chapter six

Nutrients

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6.1 *Introduction*

Nutrients are necessary for growth, maintenance, and reproduction of living organisms, and foods are their vehicles. Individual foodstuffs may contain a smaller or larger number of nutrients. This means that no individual food meets every physiological needs. The main categories of nutrients are carbohydrates, fats, proteins, vitamins and minerals.

Together, the former three are known as *macronutrients*. They are the major sources of energy and building materials for the organism. Vitamins and minerals are the so-called *micronutrients*, as they are only needed in small amounts.

Living organisms have a complex metabolic system at their disposal to maintain the concentrations of nutrients and their metabolites at proper physiological levels. If the metabolic capacity of an organism is exceeded, physiological homeostases may be disturbed, ultimately leading to adverse effects. This may be of particular importance in cases of metabolic disorder (e.g., lactose-intolerance and phenylketonuria), infection, specific physiological state (e.g., pregnancy) or drug treatment. The treatment of a metabolic disorder usually includes a diet with a low level of the nutrient concerned.

Considering the pathway from raw material to consumer in relation to the manifestation of adverse effects on human health after intake of nutrients, the following questions arise:

(a) Which components, that belong to the nutrients and are hazardous to man, occur in raw materials?
(b) Which harmful changes can nutrients undergo during the storage of raw materials?
(c) Which harmful changes can nutrients undergo during the processing of raw materials?
(d) Which harmful changes can nutrients undergo during the manufacturing, preparation, and storage of food?

In answering these questions, distinctions are made between macronutrients and micronutrients on the one hand, and between types of macronutrients on the other hand. When looking at the macronutrients, there is a difference in relevance between the four questions. The last question will be answered for all three categories of macronutrients, while the questions a, b and c are only relevant to fats. For the micronutrients, all questions will be answered.

The reactions taking place during industrial food manufacture are essentially the same as in food preparation at home. This means that for both cases the risks of harmful changes in food may be the same. However, industrial processes are evaluated and controlled extensively to prevent any possible harmful effect to human health, while inappropiate handling and preparation at home may escape notice.

In the following sections, the above questions will be answered. Section 6.2 concerns the macronutrients, whereas Section 6.3 deals with the micronutrients. Interference with the utilization and functioning of nutrients, so-called antinutritive effects, has already been discussed in Chapter 3.

6.2 *Macronutrients*

There are three categories of macronutrients: fats, carbohydrates, and proteins. The fats will be discussed first, as only for this category all of the above questions are relevant.

6.2.1 *Fats*

Dietary fats serve various needs of the living cell. First, they are concentrated energy sources. Further, they provide building blocks, i.e., polyunsaturated fatty acids, for many biological membranes. Third, oxygenation products of the essential fatty acids serve as mediators in the communication between the various cells of the organism. Fats are important to the physiology of the consumer as well as food technology and food safety.

The texture and taste of foods depend on their composition, crystal structure, melting behavior, and association with non-lipid molecules. In baking and frying, fats act as heat-transferring media. Lipids in foods contribute either directly or indirectly to the palatability by the products formed during heating. Fats may affect the quality and safety of food, even if they are present in small quantities only, because of their high reactivity.

6.2.1.1 Undesirable fat components in raw materials

The dietary fatty acids of nutritional value have (saturated or unsaturated) linear chains. The double bonds in unsaturated fatty acids follow specific patterns. The double bond(s) in fatty acids of plant origin is (are) at position 9 in the monoenes (e.g., oleic acid and palmitoleic acid), at positions 9 and 12 in the dienes (e.g., linoleic acid), at positions 6, 9, and 12 in the trienes and at positions 5, 8, 11, and 14 in the tetraenes (e.g., arachidonic acid).

In polyunsaturated fatty acids of marine origin (e.g., fish oil), the double bonds are at positions 9, 12, and 15 in the fatty acids with 18 carbon atoms (e.g., linolenic acid), at positions 5, 8, 11, 14, and 17 in the fatty acids with 20 carbon atoms, and at positions 4, 7, 10, 13, 16, and 19 in the fatty acids with 22 carbon atoms.

Unsaturated fatty acids

$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	Palmitoleic
$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	Oleic
$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	Linoleic
$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	Linolenic
$CH_3(CH_2)_4(CH{=}CHCH_2)_3CH{=}CH(CH_2)_3COOH$	Arachidonic

The specificity of the structures of dietary fatty acids suggests that any structural deviation may result in adverse effects, unless the organism can succesfully eliminate these fatty acids.

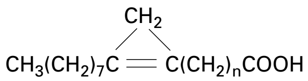
First, there is the distinction between fatty acids of the *n*-6 type (with the last double bond 6 carbon atoms from the methyl end, e.g., arachidonic acid) and fatty acids of the *n*-3 type (with the last double bond 3 carbons from the methyl end, e.g., linolenic acid). Overproduction of particular oxidation products of the *n*-6 fatty acid, arachidonic acid, the so-called eicosanoids, may lead to pathophysiological events, such as cardiovascular disorders. Acids of the *n*-3 type have been reported to inhibit the formation of *n*-6 eicosanoids. Epidemiological data on Eskimo populations, which consume large amounts of seafood (rich in *n*-3 types), suggest a reduction in cardiovascular disorders.

Secondly, several unusual fatty acids have been shown to be toxic. These include erucic acid (cis-13-docosenoic acid), sterculic acid and malvalic acid (cyclopropene fatty acids), and cetoleic acid (cis-11-docosenoic acid).

Some unusual unsaturated
fatty acids

$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$

Erucic acid



$$CH_3(CH_2)_7\overset{\overbrace{}^{CH_2}}{C{=}C}(CH_2)_nCOOH$$

n = 6: Malvalic acid
n = 7: Sterculic acid

$CH_3(CH_2)_9CH{=}CH(CH_2)_9COOH$

Cetoleic acid

Erucic acid occurs mainly in the plant family of the Cruciferae, notably in *Brassica*. The oils from rapeseeds *(B. campestris)* and mustardseeds (*B. hirta* and *B. juncea*) contain particularly high levels of erucic acid: 20 to 55%. The toxic effects are fat accumulation in the heart muscle, growth retardation, and liver damage.

Rapeseed and mustardseed oils are commercially valuable in many parts of the world, but their potential toxicity is a reason for concern. Oil refining procedures can reduce the erucic acid content to 1.4%. Selective breeding has resulted in rape varieties that produce oil with an erucic acid content of approximately 0.5%.

The cyclopropene fatty acids *malvalic acid* and *sterculic acid* are found in all plants belonging to the order of the Malvales, except *Theobroma cocoa*. Crude cottonseed oil may contain 0.6 to 1.2% cyclopropene fatty acid in the form of sterculic acid and malvalic acid. For both acids, the reproductive organs are the primary targets. Further, these substances have been found to be carcinogenic. Processing can reduce the cyclopropene fatty acid levels to 0.1 to 0.5%. Residue levels in cottonseed feed may be about 0.01%. Hydrogenation of the ring can lead to the disappearance of some of the biological effects of these acids. *Cetoleic acid* occurs in herring oil. Its toxic effects are similar to those of erucic acid.

6.2.1.2 Changes in dietary fats during storage and processing of raw materials, and during manufacturing, preparation and storage of food

6.2.1.2.1 Rancidity. Spoilage of fat- or oil-containing material is mainly characterized by rancidity, the deterioration of fats and oils in foods. It is accompanied by an unpleasant odor and taste. There are two types of rancidity, hydrolytic and oxidative. Both can occur in all steps along the way from raw material to consumer. Oxidative rancidity can lead to the formation of harmful components.

Hydrolytic rancidity results from the hydrolysis of glycerides to fatty acids and glycerol. This process may be catalyzed by lipases (enzymes present in foods or originating from microorganisms), alkali, or acid. From a food safety point of view, hydrolytic rancidity has no important direct implications. Indirectly, however, it may be involved in combined actions. In addition, foods (e.g., milk) containing short-chain fatty acids such as butyric acid, caproic acid, caprylic acid, and capric acid, may become inedible or organoleptically unacceptable because of the strong off-flavor of the free fatty acids. Sometimes, however, hydrolytic rancidity is considered desirable, for example in strong tasting cheeses. The hydrolysis of glycerides can be minimized by cold storage, proper transportation, careful packaging and sterilization.

Oxidative rancidity is caused by reactions of fatty acids with atmospheric oxygen. Oxidation of fats and oils usually results in the formation of a variety of toxic substances. Therefore, oxidative rancidity will be dealt with separately in more detail.

6.2.1.2.2 Oxidation of fats and oils, and adverse health consequences. At various stages on the way from raw material to consumer, circumstances can arise in which oxidation is either extensified or prevented, and in which the oxidation products can be removed. For the sake of quality and safety, those circumstances are taken into consideration as much as possible in modern fat and oil production.

For clarity’s sake, this subsection continues with a discussion of the various oxidation, reactions that fats and oils can undergo: autoxidation, photo-oxidation, and enzymatic oxidation. But first a detailed description of the processing of dietary fats and oils will be given in the following intermezzo.

