the normal dietary intake amounts to only 200–400 mg/day of phytosterol, margarines are enriched with phytosterols. However, as the free sterols are only poorly soluble in the fat phase, sterol esters are used in the production of margarine. Sterol esters are hydrolysed in the digestive tract. The starting material for the extraction of phytosterols is plant oils and tall oil (Swedish "tall" = pine), which accumulates as a by-product in the production of paper and pulp. Tall oil is rich in phytostanols, mainly β-sitostanol.

3.8.2.3.1 Desmethylsterols

Cholesterol, long considered to be an indicator of the presence of animal fat, also occurs in small amounts in plants (Table 3.52). Campe-, stigma- and sitosterol, which are predominant in the sterol fraction of some plant oils, are structurally related to cholesterol; only the side chain on C-17 is changed. The following structural segments (only

Table 3.52. Average sterol composition of plant oils[a]

Component	Sunflower	Peanut	Soya	Cottonseed	Corn	Olive	Palm
Cholesterol	0.5	6.2	0.5	0.5	0.5	0.5	0.5
Brassicasterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Campesterol	242	278	563	276	2655	19	88
Stigmasterol	236	145	564	17	499	0.5	42
β-Sitosterol	1961	1145	1317	3348	9187	732	252
Δ^5-Avenasterol	163	253	46	85	682	78	0.5
Δ^7-Stigmasterol	298	0.5	92	0.5	96	0.5	51
Δ^7-Avenasterol	99	34	63	18	102	30	0.5
24-Methylene-cycloartenol	204	0.5	53	0.5	425	580	0.5

[a] Values in mg/kg.

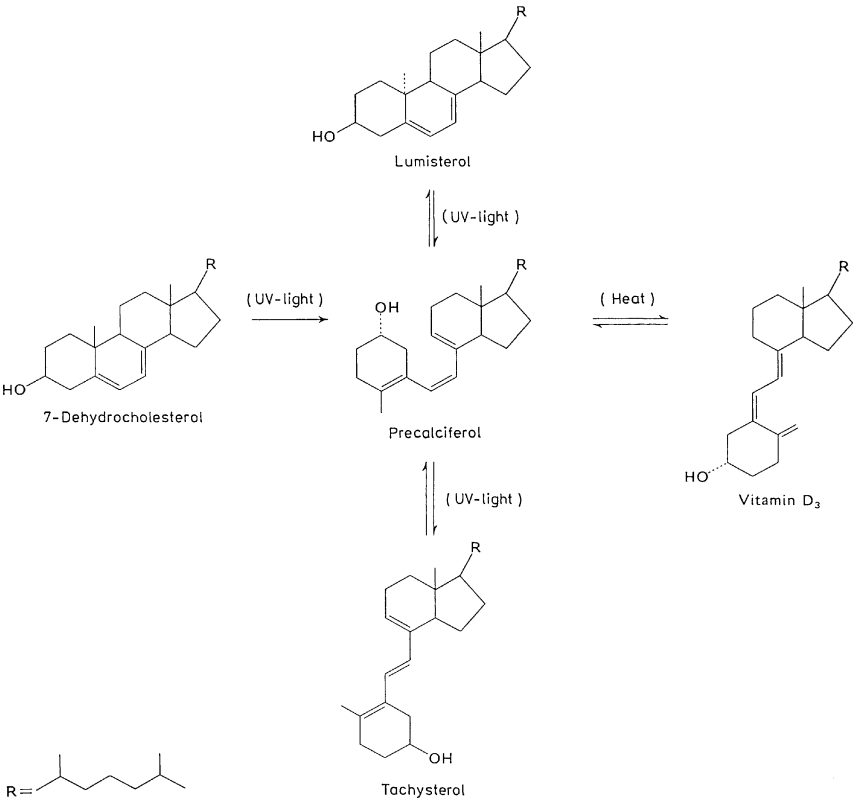
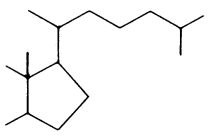
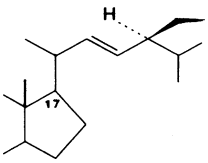


Fig. 3.42. Photochemical conversions of provitamin D_3

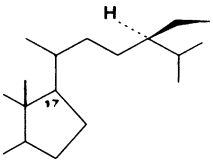
ring D and the side chain) show these differences (Formulas 3.104–3.107):



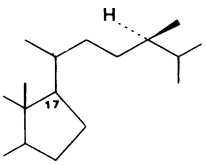
Cholesterol (3.104)



Stigmasterol (double bond at C-22) (3.106)

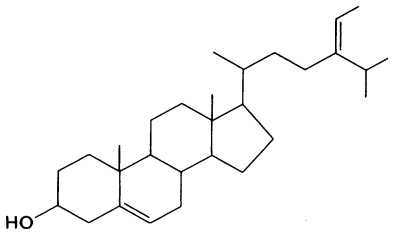


β-Sitosterol (24-α-ethyl-cholesterol) (3.105)



Campesterol (24-α-methyl-cholesterol) (3.107)

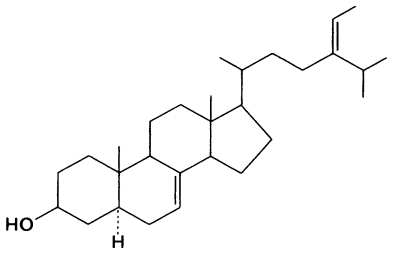
Δ^5-Avenasterol is a sitosterol derivative:



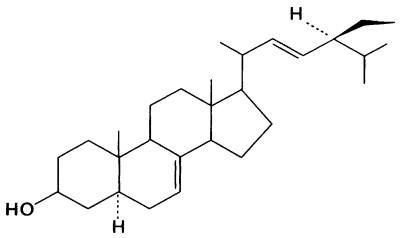
Δ^5-Avenasterol (3.108)

Steroids which, like the avenasterols, contain an ethylidene group have antioxidative activity at the temperatures used in deep frying because under these conditions, a peroxyl radical can abstract an H-atom from this group.

In addition to Δ^5-sterols, Δ^7-sterols occur in plant lipids; for example:



Δ^7-Avenasterol (3.109)



Δ^7-Stigmasterol (3.110)

Plant lipids contain 0.15–0.9% sterols, with sitosterol as the main component (Table 3.52). In order to identify blends of fats (oils), the data on the predominant steroids are usually expressed as a quotient. For example, the ratio of stigmasterol/campesterol is determined in order to detect adulteration of cocoa butter. As seen from Table 3.53, this ratio is significantly lower in a number of cocoa butter substitutes than in pure cocoa butter. The phytosterol fraction (e. g. sito- and campesterol) has to be determined in order to detect the presence of plant fats in animal fats.

