14

Natural antioxidant functionality during food processing

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14.1 Introduction

Raw materials used for food preparation already contain various components which are able to inhibit lipid oxidation, and other natural antioxidants may be added before processing. Much interest has developed during the last few decades in naturally occurring antioxidants because of the adverse attention received by synthetic antioxidants and because of the worldwide trend to avoid or minimise the use of artificial food additives. One disadvantage of natural antioxidants is their low resistance against oxygen, particularly under exposure to light, high temperature and drying. Antioxidant changes continue during storage of food products.

Food products available on the market are usually further treated in catering plants and in households during meal preparation. The most important processing technologies are listed in Table 14.1. Despite the great importance of the course of these processes, relatively little has been published on changes in antioxidants, their interactions with other food components, and the effect of these changes on food resistance against oxidation. In general, the activity of natural antioxidants is greatly affected by complex interfacial phenomena in emulsions and multicomponent foods.

14.2 Types of changes in antioxidants during food processing and storage

Antioxidants present in foods change during the processing, in a similar way to other food components. Although many studies deal with estimated
losses of food nutrients, including antioxidants, through different operations of food processing, only the residual concentration of antioxidants has been determined in most cases, rather than total antioxidant capacity of foods.²

Very different, and sometimes even opposite, effects on the intrinsic antioxidant properties of foods can occur during processing and storage,³ as is evident from Table 14.2.

**Table 14.1** Types of food processing which affect antioxidants and oxidative stability of foods

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Type of process</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated temperature</td>
<td>Water as heat transfer medium</td>
<td>Pasteurisation, Sterilisation, Blanching, Evaporation, Extrusion, Drying, Roasting, baking, Frying, Microwave heating, Infrared heating</td>
</tr>
<tr>
<td></td>
<td>Air as heat transfer medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oil as heat transfer medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Waves giving energy</td>
<td></td>
</tr>
<tr>
<td>Ambient temperature</td>
<td>Effect of enzymes</td>
<td>Fermentation</td>
</tr>
<tr>
<td></td>
<td>Effect of chemicals</td>
<td>Curing, smoking</td>
</tr>
<tr>
<td></td>
<td>Effect of time</td>
<td>Storage (also cold and frozen storage)</td>
</tr>
</tbody>
</table>

**Table 14.2** Changes in antioxidant properties of foods during processing and storage

<table>
<thead>
<tr>
<th>Resulting resistance against oxidation</th>
<th>Examples of changes in foods, affecting the antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No effect</td>
<td>In case of moderately intensive processes positive and negative influences are counterbalanced</td>
</tr>
<tr>
<td>Increased resistance against oxidation</td>
<td>Transformation of antioxidants into more active compounds, such as glycosides into aglycones, formation of novel compounds, such as Maillard products, destruction of pro-oxidants, especially photosensitizers or heavy metals, Inhibition of oxygen access, e.g. encapsulation</td>
</tr>
<tr>
<td>Decreased resistance against oxidation</td>
<td>Destruction of antioxidants by oxidation or interactions with other food components, Losses of antioxidants by evaporation, Improved access of oxygen, e.g. caused by drying, Formation of pro-oxidants or their liberation from inactive complexes</td>
</tr>
</tbody>
</table>
The most important losses of antioxidant activity occur as a result of chemical changes in antioxidants present in food materials. Naturally, the most pronounced changes result from oxidation reactions occurring rapidly on heating or slowly in storage (Table 14.3). Antioxidants are oxidised either by lipid oxidation products (mainly hydroperoxides) or directly by oxygen, either dissolved in lipidic and aqueous phases or absorbed from the air. Tocopherols have been oxidised by ferric ions and hydroperoxides even in absence of oxygen with formation of tocopherones and their mixed dimers with the polyunsaturated acid residue. Reaction products of antioxidants may retain antioxidant activity, for example tert-butylated hydroquinone (TBHQ) is oxidised either in an inactive quinone or in 2,2-dimethyl-5-hydroxy-2,3-dihydrobenzo(b) furan, which is a strong antioxidant. Other changes are mostly neglected even when they affect food resistance more than oxidation processes, such as removal of water or evaporation of volatile antioxidants or pro-oxidants.

Modification of a recipe during preparation of foods or ready meals improves the stability against oxidation especially the addition of spices. Soy sauce improved the resistance against oxidation due to its metal chelating activity.

Some examples of different changes and their effect on the resulting stability will be given in the following discussion of thermal processes, packaging, storage, etc.