Intermezzo

Processing of edible fats and oils. Oils and fats are particularly used for frying and baking. Some oils, such as olive oil, are appreciated for their distinctive flavor. Generally, however, fats and oils are expected to be rather tasteless and odorless. In addition, solid fats are expected to have well-defined physical properties and to give reproducible results. The consumer relies on the producer of oils and fats for specific properties and no impurities. It is well known that a relationship exists between certain processing techniques and the quality and safety of their products. Generally, the primary products of pressing and extraction are not suitable for use in food. Taste, color, general appearance, and maintenance of quality of crude oils and fats do not meet the required specifications. They may still contain impurities (see Table 6.1).

The impurities may originate from several sources. First, they may already have been in the crude product and left behind. Secondly, undesirable material may be formed during processing. The oxidation of lipids has been reported to lead to the formation of unwanted polymeric material. A third source of impurities may be the solvent in solvent extraction. The majority of the vegetable oils are obtained by solvent extraction with volatile petroleum ether hydrocarbons. It has been suggested that traces of carcinogenic aromatic hydrocarbons are passed from such solvents to the oils. However, this has not been confirmed. Moreover, should contamination be the case, these substances will certainly be removed during deodorization. The removal of impurities from crude oils and fats or the reduction of impurity concentrations in crude oils and fats to acceptable levels is realized by a sequence of refining processes. These are listed in Table 6.2.

It should be noted that during refining, the resistance of oils to oxidation may be reduced.

Bleaching with bleaching earth is not simply a matter of adsorption; free radicals are produced and oxidation takes place. During the process, small quantities of positional and geometric isomers of fatty acids as well as of a variety of hydroxy, keto, and epoxy derivatives are formed. Bleaching improves the quality of oils at the expense of their stability.

Deodorization is carried out under anaerobic conditions and no oxidation takes place. During this process less volatile substances are removed, including tocopherols and plant sterols. The physical properties of naturally occurring oils and fats are often not optimal for specific applications. However, in most cases they can be altered. This is especially so for melting behavior (see Table 6.3).

Saturating double bonds with hydrogen, so-called *hydrogenation*, converts oils into fats. This process requires a catalyst. Saturation of double bonds reduces the sensitivity to oxidation. The double bonds may be eliminated. Also, their position and their geometry

Table 6.1 Some impurities in crude dietary oils and fats

Impurity	Origin
Free fatty acids	Hydrolysis of triglycerides
Partial glycerides	Hydrolysis of triglycerides
Gums/Lecithin	Oil seeds such as soybean, rapeseed, sunflowerseed
Waxes	Oil seed coat, particularly sunflowerseed
Colored compounds	Oil seeds and fruit, e.g., chlorophyll in rapeseed and β-carotene in palm fruit
Insoluble materials	Oil seed fragments
Pollutants/Pesticides	Environmental pollution and pesticides used for oil seed crops
Volatile components	Breakdown of triglycerides and oxidized triglycerides
Polar components	Breakdown of triglycerides and oxidized triglycerides
Trace metals	Particularly iron and copper from processing machinery and equipment

(cis-trans) may be altered. In this way, fats with specific physical and chemical properties can be produced. For example, the combination of catalyst and reaction conditions can lead to the formation of trans-fatty acids. These are absorbed and metabolized in the same way as saturated fatty acids. Trans-fatty acid intake is 5 to 10% of the total fat intake. The trans-fatty acid content of shortenings, margarines and salad oils, made from partially hydrogenated oils, is 14 to 60%, 16 to 70% and 8 to 17%, respectively.

Usually, thorough mixing of fats and oils is not sufficient to obtain homogeneous products. The triglycerides retain their physical properties. However, *reesterification* under the influence of a specific catalyst may result in a rapid interchange of fatty acid moieties with the formation of triglycerides of random fatty acid composition. Reesterification may lead to a small reduction in stability.

Of the three types of oxidation of fats and oils, photo-oxidation and enzymatic oxidation are less important; they are dealt with in the Intermezzo on page 82.

The reactions of organic compounds with elemental oxygen under mild conditions are generally referred to as *autoxidations* (see Figure 6.1). Such oxidations often take place by themselves, if the (slightly contaminated) substrate is exposed to air. Compounds of many types, including hydrocarbons, such as (poly)unsaturated fatty acids, alcohols, phenols, and amines may undergo autoxidation. Free fatty acids are more susceptible to oxidation than the fatty acid moieties in glycerides. Hydrolysis of glycerides is catalyzed by alkali, acid, and enzymes.

The attack of oxygen on hydrocarbons, i.e., (poly)unsaturated fatty acids, may be initiated by a radical derived from an outside source (generally peroxides, see Figure 6.1). The reaction of a C–H bond with a radical proceeds more readily if the carbon is tertiary or secondary, than if it is primary, and still easier if the carbon is allylic, as in (poly)unsaturated fatty acids.

(Hydro)peroxides, as is to be expected, may act as free radical initiators. The free radicals may be produced from (hydro)peroxides in two ways:

– in reactions with metals that have at least two readily accessible oxidation states (e.g., $\mathrm{Fe}^{2+}/\mathrm{Fe}^{3+}$, $\mathrm{Cu}^{+}/\mathrm{Cu}^{2+}$):

$$\mathrm{Fe}^{2+} + \mathrm{ROOH} \rightarrow \mathrm{Fe(OH)}^{2+} + \mathrm{RO}^{\cdot}$$

– on heating or by the action of visible or ultraviolet light:

$$\mathrm{ROOR} \xrightarrow{\Delta \text{ or hv}} \mathrm{RO}^{\cdot} + .\mathrm{OR}$$

Table 6.2 Removal of impurities from crude oils and fats

Refining process	Impurity to be removed	Processing technique
Degumming	Gums, phosphoglycerides (mainly lecithin)	Hot water rinsing
Neutralization	Traces of gums and phospho-glycerides, free fatty acids, mono- and diglycerides	Treatment with aqueous alkali solutions
Bleaching	Colored components and polar substances	Treatment with bleaching earth
Filtration	Bleaching earth and insoluble material	Filtering under pressure or using a mesh filter
Deodorization	Volatile substances, pesticides and pollutants	Vacuum steam distillation

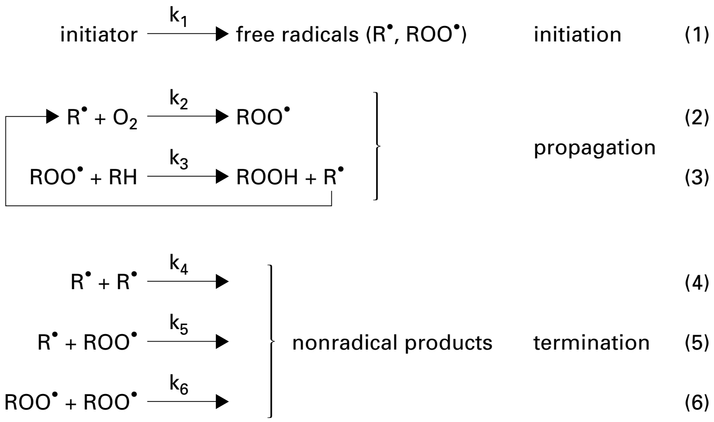


Figure 6.1 Diagrammatic representation of autoxidation.

Table 6.3 Alteration of physical properties of crude oils and fats

Process resulting in alteration	Result of processing
Hydrogenation	Rearrangement of double bonds; conversion of double bonds to single bonds
Fractionation	Separation of solid and liquid triglycerides
Reesterification	Random distribution of fatty acid moieties over the triglycerides

In the case of polyunsaturated fatty acids, the initiation is followed by a rearrangement of the double bonds (in the fatty acid radical), resulting in the formation of so-called conjugated diene radicals (see Figure 6.2). Further propagation is simple. The conjugated diene radicals may be oxygenated to peroxide radicals. The latter, in turn, may abstract hydrogen atoms again under the formation of hydroperoxides and radicals of the parent compound (see Figures 6.1 and 6.2).