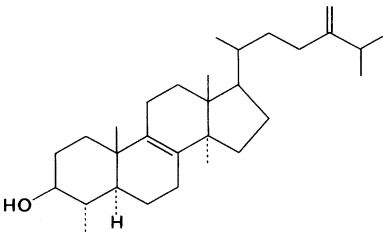
Table 3.53. Ratio of stigmasterol to campesterol in various fats

Fat	Sterols (g/kg)	% Stigmasterol / % Campesterol
Cocoa butter	1.8	2.8–3.5
Tenkawang[a]	2.15	0.42–0.55
Sal oil[a]	3	0.98
Illexao 30 – 90[b]	1.15	–[d]
Palm oil	0.67	0.43
Palm seed oil	0.81	1.28
Choclin[c]		0.38
Kaobien[c]		0.56
Coconut oil	0.75	1.47
Peanut oil, hydrogenated		0.72
Coberine[c]		0.31–0.60

[a] Cocoa butter substitutes (cf. 14.3.2.2.3).
[b] Trade name for sheasterol.
[c] Trade name for cocoa butter substitutes from the middle fraction of palm oil and shea butter.
[d] Contains no stigmasterol.

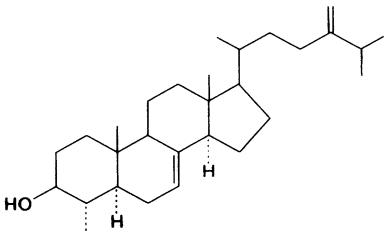
3.8.2.3.2 Methyl- and Dimethylsterols

Sterols with α-oriented C-4 methyl groups occur in oils of plant origin. The main compounds are:



(3.111)

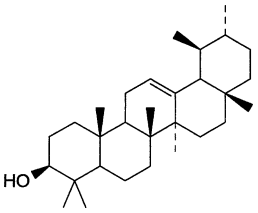
4α, 14α-Dimethyl-24-methylene-5α-cholest-8-en-3β-ol (Obtusifoliol)



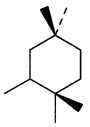
(3.112)

4α-Methyl-24-methylene-5α-cholest-7-en-3β-ol (Gramisterol)

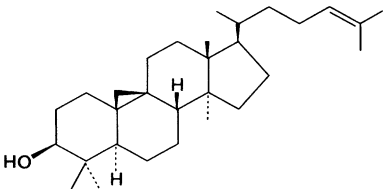
Gas chromatographic-mass spectrometric studies have also revealed the presence of 4,4-dimethylsterols in the steroid fraction of many plant oils:



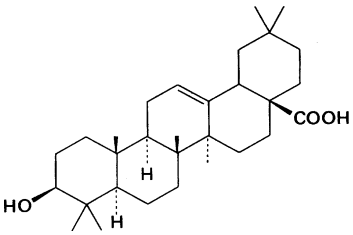
α-Amyrine (3.113a)



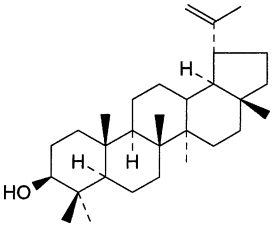
β-Amyrine (3.113b)



Cycloartenol (3.114)



Oleanolic acid (3.115)



Lup-20(29)-en-3β-ol (Lupeol) (3.116)

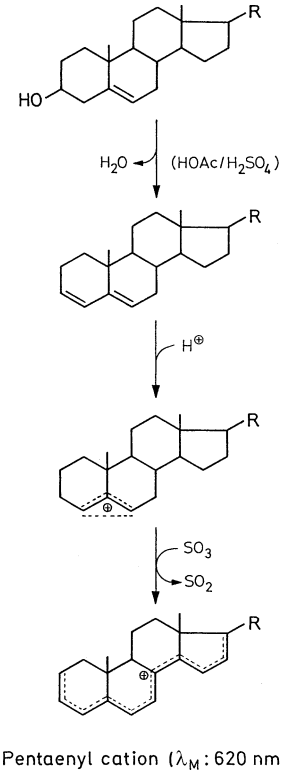


Fig. 3.43. Sterol detection according to *Lieberman–Burchard.* Reactions involved in color development

Oleanolic acid has long been known as a constituent of olive oil. Methyl- and dimethylsterols are important in identifying fats and oils (cf. Fig. 3.44).

3.8.2.4 Analysis

Qualitative determination of sterols is conducted using the *Liebermann–Burchard* reaction, in which a mixture of glacial acetic and concentrated sulfuric acids reacts directly with the fat or oil or the unsaponifiable fraction. Several modifications of this basic assay have been developed which, depending on the steroid and the oxidizing agent used, result in the production of a green or red color. The reaction is more sensitive when the SO_3 oxidizing agent is replaced by the Fe^{3+} ion. The conversion of sterols into a chromophore is based on the

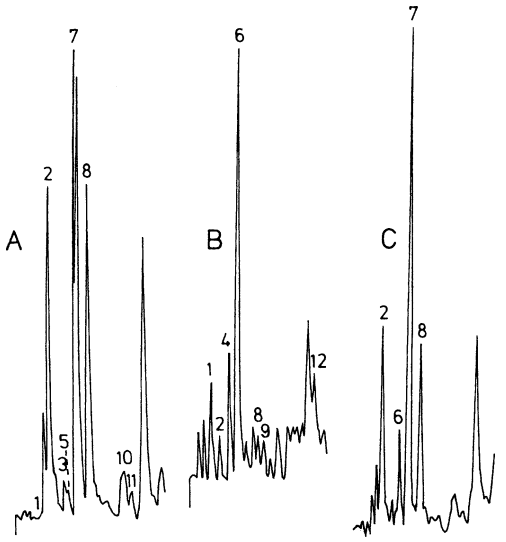


Fig. 3.44. Gas chromatographic separation of the triterpene alcohol fraction from coberine (**A**), cocoa butter (**B**) and cocoa butter +5% coberine (**C**) (according to *Gegiou and Staphylakis*, 1985). *1*, lanosterol; *2*, β-amyrine; *3*, butyrospermol; *4*, 24-methylene lanostenol; *5*, parkeol; *6*, cycloartenol; *7*, α-amyrine; *8*, lup-20(29)-en-3β-ol; *9*, 24-methylene cycloartenol; *10*, ψ-taraxasterol; *11*, taraxasterol; *12*, cyclobranol

reaction sequence given in Fig. 3.43. As shown, the assay is applicable only to sterols containing a double bond, such as in the B ring of cholesterol.