### 14.3 Changes under heating when water is the heat transfer medium

Exposure of food components to temperatures above ambient conditions (during heat processing) is a major cause of detectable changes, not only of
nutritional quality, but also of antioxidant activity. Although some processes involving higher temperatures are used in order to produce positive changes, especially of the sensory value, they often result in loss of nutritional quality, and in some cases, in losses of their resistance against lipid oxidation.

The application of moderate temperatures, up to 100 °C, reduces the negative changes of nutritional quality. Various changes under these conditions are listed in Table 14.4. Food processing by application of such temperatures results in protein denaturation and aggregation reactions. Killing micro-organisms is the main reason for moderate heating. The denaturation of enzymes, which are also proteins, is often desirable. However, various changes occur in parallel, including changes in flavour, texture and colour as well as destruction of heat-sensitive nutrients. These factors have to be considered, and thermal processes must be carefully designed, to avoid overprocessing and unnecessary reductions in product quality.

Pasteurisation and blanching are similar thermal processes utilising relatively mild thermal treatments to achieve the desired stability of food products during subsequent storage. The pasteurisation is most often associated with liquid foods, while the more complex blanching is associated with solid foods. It is generally recognised that application of a higher temperature for a shorter time will lead to improved quality retention in pasteurisation and blanching.7

Boiling is often used for processing vegetables, fruit, meat and fish. Boiling temperatures are 100 °C (or slightly higher or lower, depending on the atmospheric pressure). They are not very different from the two industrial processes described above.

### 14.3.1 Changes during pasteurisation

Losses of vitamins are a good marker of negative changes due to thermal destruction. Transformations of tocopherols (vitamin E) are the best known changes in antioxidants during thermal food processing,8 but they are only moderate during pasteurisation. Losses of ascorbic acid (an important inhibitor of oxidation) are used as an indicator of food quality and therefore the severity of pasteurisation, blanching, or the length of cooking.

<table>
<thead>
<tr>
<th>Type of process</th>
<th>Type of precursors</th>
<th>Type of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme denaturation</td>
<td>Oxidoreductases</td>
<td>Inactive enzymes</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>Heteroglycosides</td>
<td>Aglycones</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>Ascorbic acid</td>
<td>Degradation products</td>
</tr>
<tr>
<td>Extraction</td>
<td>Vitamins, phenolics</td>
<td>Loss in cooking water</td>
</tr>
</tbody>
</table>

Table 14.4 Changes in antioxidants during treatment of food with hot water or steam
These changes are due mainly to thermal destruction, and to a lesser extent, to oxidation. In fruit juices the main cause of colour deterioration is enzymatic browning of polyphenolics, catalysed by polyphenoloxidases in the presence of dissolved oxygen. Polyphenoloxidases destroy phenolic antioxidants so their rapid inactivation is desirable when the preservation of phenolics is important. Losses of ascorbic acid and carotenes are minimised by de-aeration as well. Moreover, an addition of these antioxidants before or after processing is quite common.

14.3.2 Changes during blanching
Commercial methods of blanching involve passing solid foods through an atmosphere of saturated steam or a bath of hot water, so that only the water in both physical states is the carrier of heat. Rapid heating of the food material deactivates enzymes, such as lipoxygenases, which would otherwise catalyse lipid oxidation. The primary products of lipoxygenase-catalysed oxidation – lipid hydroperoxides – would partially destroy natural antioxidants. The deactivation of polyphenoloxidases is also very useful for the protection of phenolics against enzyme-catalysed oxidation into the respective quinones, the antioxidant activity of which is very low or non-existent.

14.3.3 Changes during sterilisation
Another more severe thermal food process is referred to as a commercial sterilisation, which proceeds at higher temperatures than pasteurisation. Traditionally, this process has been used to achieve long-term shelf stability of canned foods, but it is now used for a broad range of food products. The intensity of the commercial sterilisation process is such that it results in significant changes in the quality characteristics of the product. Excepting the elimination of micro-organisms, however, these changes are usually more detrimental than positive. In canned fruits and vegetables substantial vitamin losses may occur in all water-soluble vitamins, particularly ascorbic acid, which is the most important antioxidant in these foods. Therefore, the presence of residual oxygen in the medium has to be minimised. In some foods, ascorbic acid or other antioxidants are added into the brine or syrup. The effect of processing conditions on vitamin changes in sterilised milk was discussed in detail. Although traditional sterilisation processes result in losses of sensory and nutritional quality attributes, the processes are still widely used, and could be optimised to improve quality retention regarding the specificity of any particular commodity.