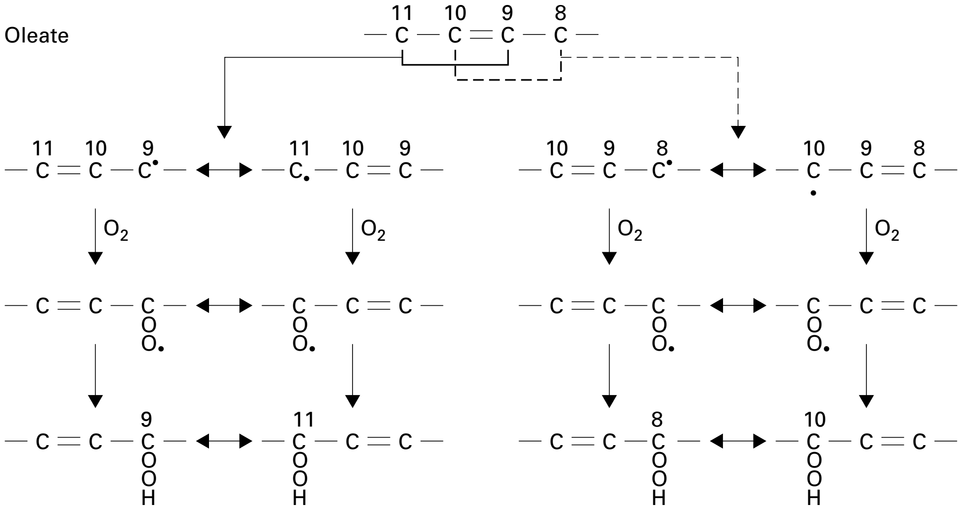


Figure 6.2 Formation of hydroperoxides.

One of the termination reactions is undoubtedly radical coupling:

$$R^{\cdot} + .R \rightarrow R{-}R$$

The chain reaction may also be terminated by radical scavenging:

$$R^{\cdot} + S{-}OH \rightarrow RH + S{-}O^{\cdot}$$
$$\text{(e.g. vitamin E)}$$

Intermezzo

Photo-oxidation and enzymatic oxidation. (Poly)unsaturated fatty acids may also undergo oxidative degradation in photoreactions (photo-oxidation) and enzymatic processes (enzymatic oxidation). The *photo-oxidations* that (poly)unsaturated fatty acids can undergo are of two types:

1.) Free-radical chain reactions, which start from the excited state of another molecule:

$$A \xrightarrow{h\nu} A^{*}$$

A = molecule of another food component, e.g., riboflavin (in milk), and A* = excited state of A.

A* may abstract an electron or a hydrogen atom from the substrate, RH:

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

RH = e.g., linoleic acid.

These radicals can undergo further reactions in the presence of oxygen to form hydroperoxides:

$$R^{\cdot} + O_2 \rightarrow R{-}OO^{\cdot} \xrightarrow{RH} R{-}OOH$$

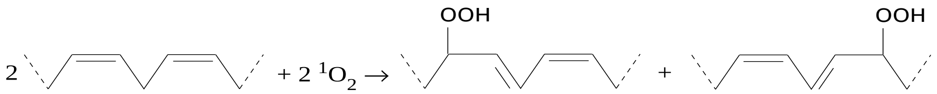
2.) Singlet oxygen ($^1O_2$) reactions in which the absorption of photons by molecules of another food component is followed by energy transfer to ground-state oxygen, leading to the formation of singlet oxygen:

$$A \xrightarrow{h\nu} A^{*}$$

A = e.g., protoporphyrin (occurring in hemoglobin, myoglobin and most of the cytochromes).

$$O_2 + A^{*} \rightarrow {}^1O_2 + A$$

Singlet oxygen can attack the double bonds in the unsaturated fatty acids (e.g., linoleic acid), yielding hydroperoxides:



As against autoxidation, tocopherols (e.g., vitamin E) can provide protection against sensitized photo-oxidation by acting as quenchers of singlet oxygen. Photodegradation of foods can be further prevented by using packaging materials that absorb the photochemically active light, and by removing endogenous photosensitizers and oxygen from the food.

Oxidative degradation of fats and oils may also be *enzyme*-mediated. The oxidation of fats and oils of plant origin may be catalyzed by lipoxygenase. The lipoxygenase-mediated oxidation is a hydroperoxide-initiated free radical chain reaction. Enzymatic oxidation also leads to the formation of hydroperoxides. Lipoxygenases can be inactivated by heat treatment.

In the three routes of oxidative degradation described above — autoxidation, photo-oxidation and enzymatic oxidation — a large variety of products is formed from fats and oils (see Figures 6.3 and 6.4).

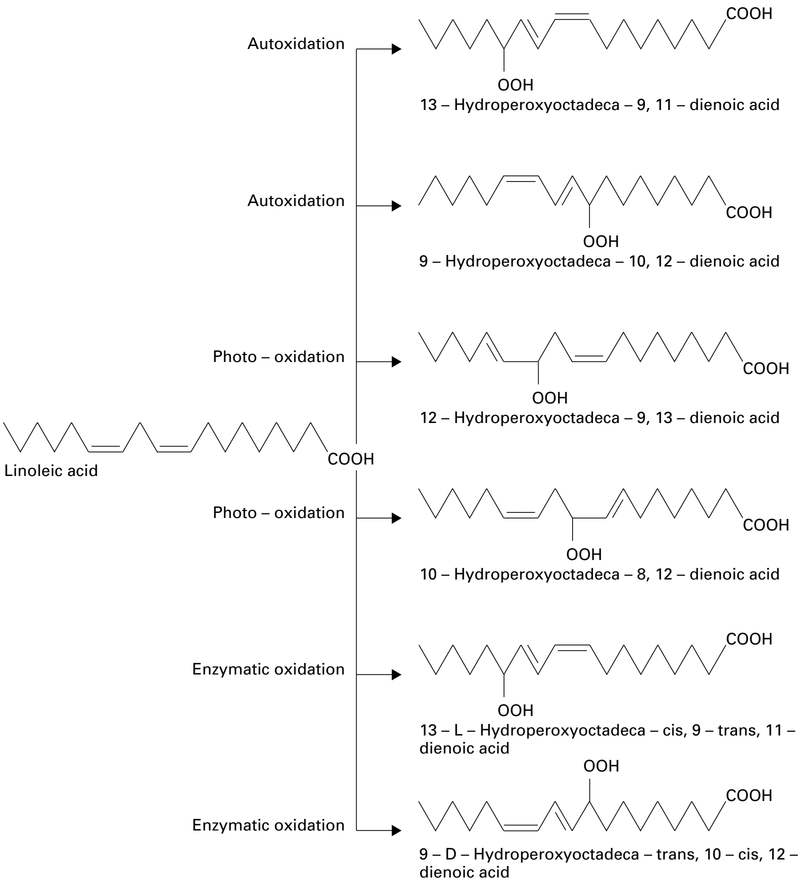
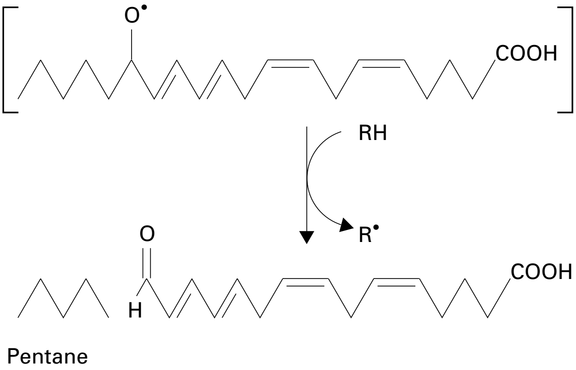


Figure 6.3 Hydroperoxide formation in autoxidation, photo-oxidation and enzymatic oxidation of linoleic acid.

The initially-formed lipid hydroperoxides are unstable. They degrade further in metal–ion catalyzed reactions to compounds such as alkanes (e.g., ethane and pentane) and (unsaturated) aldehydes (e.g., hydroxynoneal), and ketones (e.g., acetone):



Unsaturated aldehydes, in particular α,β-unsaturated carbonyl compounds, may undergo toxic conjugations (Michael additions) with biologically essential nucleophiles such as sulfhydryl compounds and DNA bases. Hydroxynonenal is known to form adducts with DNA. Further, while the hydroperoxides are relatively unvolatile, tasteless, and odorless, the products formed in the secondary degradation reactions are volatile, and play a role in the development of off-flavors.

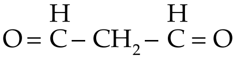
Hydroperoxides can also react with other nutritive components, such as amino acids and carotenoids. For example, methionine is oxidized to the sulfoxide, and lysine degrades to diaminopentane, aspartic acid, glycine, alanine, α-amino adipic acid and many other substances.

In all three of the oxidative degradations, termination of the chain reaction may result in dimerization of oxygen-centered radicals:

$$\mathrm{R{-}O^{\cdot} + .O{-}R \rightarrow R{-}OO{-}R}$$

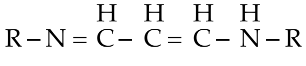
The formation of these peroxidic dimers may lead to polymerization reactions. This can be explained by both their instability and the large variety of functional groups they may contain. The dimers readily undergo homolysis, even at low temperatures. Further, homolysis may be followed by several rearrangements.

Another well-known end product of the peroxidation of polyunsaturated fatty acids such as linoleic acid and arachidonic acid is malondialdehyde:



$$\mathrm{O{=}\overset{H}{C}{-}CH_2{-}\overset{H}{C}{=}O}$$

Malondialdehyde is capable of cross-linking to primary amino groups, forming a conjugated Schiff base with the general structure:



$$\mathrm{R{-}N{=}\overset{H}{C}{-}\overset{H}{C}{=}\overset{H}{C}{-}\overset{H}{N}{-}R}$$

where R may be free amino acids, proteins or nucleic acids.