Sterols are separated as 3,5-dinitrobenzoic acid derivatives by thin layer chromatography and, after reaction with 1,3-diaminopropane, are determined quantitatively with high sensitivity in the form of a *Meisenheimer* adduct. Sterols and triterpene alcohols are silylated and then analysed by gas chromatography. One application of this method is illustrated by the detection of 5% coberine in cocoa butter (Fig. 3.44). The compounds α-amyrine and lup-20(29)-en-3β-ol (Formula 3.113a and 3.116) serve as indicators. They are present in much higher concentrations in some cocoa butter substitutes than in cocoa butter. Coberine is a cocoa butter substitute made by blending palm oil and shea butter (the shea is an African tree with seeds that yield a thick white fat, shea butter).

The content of egg (more accurately, the yolk) in pasta products or cookies can be calculated after the cholesterol content has been determined, usually by gas chromatography or HPLC. Vitamin D determination requires specific procedures in which precautions are taken with regard to the compound's sensitivity to light. A chemical method uses thin layer chromatographic separation of unsaponifiables, elution of vitamin D from the plate and photometric reading of the color developed by antimony (III) chloride. An alternative method recommends the use of HPLC.

3.8.3 Tocopherols and Tocotrienols

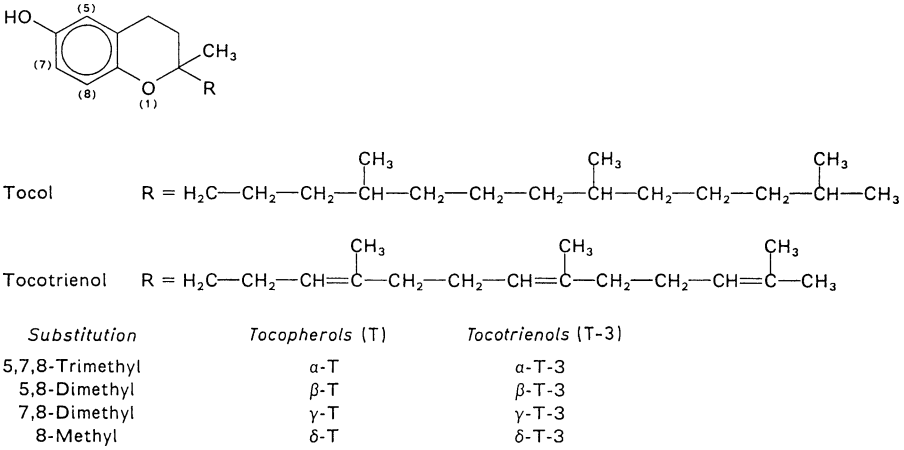
3.8.3.1 Structure, Importance

The methyl derivatives of tocol [2-methyl-2(4′, 8′, 12′-trimethyltridecyl)-chroman-6-ol] are denoted tocopherols. In addition the corresponding methyl derivatives of tocotrienol occur in food.

All four tocopherols and tocotrienols, with the chemical structures given in Fig. 3.45, are found primarily in cereals (especially wheat germ oil), nuts and rapeseed oils. These redox-type lipids are of nutritional/physiological and analytical interest. As antioxidants (cf. 3.7.3.2.1), they prolong the shelf lives of many foods containing fat or oil. The significance of tocopherols such as vitamin E is outlined in 6.2.3.

About 60–70% of the tocopherols in oilseeds are retained during the oil extraction and refining process (cf. 14.4.1 and Table 3.54). Some oils with very similar fatty acid compositions can be distinguished by their distinct tocopherol spectrum. To illustrate this, two examples are provided. The amount of β-tocopherol in wheat germ oil is quite high (Table 3.54), hence it serves as an indicator of that oil. The blending of soya oil with sunflower oil is detectable by an increase in the content of linolenic acid (cf. 14.5.2.3). However, it is possible to make a final conclusive decision about the presence and quantity of soya oil in sunflower oil only after an analysis of the composition of the tocopherols.

The tocopherol pattern is also different in almond and apricot kernel oil (Table 3.54) whose fatty acid compositions are very similar. Therefore adulteration of marzipan with persipan can be detected by the analysis of the tocopherols.



Substitution	*Tocopherols* (T)	*Tocotrienols* (T-3)
5,7,8-Trimethyl	α-T	α-T-3
5,8-Dimethyl	β-T	β-T-3
7,8-Dimethyl	γ-T	γ-T-3
8-Methyl	δ-T	δ-T-3

Fig. 3.45. Tocopherols and tocotrienols present in food

3.8.3.2 Analysis

Isolation of tocopherols is accompanied by losses due to oxidation. Therefore, the edible oil is dissolved in acetone at 20–25 °C in the presence of ascorbyl palmitate as an antioxidant. The major portion of triacylglycerols is separated by crystallization at −80 °C. Tocopherols remaining in solution are then analyzed by thin layer or gas chromatography (after silylation of the phenolic HO-group) or by HPLC (cf. Fig. 3.46). UV spectrophotometry is also possible. However, the fluorometric method based on an older colorimetric procedure developed by *Emmerie* and *Engel* is even more sensitive. It involves reduction of the Fe (III) ion to Fe (II) by tocopherols and the reaction of the reduced iron with 2,2′-bipyridyl to form an intensive red colored complex.

3.8.4 Carotenoids

Carotenoids are polyene hydrocarbons biosynthesized from eight isoprene units (tetraterpenes) and, correspondingly, have a 40-C skeleton.

Table 3.54. Tocopherols and Tocotrienols in plant oil[a]

Oil	α-T	α-T-3	β-T	β-T-3	γ-T	γ- T-3	∂-T	∂-T-3
Sunflower	56.4	< 0.02	2.45	0.2	0.4	0.02	0.09	
Peanut	14.1	< 0.02	0.4	0.4	13.1	0.03	0.92	
Soya	17.9	< 0.02	2.8	0.4	60.4	0.08	37.1	
Cottonseed	40.3	< 0.02	0.2	0.9	38.3	0.09	0.5	
Corn	27.2	5.4	0.2	1.1	56.6	6.2	2.5	
Olive	9.0	< 0.02	0.2	0.4	0.5	0.03	0.04	
Palm (raw)	20.6	39.2	< 0.1	2.5	< 0.1	42.6	2.6	10.1
Wheat germ	133.0	< 2.6	71.0	18.1	26.0		27.1	
Almond	20.7		0.3		0.9			
Apricot kernel	0.5				22.4		0.3	
Peach kernel	6.4		1.3		1.0			
Cocoa butter	0.3		< 0.1		5.3		< 0.1	
Palm oil, middle fraction	< 0.1		< 0.1		0.43		< 0.1	
Shea fat stearin	< 0.1		< 0.1		0.43		< 0.1	

[a] Average composition; indicated in mg/100 g.