Lipoxygenases were deactivated during treatments of fruit juices at 70–90°C, depending on the type of juice, for instance, the lipoxygenase activity did not substantially change in apricot and apple juices, but decreased in carrot, green bean and zucchini juices.
14.3.4 Changes during boiling
Boiling is a very common procedure for food preparation. In this case boiling water transfers heat. It is useful to add food to hot water to shorten the time for enzyme deactivation, especially the deactivation of oxidoreductases. During boiling, the antioxidant activity of proteins is affected because of their denaturation. The effect on antioxidants is similar to that occurring during sterilisation. The heat denaturation of haeme pigments in foods of animal origin could increase the pro-oxidative effect of iron and thus reduce the activity of antioxidants. During boiling, antioxidants are partially extracted and remain in the boiling water. If the boiling water is not used but discarded these antioxidants are lost.

14.3.5 Changes during evaporation
Evaporation has historically been the primary technology for liquid concentration in the food industry. Evaporation proceeds at higher temperature, which is in contrast with other methods of concentration (such as membrane filtration or freeze concentration) where the main aim is to reduce heat damage. The evaporation darkens the colour of food, for example milk, partly because of the increase in concentration of solids, but also because the reduction in water activity promotes chemical changes, such as caramelisation of sugars, Maillard browning reactions or lipid–protein interactions. The evaporation temperature also destroys some types of heat-labile vitamins, reduces the biological value of proteins, and promotes lipid oxidation.

The effect of heat on natural antioxidants during evaporation is also obviously considerable, reducing antioxidant efficiency through thermal decomposition. To minimise the degradation due to prolonged heating during evaporation, it is necessary to minimise the residence time of food products at elevated temperature and the time needed to reach the evaporation temperature. Requirements for optimal evaporation include rapid rate of heat transfer, low-temperature operation through application of reduced pressure and efficient vapour–liquid separation.

14.3.6 Changes during the extrusion cooking
Extrusion is a process which combines several unit operations, including mixing, cooking, kneading, shearing, shaping and forming. If the food is heated, the process is known as hot extrusion or extrusion cooking. Water under pressure is the medium of heat transfer. For hot extrusion processes, high temperature (most often, 120–160°C), high pressure and short time duration (less than 1 min) are typical conditions. The effect of these conditions results in products with moderate heat treatments. Therefore, nearly all of the heat-sensitive compounds remain in the final product. This high-temperature–short-time process still reduces some negative factors,
such as undesirable enzymes, micro-organisms and labile anti-nutritional components.

Nevertheless, vitamins are potentially lost during the hot extrusion process. Ascorbic acid, an extremely heat-sensitive vitamin and antioxidant, can be significantly reduced during extrusion, especially at higher temperatures. Losses of ascorbic acid and vitamin A are up to 50% depending on the time and moisture content of the food held at elevated temperatures. Losses of antioxidants rise moderately with increasing temperature and water content in the barrel. They may be prevented by addition of rosemary extract and by other antioxidants. In contrast to hot extrusion, losses in cold extrusion are minimal.

14.4 Changes in functionality of antioxidants during processes when hot air is the medium of heat transfer

Heat is transferred more slowly by hot air than by hot water, therefore, higher temperatures and/or longer processing times are often used. Changes are much more intensive on the surface than in inner layers, so that antioxidants are formed or destroyed mostly at or near the surface.

14.4.1 Changes during roasting and baking

Roasting and baking belong to common, frequently used culinary operations. Heat is applied through hot air. During these operations, outer layers of the material are heated to 120–200 °C or even more, while in inner layers, the temperature does not exceed 10 °C. Changes in antioxidants and other food components are about the same in the inner layers as in the case of boiling; but in outer layers, pyrolysis, caramelisation and Maillard reactions proceed. The most important changes are summarised in Table 14.5. They may transform antioxidants into less active, or exceptionally into more active products.

Table 14.5 Changes in antioxidants during roasting and baking

<table>
<thead>
<tr>
<th>Type of process</th>
<th>Type of precursors</th>
<th>Type of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caramelisation</td>
<td>Sugars, ascorbic acid</td>
<td>Chelating macromolecules</td>
</tr>
<tr>
<td>Maillard reaction</td>
<td>Sugars, amino acids</td>
<td>Chelating macromolecules</td>
</tr>
<tr>
<td>Strecker degradation</td>
<td>Dicarbonyls, amino acids</td>
<td>Dihydroheterocycles</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Phenolics</td>
<td>Quinones</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>Glycosides, esters</td>
<td>Aglycones, phenolic acids</td>
</tr>
</tbody>
</table>
If reducing sugars and free amino acids are present, Maillard reactions start slowly from 100–120 °C, and become very rapid at temperatures above 150 °C. Inhibitory activity of Maillard products is summarised in Table 14.6. Maillard products are formed at the maximum rate in media of intermediate humidity. An interaction product of glucose and lysine inhibits the peroxide formation in fats. Some intermediary Maillard products, such as reductones, have high antioxidant activities in aqueous solutions or emulsions. Volatile products originating in various side reactions, such as dihydrofuran, dihydropyridine or dihydropyrazine derivatives, are further oxidised into substituted furans, pyridines and pyrazines, respectively. Oxygen present in the system is thus consumed, and the oxidation of lipids or antioxidants is prevented in this way.