Enzymes may be inactivated as a result of cross-linking, either directly by a reaction or indirectly by alteration of the membrane structure. Furthermore, malondialdehyde has been demonstrated to be carcinogenic in experimental animals and mutagenic in the Ames

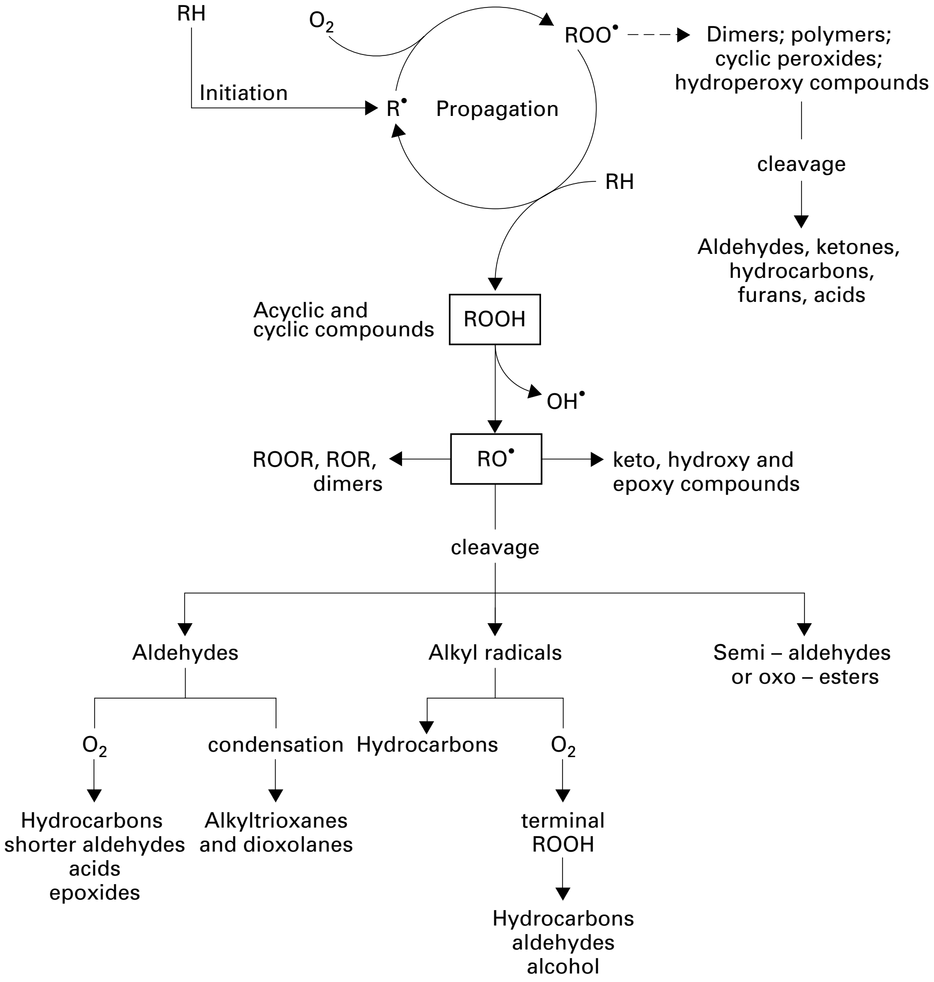


Figure 6.4 Diagrammatic representation of the degradation and polymerization reactions following autoxidation of unsaturated fatty acids.

test. The above data emphasize that peroxidation of food components may have negative effects on its nutritional value, sensoric quality and safety.

6.2.1.2.3 Effects of processing techniques on the oxidation of dietary fats and oils. The effects of various processing techniques used in food manufacture on the oxidation of dietary fats and oils can be largely predicted on the basis of tissue damage, exposure to oxygen, presence of metal ions, time, and temperature range involved.

Processing may already cause problems in the early stages of food manufacture. *Rapid freezing* of raw plant material may be accompanied by lipoxygenase-mediated oxidation. This phenomenon depends on the extent of tissue damage, and on storage temperature and time. *Blanching* and storage at low temperature may inhibit peroxidaton, but cannot prevent it.

During *dehydration* and *freeze-drying*, food lipids are extensively exposed to air as a thin film, thereby promoting autoxidation. The water content of a foodstuff is critical for the autoxidation rate. Excess water prevents extensive contact of lipids with oxygen. Further, storage temperature and time are important factors in determining the extent of oxidative deterioration. In the case of dehydration, the detection of oxidatively developed off-flavors may be facilitated, while the natural flavors and odors may disappear.

During *baking* lipids may be spread in thin films over large surfaces. Since baked products are usually consumed fairly soon after production, autoxidation will only occur to a limited extent.

Some types of *fermentation* are used for the production of substances that are undesirable in other products. Examples are the formation of short-chain acids and carbonyl compounds in cheeses and the high rancidity of a number of traditional Asian fermented fish and soy products.

Minor effects of the above processing techniques on product quality, shelf life, and vitamin content have been reported. Nutritional value and food safety do not appear to be much affected, not even under extreme conditions.

Deep-frying of foods in oils gives more rise to concern. As dealt with before, saturated and unsaturated fatty acids may undergo decomposition upon heating in the presence of oxygen. A diagrammatic summary of the thermolytic and oxidative mechanisms involved is shown in Figure 6.5.

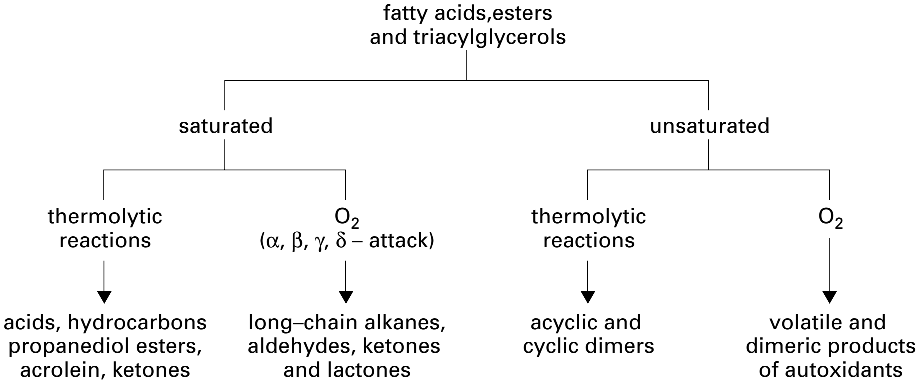


Figure 6.5 Diagrammatic representation of thermal and oxidative decompositions of lipids.

Frying of food under normal conditions may result in the formation of small amounts of stable peroxides. During industrial processing under vacuum, dimers, polymers, and cyclic products may be formed. The products that are formed when cooking oils are heated may be taken up by the fried food products. Meat, deep-fried in rapeseed oil, appeared to contain 0.63 to 1.1% of the nonvolatile oxidation products of the oil. French fried potatoes have been shown to contain secondary oxidation products of the cooking oil of high molecular mass. If oil is used in discontinuous batch-type operations, as in restaurants and at home, it is eventually discarded because of either high viscosity or excessive foaming. Discarded oils are found to contain approximately 25% polymers. Stable foams are formed if the polymeric oxidation product content is about 9%.

Generally, under normal frying conditions, oxidation of fats and oils has no harmful consequences. However, it should be noted that inappropriate heating and storage of fats and oils may lead to the formation of harmful substances at toxic levels. From the viewpoint of food safety, the conditions under which moderately rancidifying fats and oils are handled in the consumer's kitchen deserve particular attention.

6.2.2 *Carbohydrates*

In the human diet, carbohydrates are mainly present as starch. Well-known sources are cereals, potatoes and pulses.

Celluloses and other polysaccharides in the plant cell wall do not serve as energy sources. They cannot be digested by humans and contribute mainly to the dietary fiber intake. Fiber appears to play an important role in the maintenance of gastro-intestinal function, metabolism and health. Carbohydrates are useful food components because of their sweetness, solubility, cristallization behavior, water activity, hygroscopic behavior and rheological properties.

6.2.2.1 *Changes in dietary carbohydrates during manufacturing and storage of food*

Introduction. Reducing sugars may undergo a well-known (non-enzymatic) browning reaction, the so-called *Maillard reaction* in which sugars condensate with amino acids. Pentoses are usually more reactive than hexoses. The mechanism underlying this reaction has not yet been fully elucidated. The Maillard reaction is a sequence of reactions, resulting in the formation of a mixture of insoluble dark-brown polymeric pigments, known as melanoidins. In the early steps of the reaction, a complex mixture of carbonyl compounds and aromatic substances is formed. These products are water-soluble and mostly colorless. They are called *premelanoidins*.