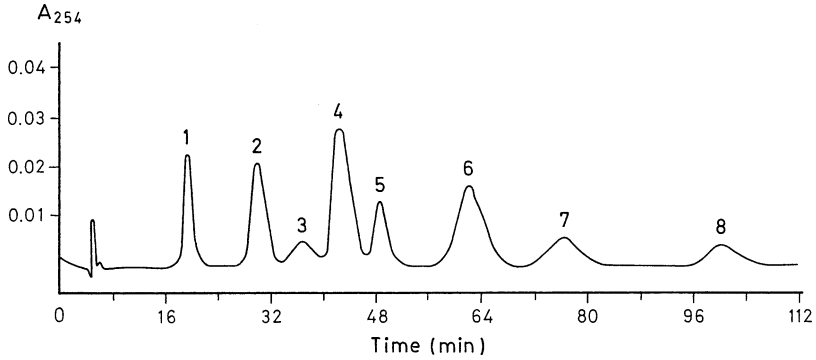


Fig. 3.46. Tocopherol and tocotrienol analysis by HPLC (according to *Cavins* and *Inglett*, 1974). 1 α-Tocopherol, 2 α-tocotrienol, 3 β-tocopherol, 4 γ-tocopherol, 5 β-tocotrienol, 6 γ-tocotrienol, 7 δ-tocopherol, and 8 δ-tocotrienol

They provide the intensive yellow, orange or red color of a great number of foods of plant origin (Table 3.55; cf. also 17.1.2.3 and 18.1.2.3.2). They are synthesized only by plants (see a textbook of biochemistry). However, they reach animal tissues via the feed (pasture, fodder) and can be modified and deposited there.

A well known example is the chicken egg yolk, which is colored by carotenoids. The carotenoids in green plants are masked by chlorophyll. When the latter is degraded, the presence of carotenoids is readily revealed (e. g. the green pepper becomes red after ripening).

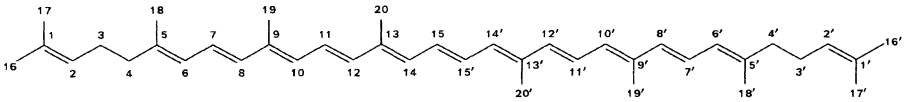
Table 3.55. Carotenoids in various food

Food	Concentration (ppm)[a]	Food	Concentration (ppm)[a]
Carrots	54	Peaches	27
Spinach	26–76	Apples	0.9–5.4
Tomatoes	51	Peas	3–7
Apricots	35	Lemons	2–3

[a] On dry weight basis.

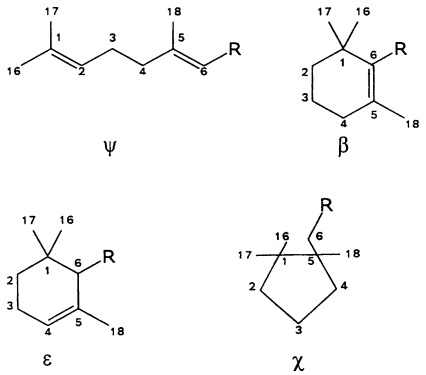
3.8.4.1 Chemical Structure, Occurrence

Other carotenoids are derived by hydrogenation, dehydrogenation and/or cyclization of the basic structure of the C_{40}-carotenoids (cf. Formula 3.117). The cyclization reaction can occur at one or both end groups. The differences in C_9-end groups are denoted by Greek letters (cf. Formula 3.118).



(3.117)

A semisystematic nomenclature used at times has two Greek letters as a prefix for the generic



(3.118)

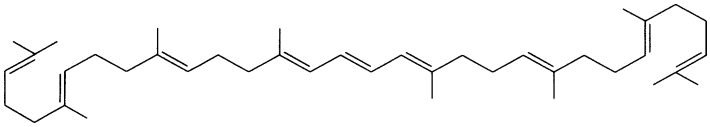
name "carotene", denoting the structure of both C_9- end groups (cf. Formulas III, IV, VI or X: cf. Formulas 3.120, 3.121, 3.122 and 3.128, respectively). Designations such as α-, β- or γ-carotene are common names.

Carotenoids are divided into two main classes: carotenes and xanthophylls. In contrast to carotenes, which are pure polyene hydrocarbons, xanthophylls contain oxygen in the form of hydroxy, epoxy or oxo groups. Some carotenoids of importance to food are presented in the following sections.

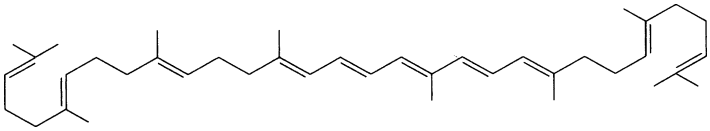
3.8.4.1.1 Carotenes

Acyclic or aliphatic carotenes

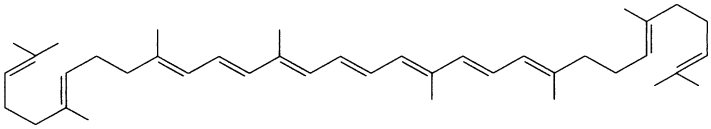
Carotenes I, II and III (cf. Formulas 3.119–3.122) are intermediary or precursor compounds which, in biosynthesis after repeated dehydrogenizations, provide lycopene (IV; see a textbook of biochemistry). Lycopene is the red color of the tomato (and also of wild rose hips). In yellow tomato cultivars, lycopene precursors are present together with β-carotene (Table 3.56).



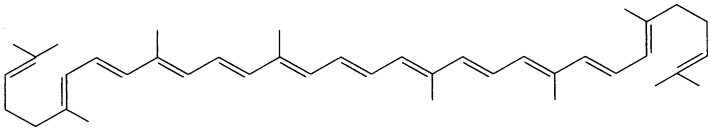
Phytoene (I) (3.119)



Phytofluene (II) (3.120)



ξ-Carotene (7,8,7′,8′-tetrahydro-ψ,ψ-carotene) (III) (3.121)

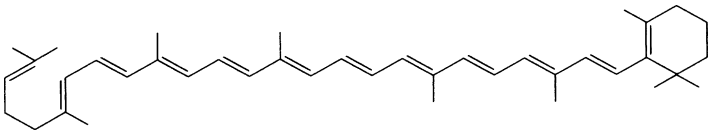


Lycopene (ψ,ψ-carotene) (IV) (3.122)

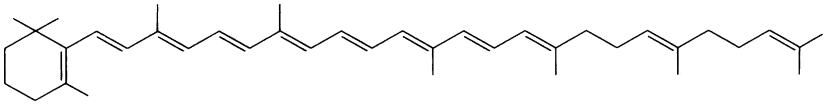
Table 3.56. Carotenes (ppm) in some tomato cultivars