Dehydroascorbic acid, produced by oxidation of ascorbic acid, can also participate in Maillard reactions, for example a pyrone derivative is formed by reaction with aspartame which shows moderate but significant antioxidant activity. Analogous reactions may proceed with other peptides or amino acids.

Maillard products also possess some chelating activity. By binding heavy metal ions or active complexes into complexes possessing no oxidation-promoting activity, they partially protect other antioxidants against destruction. An antimicrobial activity of Maillard products may be partially explained by the chelation of metal ions, essential for microorganisms. The antioxidant activity of Maillard products is stronger in emulsions than in dry systems. The mechanism of browning is very complex and until now is not yet fully understood. Some intermediary products may also act as weak pro-oxidants.

Aldehydic lipid oxidation products react with amino acids such as lysine, forming substituted pyrole derivatives possessing a moderate antioxidant activity. Maillard products were found to be active as oxidation inhibitors in biscuits, confectionery, sausages and extrusion products. Many active phytochemicals are phenolics in their nature, which undergo various reactions during thermal processing, thus affecting the flavour of

<table>
<thead>
<tr>
<th>Type of compounds</th>
<th>Precursors</th>
<th>Effect on food stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imines (Schiff bases)</td>
<td>Sugars, amino acids</td>
<td>Hydroperoxide reduction</td>
</tr>
<tr>
<td>Amino deoxy sugars</td>
<td>Schiff bases</td>
<td>Hydroperoxide reduction</td>
</tr>
<tr>
<td>Amadori, Heyns products</td>
<td>Amino deoxy sugars</td>
<td>Hydroperoxide reduction</td>
</tr>
<tr>
<td>Melanoidins</td>
<td>Premelanoidins</td>
<td>Metal chelation</td>
</tr>
<tr>
<td>Dihydrocyclic derivatives</td>
<td>Strecker compounds</td>
<td>Hydroperoxide reduction</td>
</tr>
<tr>
<td>Reductones</td>
<td>Dideoxyriulose</td>
<td>Free radical scavenging</td>
</tr>
</tbody>
</table>

Table 14.6 Inhibitory activity of Maillard reaction products
foods. Chlorogenic and caffeic acids were found substantially to modify the flavour compounds formed in browning reactions between sugars and amino acids. Pyrazines, which are formed during roasting and baking, are characterised by their nutty and roasted flavours. The presence of chlorogenic and caffeic acid significantly reduced the amount of pyrazines. It also changed the relative distribution of different pyrazines and their ratios. These processes altered the total sensory quality.

Losses of natural antioxidants in relation to processing conditions and the formation of Maillard reaction products have been studied in coffee brews and tomato puree.\textsuperscript{21} The antioxidant activities of both these evaluated food commodities were enhanced as the roasting time and temperature increased. In conclusion, although natural antioxidants are partially lost during heating, the overall antioxidant properties of heated foods can be maintained or even enhanced by the development of new antioxidants, such as Maillard reaction products.

Tocopherols and tocotrienols present in flour are partially destroyed (by about 25\% in the case of $\alpha$-tocopherol) during cooking and baking. In rye bread, prepared by traditional technology, a loss of 50\% $\alpha$-tocopherol was observed.\textsuperscript{22}

14.4.2 Changes during drying

During evaporation the water content of the material is reduced to about 30–60\%. The drying is an operation when the water content is reduced to about 6–12\%, so that a solid product results. In original foods, lipid droplets, liposomes or membranes are protected by layers of hydrated proteins against oxygen access from the air. Owing to the dehydration, this protective layer is damaged so that lipids are exposed to the free access of oxygen, being transformed into a thin film on the surface of non-lipidic particles. Therefore, the oxidation of lipids (including sterols) is much more rapid in dry foods than in original water-rich foods, even at ambient temperature or under refrigerated storage. High concentration of free lipidic radicals involves heavy losses of antioxidants, and their low efficiency. Relative antioxidant activities are changed by water removal as they are often different in bulk oil and in an emulsion.\textsuperscript{23} These oxidation reactions occur only during storage of dry foods. During the drying process, lipid oxidation is restricted because of short drying time (only a few minutes or still less in modern drying equipments), and the presence of water vapour in the atmosphere. Antioxidants are usually not damaged during drying, and their evaporation is only moderate.