Initial steps of the Maillard reaction. The first step in the Maillard reaction is the condensation of sugars with amino groups of amino acid moieties. The initial products, glycosylamines, are quite unstable and undergo Amadori rearrangement (Figure 6.6). The course of the condensation depends on the water content of the food. Further, these reactions proceed more rapidly upon heating, especially under neutral to alkaline conditions.

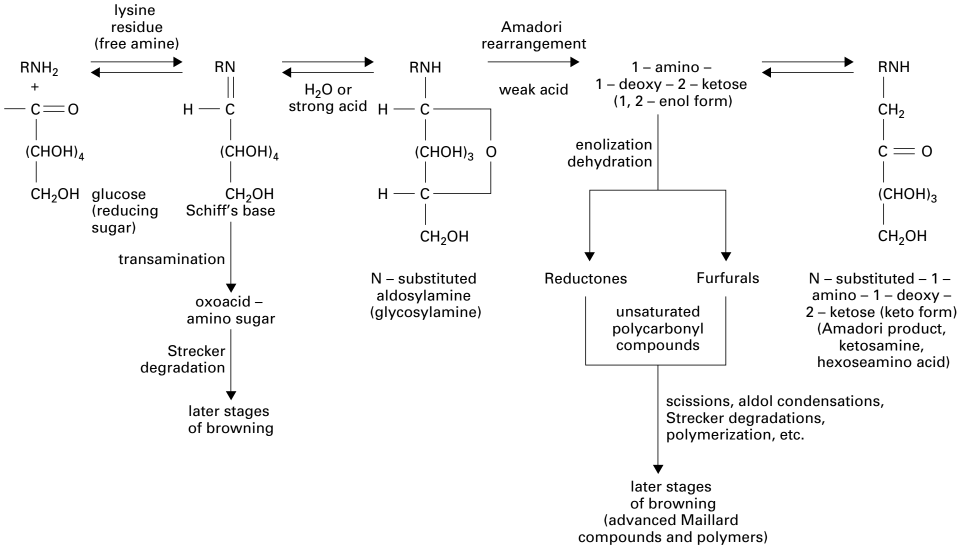


Figure 6.6 Condensation of glucose with lysine, followed by Amadori rearrangement.

The classic example of a foodstuff in which the initial steps may occur is drum-dried milk powder in which a 10 to 40% decrease in availability of lysine has been reported. If spray-drying is used, the availability of lysine does not decrease.

Subsequent steps of the Maillard reaction. In the following steps, reactive unsaturated polycarbonyl compounds such as reductones, and heterocyclic compounds such as pyrazines, are formed. These compounds bind to α-terminal, ε-amino and other amino groups of different polypeptide chains to form colored, high-molecular mass, highly cross-linked carbohydrate–protein polymers of low solubility, low digestibility, and low nutritional value. These steps may be followed by breakage of the polypeptide chains, and decarboxylation and ultimately deamination of the amino acid moieties (Strecker degradation, Figure 6.7).

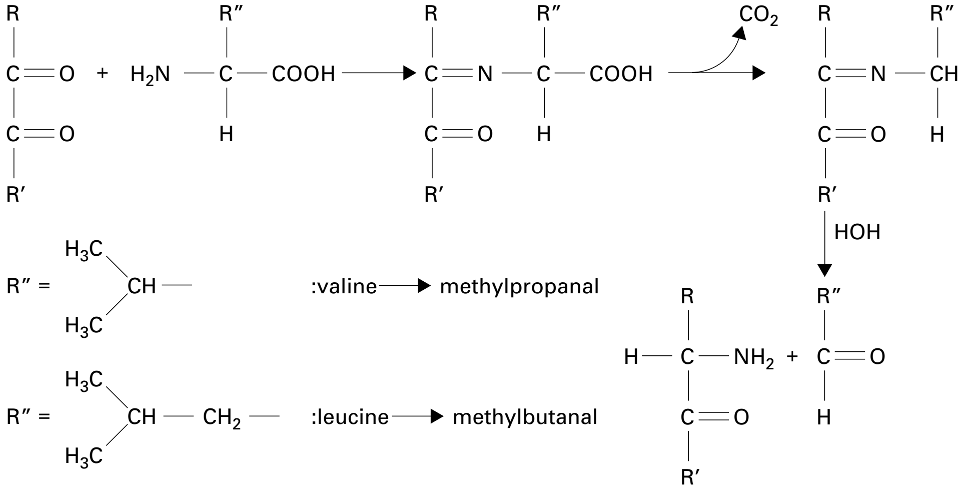


Figure 6.7 Strecker degradation of amino acids.

The last steps of the Maillard reaction may take place to a considerable extent on heating of the food, especially chocolate and baked products such as bread, biscuits, and cakes. The initial steps of the Maillard reaction do not cause marked changes in the color and flavor of the foodstuff. However, as mentioned above, the course of the Maillard reaction depends on the water content of the food. As a result, the deterioration may be intensified by concentration and dehydration of protein-containing food, e.g., concentration and drum-drying of milk, dehydration of egg white, and drying of oilseed products. The last steps are largely responsible for the desired color and flavor of baked products.

Animal studies have indicated that premelanoidins inhibit growth, disturb reproduction and cause liver damage. Further, certain types of allergic reactions have been attributed to Maillard reaction products. Maillard reactions can be prevented by using the additive power of the carbonyl group in reducing sugars. A reaction characteristic of aldehydes and some ketones is addition of sodium bisulfite. The addition products are crystalline salts, very soluble in water. Further, Maillard reactions may be inhibited by regulating the temperature, pH, and water content.

6.2.3 *Proteins*

Proteins are the source of essential amino acids. They are the only dietary source of nitrogen for protein synthesis. Denaturation of protein occurs when food is heated during preparation. This is a desirable effect, as denaturated protein is more readily digested. Apart from their nutritional value, proteins are important to the physical properties of foods. Their solubility and dispersability, hygroscopic behavior, viscosity, and stabilizing properties determine the structure and texture of foods.

6.2.3.1 *Changes in proteins during processing of raw materials, and during manufacturing, preparation and storage of food*

A technique that is increasingly used in the processing of proteins is treatment with alkali. It involves solubilization and purification of proteins. Protein concentrates are prepared this way. Alkali cooking of maize is a traditional Mexican technique to increase its digestibility. Treatment with sodium hydroxide is advocated for the peeling of grain. The use of gaseous ammonia has been proposed to free peanut and cottonseed products from aflatoxin.

Intensive treatment of proteins with alkali is known to result in advanced degradation of several amino acids, cystine, arginine, threonine, and serine being the most sensitive. Under mild alkaline conditions and at moderate temperatures, products may be formed that have been found to be nephrotoxic in rats, e.g., lysinoalanine (LAL), ornithinoalanine (OAL) and lanthionine. LAL is the condensation product of dehydroalanine and lysine (Figure 6.8). Dehydroalanine is formed on alkaline desulfurization of cystine. Sulfur is released as H_2S. Treatment of serine and phosphoserine moieties with alkali also yields dehydroalanine.

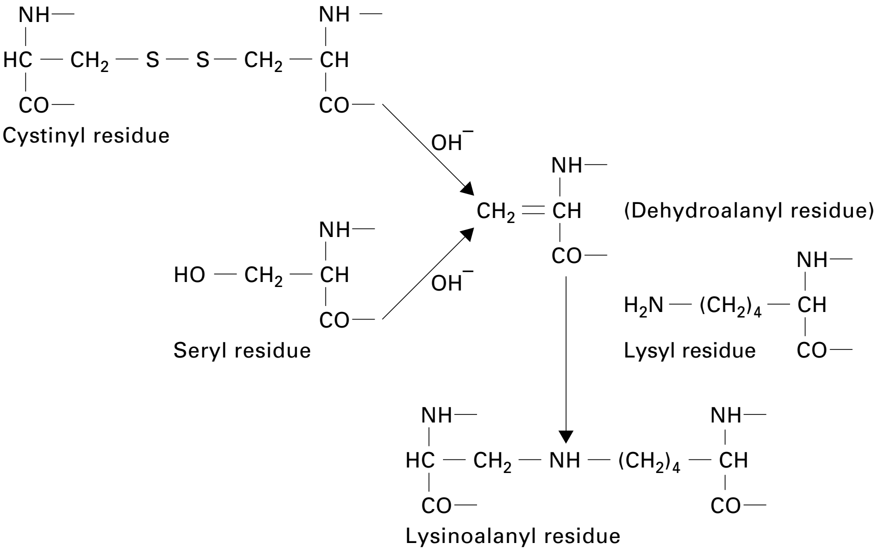


Figure 6.8 Formation of lysinoalanine (LAL).