Cultivar	Phytoene (I)	Phytofluene (II)	β-Carotene (VII)	ξ-Carotene (III)	γ-Carotene (V)	Lycopene (IV)
Campbell	24.4	2.1	1.4	0	1.1	43.8
Ace Yellow	10.0	0.2	trace	0	0	0
High Beta	32.5	1.7	35.6	0	0	0
Jubilee	68.6	9.1	0	12.1	4.3	5.1

Monocyclic Carotenes

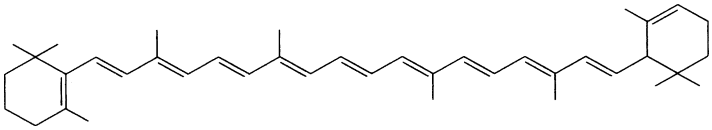


γ-Carotene (ψ, β-carotene) (V) (3.123)

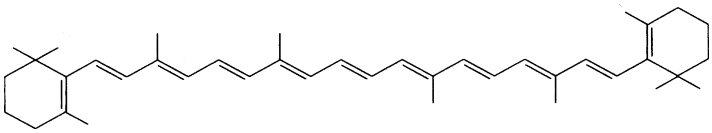


β-Zeacarotene (Va) (3.124)

Bicyclic Carotenes



α-Carotene (β, ε-carotene) (VI) (3.125)

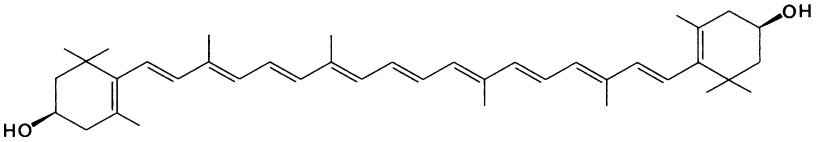


β-Carotene (β, β-carotene) (VII) (3.126)

The importance of β-carotene as provitamin A is covered under 6.2.1.

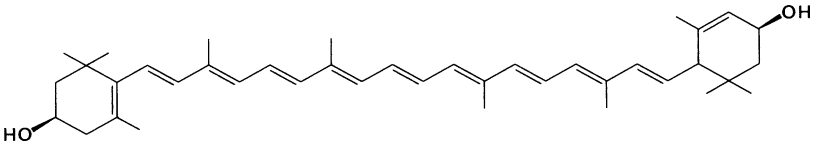
3.8.4.1.2 Xanthophylls

Hydroxy Compounds



Zeaxanthin (β, β-carotene-3, 3′-diol) (VIII) (3.127)

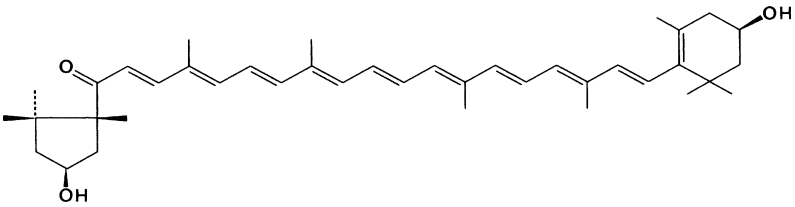
This xanthophyll is present in corn (*Zea mays*).



Lutein (β, ε-carotene-3, 3′-diol) (IX) (3.128)

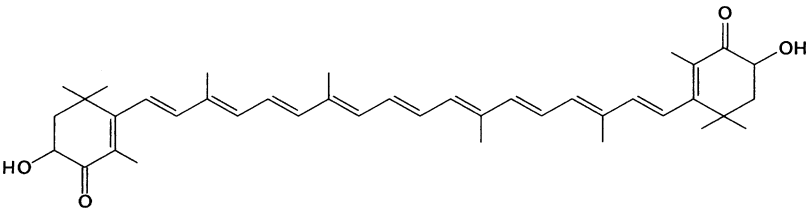
This xanthophyll occurs in green leaves and in egg yolk.

Keto Compounds



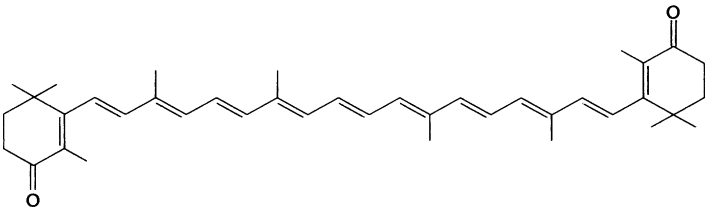
Capsanthin (3,3′-dihydroxy-β,ϰ-carotene-6′-one) (X) (3.129)

This xanthophyll is the major carotene of paprika peppers.



Astaxanthin (XI) (3.130)

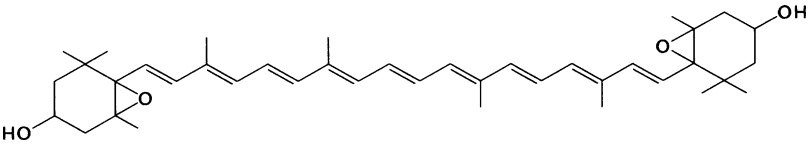
Astaxanthin is present in crab and lobster shells and, in combination with proteins, provides three blue hues (α-, β- and γ-crustacyanin) and one yellow pigment. During the cooking of crabs and lobsters, the red astaxanthin is released from a green carotenoid-protein complex. Astaxanthin usually occurs in lobster shell as an ester, e. g., dipalmitic ester.



Canthaxanthin (XII) (3.131)

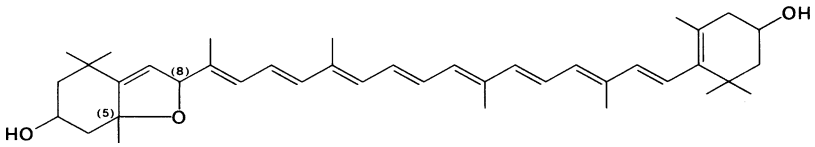
This xanthophyll is used as a food colorant (cf. 3.8.4.5).

Epoxy Compounds



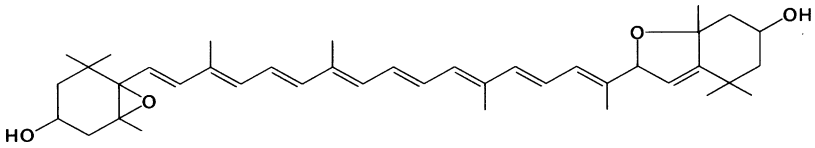
Violaxanthin (zeaxanthin-diepoxide) (XIII) (3.132)

Violaxanthin is present in orange juice (cf. Table 3.57) and it also occurs in green leaves.