Freezing is a process similar to drying in that water is being removed in the form of ice crystals. Lipids are again distributed as a thin film or in droplets, exposed to the access of air. Oxidative changes in lipids and antioxidants may be greater in frozen foods than in refrigerated foods.
14.5 Processes where the energy is transferred as waves

Microwave and infrared energy are both transmitted as waves which penetrate food, and are then converted to heat. Nowadays, microwave cooking is the most versatile method all over the world. It is more energy efficient and reduces the cooking time compared to conventional heating. For industrial applications, microwave heating can be used for many processes, including tempering, dehydration, blanching, cooking, pasteurisation, and sterilisation.

Microwaves induce molecular friction in water molecules to produce heat, whereas infrared energy is simply absorbed and converted to heat. Heating by microwaves is determined in part by the moisture content of the food, whereas the extent of heating by infrared energy depends on the surface characters and colour of food. Some studies were published on the effect of microwaves on food constituents and on the nutrient retention in food. Several publications dealing with certain aspects related to edible fats, such as olive oil or other fats or \( \alpha \)-tocopherol also appeared.

The effect of microwaves and air-drying of grape pomace on the physical and chemical parameters of grapeseed oil were investigated. The results of microwave conditioning demonstrate the impact in producing oil from grapeseeds. Microwave treatment improved oil yield and increased viscosity, peroxide value, saponification value, and content of conjugated dienes. The content of \( \gamma \)-tocotrienol (the major representative of this class in grape-seed oil) increased, while concentrations of \( \delta \)-tocopherol with high antioxidant activity decreased on heat treatment of grapeseed.

Tocopherols and other antioxidants were partially destroyed during microwave cooking of oilseeds. During the microwave heating, tocopherols are destroyed in the order of:

\[
\delta\text{-tocopherol} > \beta\text{-tocopherol} > \gamma\text{-tocopherol} > \alpha\text{-tocopherol}
\]

About 10% were decomposed during the first 6 min of microwave heating, which is the optimum time. During this short period, the lipid fraction was not affected. The degree of tocopherol decomposition increased to 40% during the next 6 min of microwave heating.

The influence of microwave and conventional heating on the quality of lipids in refined cottonseed and hydrogenated palm oils was studied. Exposing the oil samples to microwaves caused some hydrolysis of triacylglycerols into free fatty acids and accelerated the formation of hydroperoxides and secondary oxidation products. In general, the peroxide values of lipids heated by microwaves were nearly twice as high as those produced by conventional heating.

The oxidation stability of lard, rapeseed and sunflower oils was investigated during 10 min microwave and conventional heating experiments. It was observed that the microwave heating accelerates their oxidation two or three times faster than conventional heating. This effect started when the
temperature of examined fat and oils rose over 100 °C. Below 100 °C, the rate of oxidation was relatively slow.

Some attempts were made to minimise the undesirable effect of microwave heating by the use of BHT and natural antioxidants, such as sage and rosemary extracts. Addition of antioxidants was only effective for lard (Fig. 14.1), which contains no natural antioxidants in its native form. For sunflower oil the effect was only slight (Fig. 14.2), no significant effect was observed for rapeseed oil.

The maximum temperature of fat samples was about 155 °C after 10 min of microwave heating, and the same value was adjusted for conventional heating. No undesirable effect was monitored below the temperature of 100 °C.

14.6 Changes during processes where oil is the heat transfer medium

Deep fat frying is one of the most commonly used procedures for the preparation and production of foods. The presence of food moisture and atmospheric oxygen cause various chemical changes at high temperatures, such as polymerisation and degradation, for example. These reactions lead to changes in functional, sensory and nutritional quality of fried foods.
During deep fat frying, food is added to an oil bath preheated at 130–200 °C for 2–10 min. Heat is transferred by frying oil. Changes in antioxidants during frying are listed in Table 14.7. At high frying temperatures antioxidants may be partially lost by evaporation, especially in combination with water vapour originating from water present in fried material. Steam enhances losses of nonpolar volatile material. Synthetic antioxidants, such as BHT or BHA, are lost relatively easily, for example 80 % BHT escaped.