The extent of LAL formation depends on the nature of the protein, the duration and temperature of the reaction, and the alkali concentration. Different legume proteins from mung beans, cow peas, and peanuts have been shown to produce considerable amounts of LAL when treated with 0.05 to 0.075 *N* NaOH at 20°C for 30 min. The LAL content may range from 200 to 800 mg/100 g of protein. Some legumes, such as kidney beans, lima beans, and vetch were stable under these conditions. After treatment with alkali at 80°C, however, all legumes contained LAL. Their LAL content decreased on prolonged treatment at higher temperatures. In several other foods, LAL is formed during cooking in the absence of alkali (Table 6.4). Chicken meat which was free from LAL before cooking, contained 200 µg/g after cooking in a microwave oven. Egg white free from LAL when fresh, contained 270 to 370 µg/g after boiling for 10 to 30 min, and 1.1 mg/g if pan-fried at 150°C for 30 min.

Table 6.4 Lysinoalanine content of heated proteins and some protein-containing food products

Protein or food	LAL (μg/g)
Sausage after boiling in water for 10 min	50
Corn chips	390
Pretzels	500
Tortillas	200
Evaporated milk	590–860
Simulated cheese	1070
Egg white solids	160–1820
Hydrolyzed vegetable proteins	40–500
Whipping agent	6500–50,000
Soya protein isolates	0–370

The reactions of proteins with oxidation products of oils and fats and with reducing sugars (Maillard reaction) have already been discussed in Sections 6.2.1.2.2 and 6.2.2.1 respectively.

6.2.4 *Pyrolysis products occurring in food*

Decomposition of a compound into smaller, more reactive structures by the action of heat alone is known as *pyrolysis*. The fragmentation is usually followed by combination of the smaller structures to more stable compounds, provided the conditions do not allow the conversion to CO and CO_2.

Since pyrolysis products may occur in all three of the macronutrient categories and the formation of these products proceeds largely by the same mechanism, this subsection deals with all three categories. The formation of pyrolysis products depends on the type of parent compound and the temperature. In the case of food, hazardous compounds are formed from about 300°C. In the following paragraphs, some well-known types of pyrolysis products occurring in food are discussed.

Polycyclic Aromatic Hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs) are likely to be formed from degradation products consisting of two- or four-carbon units, such as ethylene and butadiene radicals (Figure 6.9).

The most potent carcinogenic PAH is benzo[*a*]pyrene (3,4-benzpyrene). Benzo[*a*]pyrene has been demonstrated in pyrolysis products of food. It has been identified in the charred crusts of biscuits and bread, in broiled and barbecued meat, in broiled mackerel and in industrially roasted coffees. The levels found in broiled meat ranged from 0.17 to 10.5 ppb. Fat is an important "precursor" for the formation of PAHs (in meat and fish). Broiling of high-fat hamburgers led to the production of 43 ppb of PAHs, of which 2.6 ppb was benzo[*a*]pyrene. In the lean product, only 2.8 ppb of PAH were found, and no benzo[*a*]pyrene.

Starch may also undergo pyrolysis. On heating of starch from 370 to 390°C, 0.7 ppb benzo[*a*]pyrene was formed. The range of 370 to 390°C is readily reached in cooking procedures, e.g., at the surface of bread during baking and in boiling cooking fats.

The conditions during the preparation of food may affect the levels of PAH formed. In T-bone steaks cooked close to the charcoal and relatively long, benzo[*a*]pyrene levels up to 50 ppb have been detected. These levels could be considerably reduced by cooking at a larger distance from the charcoal. PAHs are abundantly found in smoked food (Table 6.5). They originate from the combustion of wood and other fuels.

Heterocyclic pyrolysis products from amino acids. Some potent mutagens are produced on pyrolysis of amino acids. Tryptophan has been shown to be the "precursor" of the

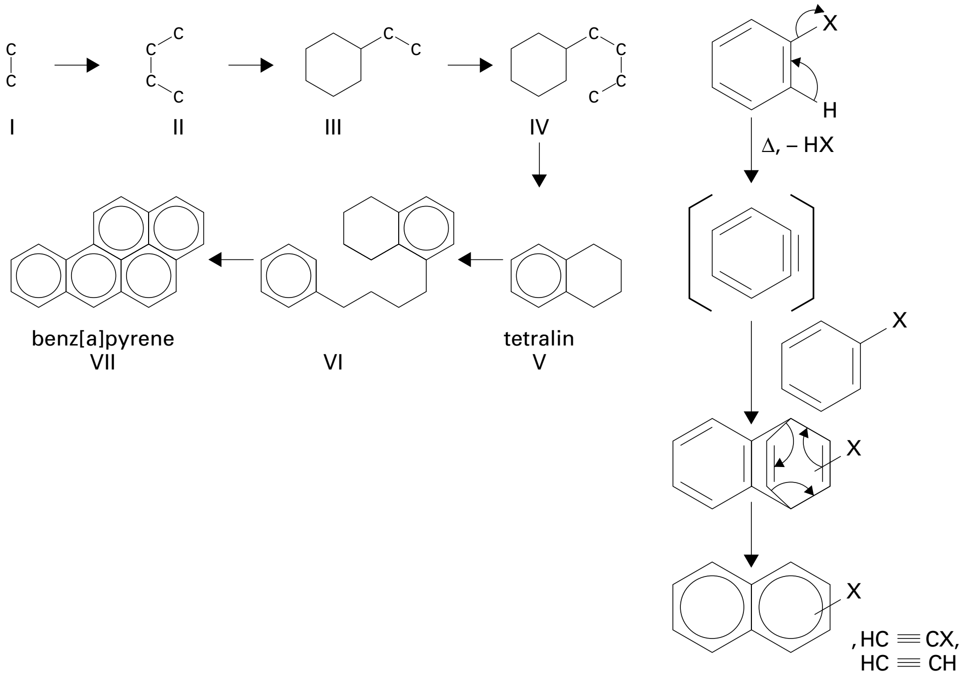
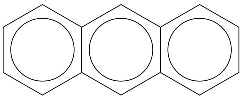
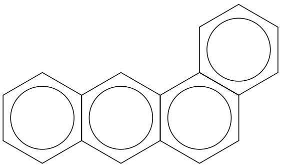


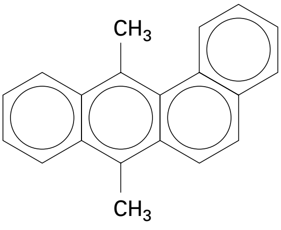
Figure 6.9 Proposed routes for the formation of PAH.



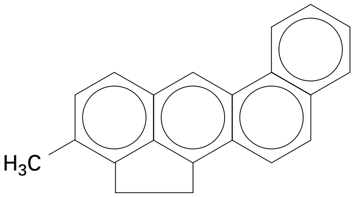
Anthracene



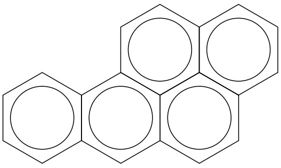
Benz[a]anthracene



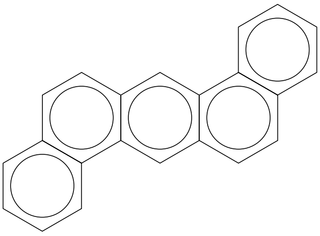
7, 12 – Dimethylbenz[a]-
anthracene



3 – Methylcholanthrene



Benz[a]pyrene

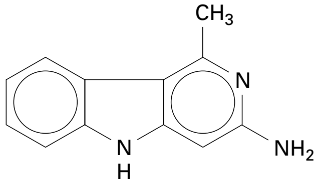


Dibenz[a,h]anthracene

Table 6.5 Benzo[*a*]pyrene content of smoked and other foods

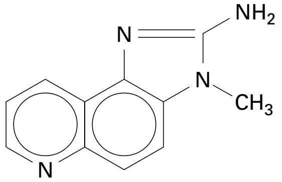
Food	Benzo[*a*]-pyrene (ppb)	Food	Benzo[*a*]-pyrene (ppb)
Smoked fish		Barbecued meats	
Eel	1.0	(Charcoal broiled)	
Herring	1.0	Hamburgers	11.2
Sturgeon	0.8	Pork chop	7.9
Chubs	1.3	Chicken	3.7
White fish	6.6	Sirloin steak	11.1
Kippered cod	4.5	T-bone steak	57.4
Smoked meats		T-bone steak	4.4
Ham	0.7–55.0	(flame broiled)	
Mutton		Ribs	10.5
close to stove	107.0	Other steaks	5.8–8.0
distant from stove	21.0	Other foods	
Lamb	23.0	Spinach	7.4
Sausage (with skin)		Kale	12.6–48.1
cold smoked	2.9	Yeast	1.8–40.4
hot smoked	0.7	Tea	3.9–21.3
Salami	0.8	Coffee	0–15.0
Bacon	3.6	Cereals	0.2–4.1
		Soybean	3.1

mutagens 3-amino-1,4-dimethyl-5H-pyrido[4-*b*]indole and 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole. Pyrolysis of phenylalanine may lead to the formation of the mutagenic substance 2-amino-5-phenylpyridine.