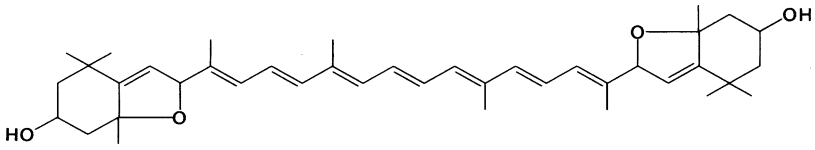
Mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol) (XVI) (3.133)

This epoxy carotenoid is present in oranges (cf. Table 3.57).



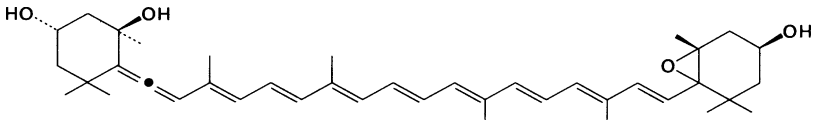
Luteoxanthin (XIV) (3.134)

Luteoxanthin is the major carotenoid of oranges (cf. Table 3.57).



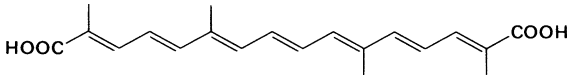
Auroxanthin (XV) (3.135)

This carotenoid is a constituent of oranges (cf. Table 3.57).



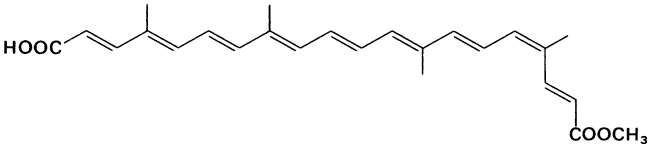
Neoxanthin (XX) (3.136)

Dicarboxylic Acids and Esters



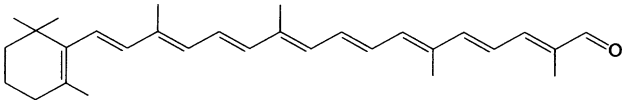
Crocetin (XVII) (3.137)

This carboxylic acid carotenoid is the yellow pigment of saffron. It occurs in plants as a diester, i. e. glycoside with the disaccharide gentiobiose. The diester, called crocin, is therefore water-soluble.



Bixin (XVIII) (3.138)

Bixin is the main pigment of annato extract. Annato originates from the West Indies and the pigment is isolated from the seed pulp of the tropical bush *Bixa orellana.* Bixin is the monomethyl ester of norbixin, a dicarboxylic acid homologous to crocetin.



β-apo-8′-carotenal* (XIX) (3.139)

Carotenoids are, as a rule, present in plants as a complex mixture. For example, the orange has more than 50 well characterized compounds, of which only those that exceed 5% of the total carotenoids are presented in Table 3.57.
Hydroxy-carotinoids are often present as esters of fatty acids; e. g., orange juice contains 3-hydroxy-β-carotene (cryptoxanthin) esterified with lauric, myristic and palmitic acid. The quantitative analysis of this ester fraction is used as proof of an adulteration of orange juice with mandarin juice.

Table 3.57. Major carotenoid components in orange juice

Carotenoid	As percent of total carotenoids
Phytoene (I)	13
ξ-Carotene (III)	5.4
Cryptoxanthin (3-Hydroxy-β-carotene)	5.3
Antheraxanthin (5,6-Epoxyzeaxanthin)	5.8
Mutatoxanthin (XVI)	6.2
Violaxanthin (XIII)	7.4
Luteoxanthin (XIV)	17.0
Auroxanthin (XV)	12.0

[c] The prefix "apo" indicates a compound derived from a carotenoid by removing part of its structure.

3.8.4.2 Physical Properties

Carotenoids are very soluble in apolar solvents, including edible fats and oils, but they are not soluble in water. Hence, they are denoted "lipochromes". Carotenoids are readily extracted from plant sources with petroleum ether, ether or benzene. Ethanol and acetone are also suitable solvents.
The color of carotenoids is the result of the presence of a conjugated double bond system in the molecules. The electron excitation spectra of such systems are of interest for elucidation of their structure and for qualitative and quantitative analyses.
Carotenoids show three distinct maxima in the visible spectrum, with wavelength positions dependent on the number of conjugated double

Table 3.58. Absorption wavelength maxima for some carotenoids

Compound	Conjugated double bonds	Wavelength, nm (petroleum ether)		
A. Effect of the number of conjugated double bonds				
Phytoene (I)	3	275	285	296
Phytofluene (II)	5	331	348	367
ξ-Carotene (III)	7	378	400	425
Neurosporene	9	416	440	470
Lycopene (IV)	11	446	472	505
B. Effect of the ring structure				
γ-Carotene (V)	11	431	462	495
β-Carotene (VII)	11	425[a]	451	483

[a] Maximum absorption wavelength is not unequivocal (cf. Fig. 3.47).

bonds (Table 3.58). The fine structure of the spectrum is better distinguished in the case of acyclic lycopene (IV) than bicyclic β-carotene, since the latter is no longer a fully planar molecule. The methyl groups positioned on the rings interfere with those on the polyenic chain. Such steric effects prevent the total overlapping of π orbitals; consequently, a hypsochromic shift (a shift to a shorter wavelength) is observed for the major absorption bands (Fig. 3.47a).

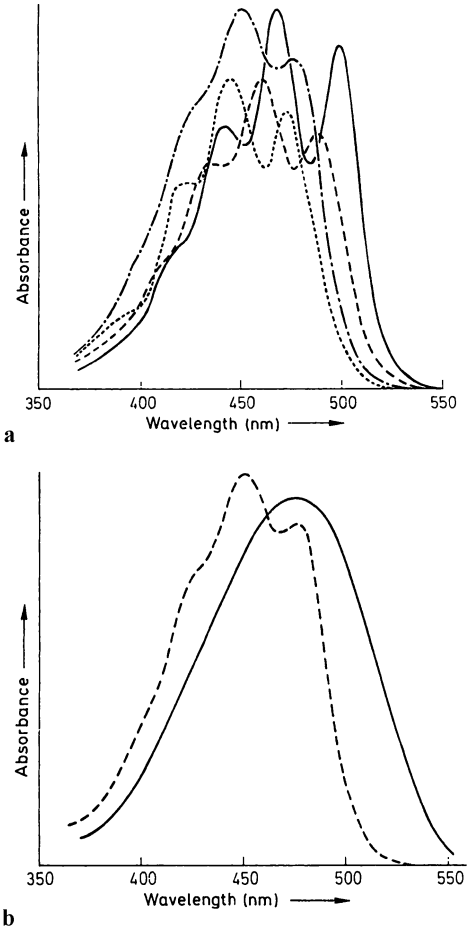


Fig. 3.47. Electron excitation spectra of carotenoids (according to *Isler*, 1971). **a** — Lycopene (IV), – – – – – γ-carotene (V), ····· α-carotene (VI), – · – · – β-carotene (VII); **b** Canthaxanthin (XII) before — and after – – – – oxo groups reduction with $NaBH_4$

Oxo groups in conjugation with the polyene system shift the major absorption bands to longer wavelengths (a bathochromic effect) with a simultaneous quenching of the fine structure of the spectrum (Fig. 3.47b). The hydroxyl groups in the molecule have no influence on the spectra.