14.2 Oxidation stability of sunflower oil in the presence of antioxidants at 0.1 % (w/w); MH = microwave heating; CH = conventional heating.

Table 14.7 Changes in antioxidants during deep frying

<table>
<thead>
<tr>
<th>Type of change</th>
<th>Precursors</th>
<th>Effect on stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaporation</td>
<td>Water in fried food</td>
<td>Steam distillation of antioxidants</td>
</tr>
<tr>
<td>Oxidation Interchange of frying oil</td>
<td>Polyunsaturated acids, Proteins, lipids</td>
<td>Destruction of phenolics</td>
</tr>
<tr>
<td>with fried substance</td>
<td></td>
<td>Formation of chelating lipoproteins, reduction in protein activity</td>
</tr>
<tr>
<td>Reaction with fried material</td>
<td>Original antioxidants, Lipid oxidation products, Antioxidants, lipids</td>
<td>Decrease in their pro-oxidative activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrease in pro-oxidative activity of oxidation products, but decrease in antioxidant activity</td>
</tr>
<tr>
<td>Polymerisation</td>
<td>Frying oil oxidation products, antioxidants</td>
<td>Activity decrease</td>
</tr>
</tbody>
</table>
during french frying, and BHA almost entirely disappeared during frying of four batches.\textsuperscript{35} BHA does partially dimerise during frying, forming dimers with carbon-to-carbon and ether linkages.\textsuperscript{36} The dimers retain certain antioxidant activity. Because of volatility of common synthetic antioxidants, less volatile synthetic antioxidants suitable as additives to frying oils have been developed. They are more polar, containing hydroxyl groups in side chains, such as a methylol group – CH\textsubscript{2}OH instead of a methyl group, or are dimers or trimers of common antioxidants. Following our opinion, they are not used in industry or are only used at a very limited extent. Losses of natural antioxidants are only small as their volatility is much lower than that of common synthetic antioxidants. They are also changed by oxidation reactions, for example carnosol is converted into miltirone (a quinone) and dehydrorosmarinquinone.\textsuperscript{37}

Another characteristic of deep frying is the low level of oxygen in frying oil. All oxygen originally dissolved in frying oil has already been consumed by oxidation in the time taken to heat frying oil to frying temperatures. Additional oxygen can enter frying oil only by diffusion from air, which is sometimes prevented by special metal cover sheets on the frying bath. Under these conditions autoxidation chains are short so that antioxidants are used up relatively rapidly. While carotene is rapidly oxidised with full access of oxygen, at low oxygen pressure, such as occurs during deep frying, carotenes act as free radical scavengers. Tocopherols in frying oils are decomposed by both direct oxidation with oxygen and by reaction with oxidised fatty acids.\textsuperscript{38} The more unsaturated the frying oil, the more rapid the destruction of tocopherols under frying conditions. The concentration of free lipidic radicals is high in frying oils so that polymerisation is easy between two lipidic free radicals, for example:

\begin{equation}
2 \text{R-O}^* \rightarrow \text{R-O-O-R} \quad [14.1]
\end{equation}

or

\begin{equation}
\text{R-O}^* + \text{R}^* \rightarrow \text{R-O-R} \quad [14.2]
\end{equation}

and also between a lipidic free radical and an antioxidant free radical, such as:

\begin{equation}
\text{R-O}^* + \text{A}^* \rightarrow \text{R-O-A} \quad [14.3]
\end{equation}

which results in higher formation of mixed dimers. The tocopheryl free radicals reacted in three ways under deep frying conditions\textsuperscript{39}:

1. They were oxidised with molecular oxygen into the respective free peroxy radicals.

\begin{equation}
\text{A}^* + \text{O}_2 \rightarrow \text{A-OO}^* \quad [14.4]
\end{equation}

2. They could abstract hydrogen from lipids, forming lipid radicals (this reaction is very slow).
Two tocopheryl radicals could react with one another forming a dimer (reaction [14.6]).

If the concentration of antioxidants is high (it is more common in the case of natural antioxidants), like those of tocopherols in soybean or rapeseed oils used as frying oils, the concentration of antioxidant free radicals increases so that the chance of dimeric compound products being formed by interaction of antioxidant free radicals rises in spite of their low reactivity:

\[ \text{A}^* + \text{A}^* \rightarrow \text{A-A} \]  

[14.6]

Spirodimers and trimers of tocopherol radicals have been detected and these dimers still possess moderate antioxidant activity.