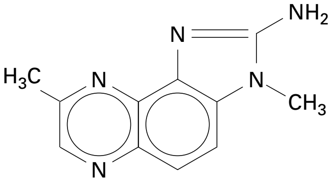


3 – Amino – 1 – methyl –
5H – pyrido (4, 3 – b) indole

These mutagens and several structurally related substances have been isolated from the surface of protein-containing food cooked at 250°C and higher. Mutagens of the aminoimidazoazoarene type have also been isolated from different types of protein-rich foods heated at about 200°C. They include, for example, quinolines and quinoxalines:



2 – Amino – 3 – methylimidazo
(4,5 – f) quinoline



2 – Amino – 3,8 – dimethylimidazole
(4,5 – f) quinoxaline

6.3 Micronutrients

This section discusses various relevant aspects of the micronutrients *vitamins* and *minerals*. There are 13 known vitamins. They are usually divided into two groups: lipophilic and hydrophilic vitamins. Generally, vitamins play no part in food technology, with the exception of the vitamins C and E which can function as antioxidants.

The minerals may be divided into two groups based on the levels at which they occur in the body. The elements that are present in considerable quantities are sodium, potassium, calcium, chlorine, magnesium, and phosphorus. Their combined mass is about 3 kg in adult men. The second group comprises the so-called trace elements. They include sulfur, iron, fluorine, iodine, zinc, copper, selenium, manganese, molybdenum, chromium, and cobalt. They are required in very small amounts only. Their combined content is roughly 30 g. Minerals are important in food processing because of their effects on the course of enzymatic as well as non-enzymatic processes. Further, they can affect the texture of foods by reacting with polysaccharides (gel formation). Minerals are also important flavorings.

Health risks due to micronutrients are usually associated with deficiencies in the diet. This has already been mentioned in Chapter 3. Dietary excess of micronutrients does not cause great problems because the minerals and the majority of the vitamins are water-soluble and are readily eliminated by excretion as well as metabolism. Only the intake of the lipid-soluble vitamins A and D may lead to toxic effects, as they accumulate easily in the body. Intake of vitamins in excess of the required amounts results in the toxic syndrome of hypervitaminosis. Selenium is of special importance because the margin between the physiological need and the toxic dose is very small. Dietary intake is estimated at 56 mg/day in regions poor in selenium and at 326 mg/day in regions rich in selenium. Selenium is the only mineral that accumulates in plants in considerable amounts. It is incorporated in amino acids by replacing sulfur. This has been reported to lead to intoxications in animals feeding on selenium-rich plants.

In view of the large intake level range for micronutrients indicated above, the nutritional state of an individual can affect cellular processes other than the specific metabolic reactions in which the vitamins and minerals play essential physiological roles. Micronutrients may influence cellular differentiation, hormone metabolism and regulation, immunological control, and metabolic activation and inactivation of protoxins. Vitamins A, B_2 and C have been found to protect against carcinogenesis in a number of human as well as animal studies. Copper, zinc, and especially selenium can act in a dualistic way. These minerals may cause enhancement of and provide protection against carcinogenesis, depending on the toxic substance involved.

6.3.1 Hypervitaminosis

6.3.1.1 Vitamin A

The major dietary sources of vitamin A are carotenoids, particularly β-carotene, occurring in red, orange, and green plants, and retinyl esters, present in animal tissues. The active form of vitamin A is retinol. Carotenoids act as provitamins. They are converted to retinol in the intestinal mucosa. After absorption in the intestines, retinol is transported to the liver bound to plasma proteins, where it is stored as retinyl fatty acid esters.

Generally, carotenoids are not considered to be toxic, as their conversion to retinol is rather inefficient. On very high intake, carotene accumulates in the body. The toxic symptoms of high vitamin A intake are yellow pigmentation of the skin, headache, dizziness, vomiting, and diarrhea followed by swelling of the skin, which eventually cracks and peels. In most cases, these symptoms disappear within a few days after termination of the high intake.

Table 6.6 Effect of the degree of maturity on the ascorbic acid content of a tomato variety

Weeks after bloom	Average weight (g)	Color	Ascorbic acid (mg %)
2	33.4	green	10.7
3	57.2	green	7.6
4	102.5	green-yellow	10.9
5	145.7	yellow-red	20.7
6	159.9	red	14.6
7	167.6	red	10.1

The natural foods that contain sufficient retinol to induce toxic effects in man are the livers of animals at the top of long food chains, such as marine fish and polar bears. Cases of acute toxicity have been reported by researchers and fishermen in polar regions after eating generous liver portions, containing up to 100,000 IU (1.0 IU equals 0.3 μg retinol) of vitamin A per gram. The toxic intake by adults is estimated at 2 to 5 million IU. Single intakes of 30 million IU have been reported.

An insidious cause of vitamin A intoxication today is the chronic consumption of vitamin supplements. Capsules containing retinol doses of 25,000 IU are easily obtainable, while the recommended daily intake is 5000 IU. Daily intakes of 20,000 to 40,000 IU for a number of years have been found to lead to toxic symptoms.

6.3.1.2 Vitamin D

Vitamin D can be formed from 7-dehydrocholesterol in the skin under the influence of ultraviolet light. The product is cholecalciferol (vitamin D_3). This passes to the liver where it is hydroxylated to 25-hydroxycholecalciferol (25-OHD). After transport to the kidney, 25-OHD is metabolized to 1,25-dihydroxycholecalciferol, a more potent form. The synthesis of vitamin D in the skin is carefully regulated. However, high intakes of this vitamin may result in hypercalcemia. This may be accompanied by tissue injury and hypersensitivity. Daily intakes in excess of 50,000 IU (1.25 mg) have been found to cause toxic effects. The normal dietary intake is estimated at 1000 IU per day.

6.3.2 Changes in raw materials during storage and processing, and in food during manufacture, preparation and storage

For the micronutrients, no changes leading to the formation of harmful substances are known. On the other hand, all foods lose vitamins and minerals to some extent when stored and processed, either industrially or at home. The food industry tries to minimize these losses by careful regulation of the various processing steps.

Apart from the above, the micronutrient content of food largely depends on a number of (pre-storage and pre-processing) factors, including genetic variation, degree of maturity, soil conditions, use and type of fertilizer, climate, availability of water, light (day length and intensity), and post-harvest or post-mortem handling. Data on these factors, however, are scarce. As an example, Table 6.6 shows the effect of maturing of tomatoes on their ascorbic acid content. The maximum vitamin content is already reached before maturity.

6.3.2.1 Vitamins

Vitamins are reactive substances. They may be sensitive to light, heat, moisture, oxidizing and reducing agents, acid, alkali and (traces of) heavy metals. It should be noted that each vitamin reacts in a different way, as is shown in Table 6.7.

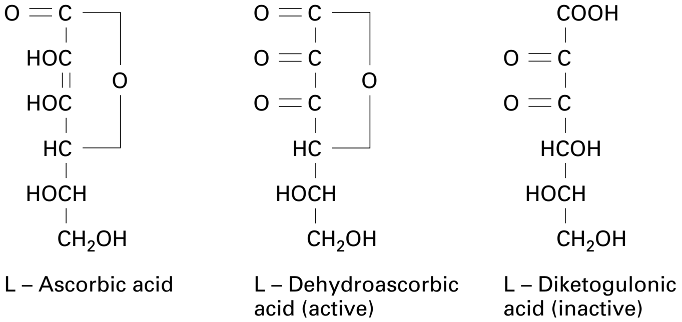
Effects of food handling on vitamin content will be illustrated for vitamin C (ascorbic acid). This vitamin is probably the most extensively investigated with regard to its behav-

Table 6.7 Sensitivity of vitamins to chemical and physical factors

Type of vitamin	Light	Heat	Humidity	Oxidizing agents	Reducing agents	Acids	Bases
Vitamin A	XX	X	0	XX	0	X	0
Vitamin D	XX	X	0	XX	0	X	X
Vitamin E	X	X	0	X	0	0	X
Vitamin B_1	X	XX	X	XX	0	0	XX
Vitamin B_2	XX	0	0	0	X	0	XX
Vitamin B_6	X	0	0	0	X	X	X
Vitamin B_{12}	X	0	0	0	X	X	XX
Panthotenic acid	0	X	X	0	0	XX	XX
Folic acid	X	0	0	XX	XX	X	X
Vitamin C	0	X	X	XX	0	X	XX

Note: 0 = hardly or not sensitive; X = sensitive; XX = very sensitive.

ior during food processing. Ascorbic acid has an enediol structure, in which the double bond is conjugated with a carbonyl group. Due to this structure, it has both acidic and (strong) reducing properties. The natural form is the L-isomer. The D-isomer has about 10% of the activity of the L-isomer. The latter is used as food antioxidant.



Ascorbic acid is easily and reversibly oxidized to dehydroascorbic acid. The oxidation is catalyzed by metal ions. Subsequent hydrolysis of dehydroascorbic acid to 2,3-diketogulonic acid results in irreversible loss of vitamin acitivity. The latter compound may undergo anaerobic degradation. The primary degradation products are xylosone and 4-desoxypentosone. These in turn may produce ethyl glyoxal, reductones, furfural, and 2,5-dihydrofuroic acid (Figure 6.10).