A change of solvent system alters the position of absorption maxima. For example, replacing hexane with ethanol leads to a bathochromic shift.

Most of the carotenoids in nature and, thus, in food are of the trans-double bond configuration. When a mono-cis- or di-cis-compound occurs, the prefix "neo" is used. When one bond of all trans-double bonds is rearranged into this cis-configuration, there is a small shift in absorption maxima with a new minor "cis band" shoulder on the side of the shorter wavelength.

3.8.4.3 Chemical Properties

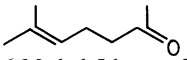
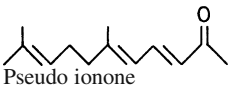
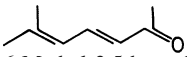
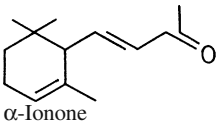
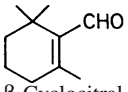
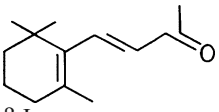
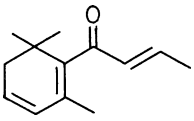
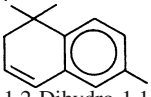
Carotenoids are highly sensitive to oxygen and light. When these factors are excluded, carotenoids in food are stable even at high temperatures. Their degradation is, however, accelerated by intermediary radicals occurring in food due to lipid peroxidationo (cf. 3.7.2). The cooxidation phenomena in the presence of lipoxygenase (cf. 3.7.2.2) are particularly visible. Changes in extent of coloration often observed with dehydrated paprika and tomato products are related to oxidative degradation of carotenoids. Such discoloration is desirable in flours (flour bleaching; cf. 15.4.1.4.3).

The color change in paprika from red to brown, as an example, is due partly to a slow *Maillard* reaction, but primarily to oxidation of capsanthin (Fig. 3.48) and to some as yet unclear polymerization reactions.

3.8.4.4 Precursors of Aroma Compounds

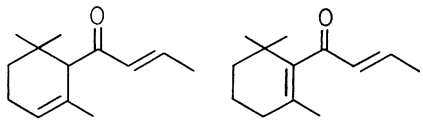
Aroma compounds are formed during the oxidative degradation of carotenoids. Such compounds, their precursors and the foods in which they occur are listed in Table 3.59. The mentioned ionones and β-damascenone

Table 3.59. Aroma compounds formed in oxidative degradation of carotenoids

Precursor[a]	Aroma compound	Odor threshold (μ/l, water)	Occurrence
Lycopene (I)	 6-Methyl-5-hepten-2-one	50	Tomato
	 Pseudo ionone	800	Tomato
Dehydrolycopene	 6-Methyl-3,5-heptadien-2-one	380	Tomato
α-Carotene (VI)	 α-Ionone	R(+): 0.5–5 S(−): 20–40	Raspberry, black tea carrots, vanilla
β-Carotene (VII)	 β-Cyclocitral	5	Tomato
	 β-Ionone	0.007	Tomato, raspberry, blackberry, passion fruit, black tea
Neoxanthin (XX)	 β-Damascenone	0.002	Tomato, coffee, black tea, wine, beer, honey, apple
	 1,2-Dihydro-1,1,6-trimethylnaphthalene	2	Wine, peach, strawberry

[a] Roman numerals refer to the chemical structures presented in 3.8.4.1.

belong to the class of C_{13}-norisoprenoides. Other than β-ionone, α-ionone is a chiralic aroma compound whose R-enantiomer is present almost in optical purity in the food items listed in Table 3.59. α- and β-Damascone (Formula 3.140), present in black tea are probably derived from α- and β-carotene. Chiro-specific analysis (cf. 5.2.4) indicated that α-damascone occurs as racemate.



(3.140)

The odor thresholds of the R- and S-form (about 1 μg/kg, water) differ rarely.

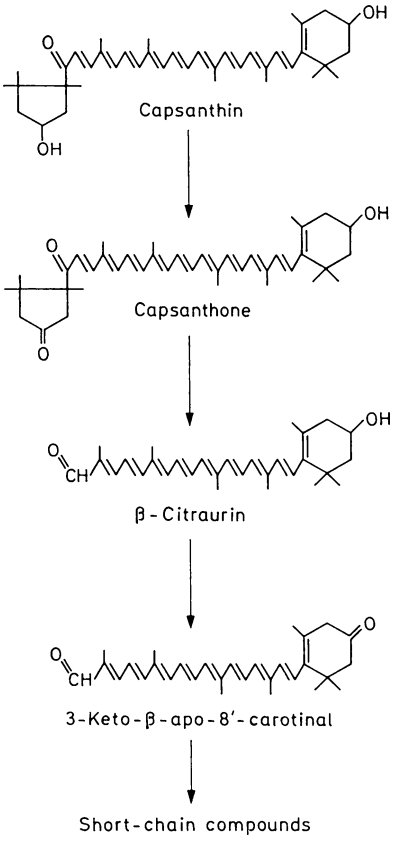
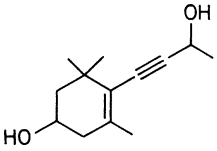


Fig. 3.48. Oxidative degradation of capsanthin during storage of paprika (according to *Philip* and *Francis*, 1971)

Of all C_{13}-norisoprenoids, β-damascenone and β-ionone, smelling like honey and violets respectively, have the lowest odor threshold values (Table 3.59). Precursor of β-damascenone is neoxanthine, out of which the *Grasshopper ketone* (I in Formula 3.142) is formed by oxidative cleavage. The oxygen function migrates from the C-9 to the C-7 position by reduction of I to form an allentriol, elimination and attachment of HO-ions. In acid medium, 3-hydroxy-β-damascone and β-damascenone result from the intermediate (II).