The stabilisation of frying oils with flavonoids or phenolic acids is less efficient as they are only sparingly soluble in oil and their antioxidant activity is thus evident only on interfaces of frying oil and fried material. Destruction of tocopherols was retarded by addition of rosemary extract to frying oil because carnosic acid decomposed faster than tocopherols.\(^{40}\)

Substituted polysiloxanes are often used as a permitted non-toxic additive to frying oils as they prevent the access of air oxygen into frying oil by diffusion. Silicones were used earlier for the same purpose. The concentration of non-oxidised lipid free radicals \( \text{R}^* \) increases because of lack of oxygen and the possibility of their interaction with one another or with an antioxidant free radical increases. Non-polar dimers were detected in frying oils.

During foodservice frying of french fries, tocopherols are oxidised in frying oil, in parallel with oxidation of fatty acids.\(^{41}\) An addition of antioxidants to frying oil reduced losses of tocopherols. The content of ascorbic acid in french fries decreased with decreasing tocopherol content. Citric acid had no synergistic effect on the inhibition of tocopherol decomposition. Catechins were decomposed during the destruction of tocopherols.\(^{42}\) Catechins were destroyed more easily than gallocatechins and their gallates.

In the case of frying fish, polyunsaturated fish lipids are partially released into frying oil, which thus becomes more unsaturated and more easily oxidised. The consumption of both native tocopherols and added synthetic or natural antioxidants thus increases. The sensory value of frying oil and fried products thus deteriorates. Therefore, it is dangerous, with respect to quality, to use frying oil for frying chicken or potatoes immediately after frying fish.

Fried food contains heavy metals in far higher concentrations than frying oil. During deep frying, heavy metal derivatives are partially released into frying oil, where they enhance the deterioration. Fortunately, their activity is only low under frying conditions. Nevertheless, it is advisable to add some metal scavengers to frying oil, preferably citric acid.

In a similar way, an interchange between frying oil and fried food occurs...
in the case of antioxidants. Many food components, such as proteins and phenolics, stabilise food under frying conditions. Fried food rich in lipids and antioxidants (e.g. tocopherols) loses a part of its lipid fraction into frying oil together with antioxidants dissolved in the lipid phase. These antioxidants improve the resistance of frying oil against oxidation, but the stability of fried food on further storage decreases by loss of antioxidants.

Quite a different operation proceeds at extremely high temperatures, i.e. the deodorisation of edible oils. Deodorisation is the last step in oil refining, used for removal of volatile sensory objectionable substances. Fortunately, the process takes place in superheated steam free of oxygen and under substantially reduced pressure, so that the oxidation reactions are excluded or remain negligible. At deodorisation temperatures of 220–250 °C, tocopherols are partially evaporated and are collected in deodorisation condensate. Tocopherol losses depend on the temperature, and may amount to 30 % of the original content. Relative tocopherol losses depend on the degree of methylation of the chroman ring of tocopherols, and on the resulting volatility of tocopherols (Table 14.8).

<table>
<thead>
<tr>
<th>Tocopherol</th>
<th>Alkali refining</th>
<th>Physical refining</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>61</td>
<td>59</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Total tocopherols</td>
<td>64</td>
<td>62</td>
</tr>
</tbody>
</table>

14.7 Changes during processes not requiring heat application

Under very common processing conditions, the reaction proceeds at ambient temperature or at a temperature not substantially different, without or with only negligible heat application.

14.7.1 Effect of smoking and curing meat products

Smoke contains numerous phenolic compounds, produced by pyrolysis of phenolics and lignin. They are mainly bound to meat proteins, but nevertheless, they increase the resistance to oxidation and protect tocopherols against oxidation. Lipoxygenases are partially deactivated in the process.

Curing consists of application of nitrates, nitrites and salt to protect meat against bacterial spoilage, and to prevent browning changes of the natural colour. Nitric oxide is an inhibitor of oxidases, such as lipoxygenases and cyclo-oxygenases, and deactivate haemoglobin by its conversion into
In presence of ascorbic acid (added to protect meat against discoloration), nitrite is reduced to NO, which reacts with ferricytochrome c, forming ferrocytochrome c nitroso derivatives.

Nitrite can be regarded as an antioxidant, reducing the oxidation rate of polyenoic fatty acids, but its activity decreases by long cooking or heating to high temperatures. Cooked ground pork and beef, treated with nitrite was stored at 4 °C, and the antioxidant activity was found to be 1.5–3 times better than in untreated meats.

14.7.2 Changes in antioxidants during fermentation processes
Fermentation processes are enzymatic reactions taking place for a relatively long time at temperatures close to the ambient temperature so that lipids are damaged by oxidation processes to a negligible degree, and thus, antioxidants are not damaged by oxidation as well. Oxygen is partially replaced by carbon dioxide in most fermentation processes.