The presence of ascorbic acid, dehydroascorbic acid and the degradation products in amino group-containing food can give rise to Maillard-type browning reactions.

Ascorbic acid is readily lost by leakage from cut or bruised surfaces of foods. Loss by leakage is most prominent during cooking (Figure 6.11). In processed foods, the most considerable losses result from oxidative degradation, especially upon irradiation with sunlight. This is most frequently seen in foods which are high in ascorbic acid, such as fresh fruits. Warm foods should not be left in open metal containers before eating in order to prevent oxidative degradation catalyzed by the metal of the container.

6.3.2.2 *Minerals*

Losses of minerals, result from physical removal or interaction with other food components rather than from chemical degradation. Primarily responsible for loss are processing

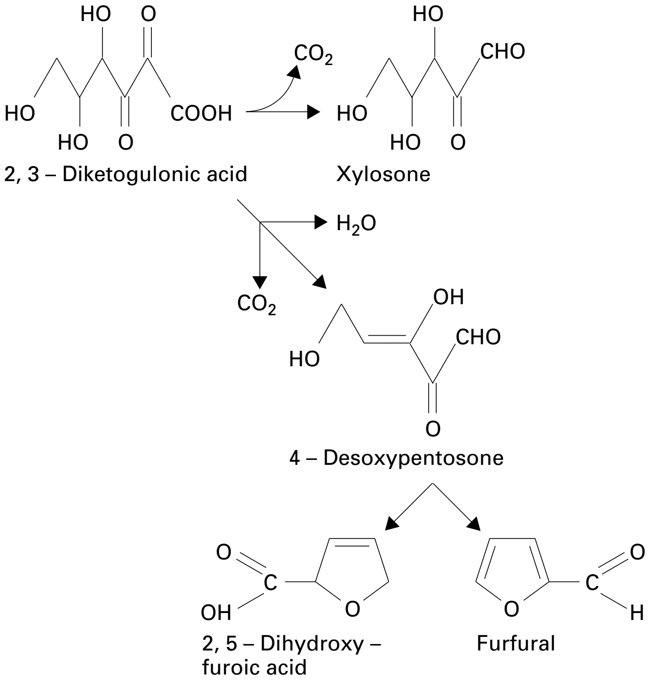


Figure 6.10 Degradation reactions of 2,3-diketogulonic acid.

techniques in which contact with water is possible. These include leaching (of water-soluble materials), blanching, and cooking. Table 6.8 shows the effect of blanching on mineral loss from spinach.

As can be seen from Table 6.8, the differences in loss between the minerals can be attributed to differences in water solubility.

In the case of cereals, milling appears to be a major cause of mineral loss. In general, the overall loss of minerals during storage and processing of foodstuffs has no negative effect on the dietary mineral intake levels. A varied diet still provides the proper amounts of minerals.

6.4 Summary

Nutrients are the major food components. They are necessary for growth, maintenance, and reproduction of living organisms. The main categories of nutrients are carbohydrates, fats, proteins, vitamins, and minerals. The first three categories, the so-called macronutrients, are

Table 6.8 Effect of Blanching on the Mineral Loss from Spinach

Mineral	Unblanched g/100 g	Blanched g/100 g	Loss (%)
Potassium	6.9	3.0	56
Sodium	0.5	0.3	43
Calcium	2.2	2.3	0
Magnesium	0.3	0.2	36
Phosphorus	0.6	0.4	36
Nitrate	2.5	0.8	70

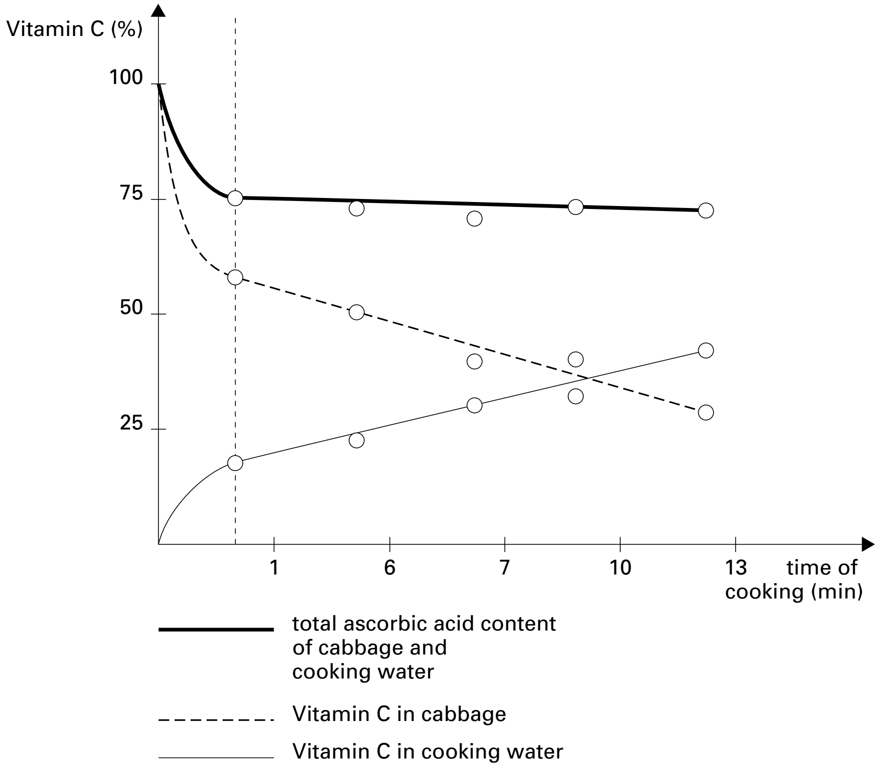


Figure 6.11 Ascorbic acid loss during cooking of cabbage.

the major sources of energy and building materials to the organism. Vitamins and minerals are known as micronutrients, since they are only needed in small amounts. Micronutrients play essential roles in specific metabolic reactions.

Living organisms have a complex metabolic system at their disposal to maintain the concentrations of nutrients and their metabolites at physiological levels. If the metabolic capacity of an organism is exceeded, physiological homeostases may be disturbed, ultimately leading to adverse effects.

On the way from raw material to consumer, however, conditions may be encountered under which nutrients can undergo harmful changes, i.e., during storage and processing of the raw materials, and during manufacturing, preparation, and storage of the actual good food.

Fats are highly reactive. Their reaction products can affect both the quality and the safety of food. Only a few unusual fatty acids have been shown to be toxic themselves, e.g., erucic acid and sterculic acid. Deterioration of fats is mainly associated with hydrolytic and oxidative rancidity. The former has no important consequences from a food safety point of view. Oxidation of fats and oils usually leads to the formation of a variety of toxic substances. Three types of oxidation can be distinguished: autoxidation, photo-oxidation, and enzymatic oxidation. Toxic oxidation products are hydroperoxides, unsaturated aldehydes (e.g., hydroxynonenal), and malondialdehyde. Hydroxynonenal is known to form adducts with DNA. Malondialdehyde has been demonstrated to be carcinogenic in experimental animals and to be mutagenic in the Ames test. Oxidative degradation of fats and fat-containing foods during storage and processing depends on the extent to which the structures of the raw materials and foodstuffs have been damaged on exposure to oxygen and light, and on the time and temperatures involved.

Carbohydrates may undergo a well-known (non-enzymatic) browning reaction, the Maillard reaction. They condensate with amino acids. The Maillard reaction is a sequence of reactions, ultimately leading to the formation of a mixture of insoluble dark-brown polymeric pigments, the so-called melanoidins. In the early steps of the reaction, a complex mixture of carbonyl compounds and aromatic substances is formed. These are known as premelanoidins. They have been found to inhibit growth, to disturb reproduction and to cause liver damage. Further, certain types of allergic reactions have been attributed to products of the Maillard reaction.

A technique that is increasingly used in the processing of proteins is treatment with alkali. Severe treatment with alkali can result in advanced degradation of amino acids. Under mild alkaline conditions and at moderate temperatures, products may be formed that have been found to be nephrotoxic in rats, e.g., lysinoalanine.

All three macronutrient categories can undergo pyrolysis during food preparation. Well-known types of toxic pyrolysis products are polycyclic aromatic hydrocarbons and heterocyclic compounds. The former are likely to be formed from degradation products consisting of two- or four-carbon units. A highly potent carcinogenic of this type of pyrolysis products in food is benz[*o*]pyrene. Heterocyclic products are formed by pyrolysis of amino acids. Pyrolysis of tryptophan appears to lead to the formation of the mutagen 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole.

Vitamins and minerals are essential dietary components. Health risks due to micronutrients are usually associated with deficiencies in the diet. Only intake of the lipid-soluble vitamins A and D may lead to toxic effects, as they accumulate easily in the body. Intake of vitamins in excess of the required amounts results in the toxic syndrome called hypervitaminosis. During storage and processing of raw materials and food, micronutrients are lost to some extent, either in industry or at home.

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