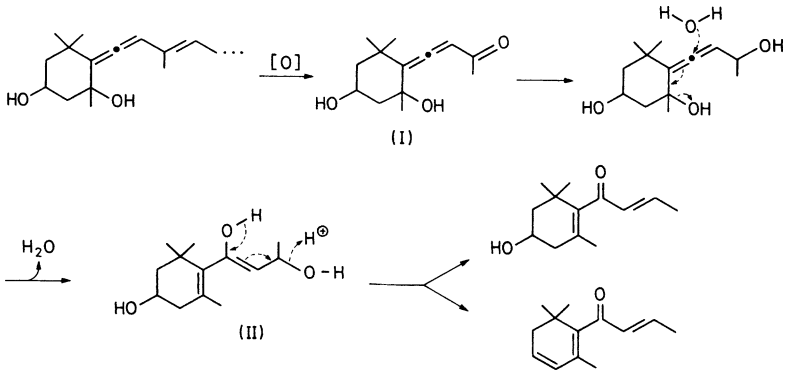


(3.141)

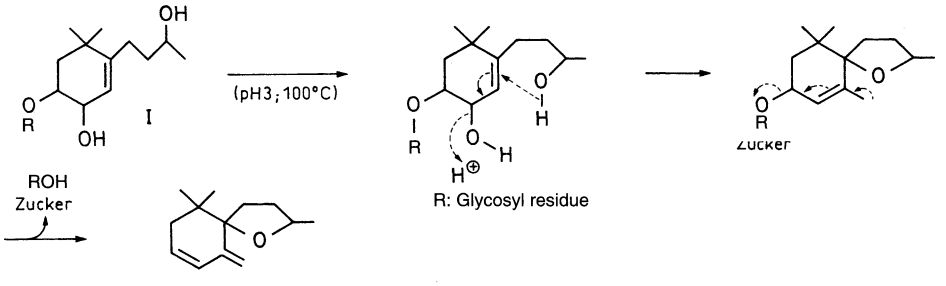
Besides the *Grasshopper ketone*, another enindiol (Formula 3.141) was identified in grape juices. When heated (pH 3), this enindiol yields 3-hydroxy- β-damascone as main and β-damascenone as minor product.

Hydroxylated C_{13}-norisoprenoids (i. e. *Grasshopper ketone*, 3-hydroxy-β-damascone) often occur in plants as glycosides, and can be liberated from these by enzymatic or acid hydrolysis and then transformed into aroma compounds. Therefore the aroma profile changes when fruits are heated during the production of juice or marmalade. An example is the formation of vitispirane (II in Formula 3.143) by hydrolysis of glycosidic bound 3-hydroxy-7,8-dihydro-β-ionol (I) in wine. The odor threshold of vitispirane is relatively high (800 μg/kg, wine) but is clearly exceeded in some port varieties.

1,2-Dihydro-1,1,6-trimethyl naphthalene (Table 3.59) can be formed by a degradation of neoxanthin and other carotinoids during the storage of wine. It smells like kerosene (threshold 20 μg/kg, wine). It is thought that this odorant contributes considerably to the typical aroma of white wine that was stored for a long period in the bottle. The compound may cause an off-flavor in pasteurized passion fruit juice.



(3.142)



(3.143)

3.8.4.5 Use of Carotenoids in Food Processing

Carotenoids are utilized as food pigments to color margarine, ice creams, various cheese products, beverages, sauces, meat, and confectionery and bakery products. Plant extracts and/or individual compounds are used.

3.8.4.5.1 Plant Extracts

Annato is a yellow oil or aqueous alkaline extract of fruit pulp of *Raku* or *Orleans* shrubs or brushwood (*Bixa orellana*). The major pigments of annato are bixin (XVIII) and norbixin, both of which give dicarboxylic acids upon hydrolysis. Oleoresin from paprika is a red, oil extract containing about 50 different pigments. The aqueous extract of saffron (more accurately, from the pistils of the flower *Crocus sativus*) contains crocin (XVII) as its main constituent. It is used for coloring beverages and bakery products.

Raw, unrefined palm oil contains 0.05–0.2% carotenoids with α- and β-carotenes, in a ratio of 2:3, as the main constituents. It is of particular use as a colorant for margarine.

3.8.4.5.2 Individual Compounds

β-Carotene (VII), canthaxanthin (XII), β-apo-8′-carotenal (XIX) and the carboxylic acid ethyl ester derived from the latter are synthesized for use as colorants for edible fats and oils. These carotenoids, in combination with surface-active agents, are available as micro-emulsions (cf. 8.15.1) for coloring foods with a high moisture content.

3.8.4.6 Analysis

The total lipids are first extracted from food with ispropanol/petroleum ether (3:1 v/v) or with acetone. Alkaline hydrolysis follows, removing the extracted acyl-lipids and the carotenoids from the unsaponifiable fraction. This is the usual procedure when alkali-stable carotenoids are analyzed. Although carotenoids are generally alkali stable, there are exceptions. When alkali-labile carotenoids are present, the acyl lipids are removed instead by a saponification method using column chromatography as the separation technique.

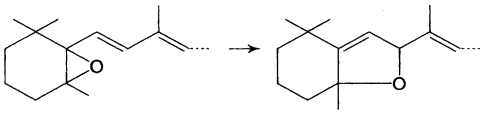
A preliminary separation of the lipids into classes of carotenoids is carried out when a complex mixture of carotenoids is present. For example, column chromatography is used with Al_2O_3 as an adsorbent (Table 3.60). Additional separation into classes or individual compounds is achieved

Table 3.60. Separation of carotenoids into classes by column chromatography using neutral aluminum oxide (6% moisture) as an adsorbent P: Petroleum ether, D: diethyl ether

Elution with	Carotenoids in effluent
100% P	Carotenes
5% D in P	Carotene-epoxides
20–59% D in P	Monohydroxy-carotenoids
100% D	Dihydroxy-carotenoids
5% Ethanol in D	Dihydroxy-epoxy-carotenoids

by HPLC and thin layer chromatography. Thin layers made of MgO or $ZnCO_3$ are suitable. These adsorbent layers permit separation of carotenoids into classes according to the number, position and configuration of double bonds.

Identification of carotenoids is based on chromatographic data and on electron excitation spectra (cf. 3.8.4.2), supplemented when necessary with tests specific to each group. For example, a hypsochromic effect after addition of $NaBH_4$ suggests the presence of oxo or aldehyde groups, whereas the same effect after addition of HC1 suggests the presence of a 5,6-epoxy group. The latter "blue hue shift" is based on a rearrangement reaction:



(5,6-Epoxide) (5,8-Epoxide)

(3.144)

Such rearrangements can also occur during chromatographic separations of carotenoids on silicic acid. Hence, this adsorbent is a potential source of artifacts.

Epoxy group rearrangement in the carotenoid molecule can also occur during storage of food with a low pH, such as orange juice.

Elucidation of the structure of carotenoids requires, in addition to VIS/UV spectrophotometry, supplemental data from mass spectrometry and IR spectroscopy. Carotenoids are determined photometrically with high sensitivity based on their high molar absorbancy coefficients. This is often used for simultaneous qualitative and quantitative analysis. New separation methods based on high performance liquid chromatography have also proved advantageous for the qualitative and quantitative analysis of carotenoids present as a highly complex mixture in food.

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