Hydrolytic processes could cause cleavage of esters or glycosides of phenolic antioxidants into the respective acids or aglycones. They are usually more active as antioxidants than the original compounds, such as quercetin and myricetin, which are more active than the respective glycosides. Polyvalent organic acids with a synergistic activity may be formed.

Proteins are partially hydrolysed into free amino acids, which contribute as synergists to the stability of fermented products against oxidation. Soy, fish proteins, egg albumin and casein were hydrolysed by proteases into products inhibiting lipid oxidation, being synergists of tocopherols. Soybean fermentation can release potent antioxidants from their inactive precursors, for example during the preparation of tempeh or natto.

In food processing, technical enzymes are used to reduce processing costs, to increase yields of extracts from raw materials and to improve the shelf life and sensory characteristics of foods. From the point of view of food deterioration through oxidation there are important oxidases. Glucose oxidase transforms glucose into gluconic acid in the presence of oxygen. It is used to remove sugar, to stabilise egg products, and to increase the shelf life of bottled beer, soft drinks, and other oxygen-sensitive foods. It has an advantage over chemical antioxidants because it does not lose its activity with time, as it is not itself oxidised. Catalase decomposes hydrogen peroxide to form water and oxygen. It is used to provide oxygen for desugar-
tages from the standpoint of antioxidant preservation are compared in Table 14.9.

The most frequent application is to improve the oxidative stability of meat products. The use of antioxidant additives is best combined with adequate packaging techniques. Although muscle contains a multicomponent antioxidant defence system, processing operations alter the oxidative balance of muscle foods. The denaturation of muscle proteins is the main reason for this imbalance. Cooking meat releases iron, and salting changes its distribution. The deleterious effect of salt was recognised early, and encapsulated salt was suggested as a solution. To maintain a balance between pro-oxidants and antioxidants in muscle foods, modified atmosphere packaging, vacuum packaging or oxygen scavengers (such as addition of glucose and glucose oxidase) can help to control oxygen content. The most important step before packaging is to remove oxygen quickly. Additives can decrease the catalytic activity of iron. Decreasing the iron release from proteins is another good method of control. Protecting endogenous antioxidants, and increasing their content through dietary supplements and additives also help to minimise rancidity in meat products.

Foods of both animal and plant origin contain phospholipids which have antioxidant activity in lard or in sunflower oil. The more polar, ethanol-soluble fraction is more active than the ethanol-insoluble fraction. A synergism exists between \( \alpha \)-tocopherol and phospholipids, especially phosphatidylethanolamine, on storage of sardines and mackerel. When tocopherol becomes almost exhausted, rapid oxidation of lipids starts. A possible explanation is a reaction between phosphatidylethanolamine with \( \alpha \)-tocopheryl quinone. Phospholipids decompose lipid hydroperoxides, thus enhancing the stability of stored foods. Phosphatidylcholine is oxidised with formation of trimethylamine oxide, which reacts with another lipid hydroperoxide to form a keto derivative. Both phosphatidylcholine and phosphatidylethanolamine decompose lipid hydroperoxides in a non-radical way.

### Table 14.9 Methods of food storage and their effect on antioxidant functionalities

<table>
<thead>
<tr>
<th>Process</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple storage with free access of air</td>
<td>Low price, simplicity</td>
<td>Easy destruction of antioxidants</td>
</tr>
<tr>
<td>Vacuum packaging</td>
<td>Good protection, when low residual oxygen</td>
<td>High price</td>
</tr>
<tr>
<td>Packaging under inert gas</td>
<td>Good protection, when low residual oxygen</td>
<td>High price</td>
</tr>
<tr>
<td>Use of oxygen scavengers</td>
<td>Satisfactory, but no complete protection</td>
<td>Higher price, regulations should be observed</td>
</tr>
<tr>
<td>Addition of antioxidants</td>
<td>Satisfactory, but no complete protection</td>
<td>Higher price, regulations should be observed</td>
</tr>
</tbody>
</table>

The more polar, ethanol-soluble fraction is more active than the ethanol-insoluble fraction. A synergism exists between \( \alpha \)-tocopherol and phospholipids, especially phosphatidylethanolamine, on storage of sardines and mackerel. When tocopherol becomes almost exhausted, rapid oxidation of lipids starts. A possible explanation is a reaction between phosphatidylethanolamine with \( \alpha \)-tocopheryl quinone. Phospholipids decompose lipid hydroperoxides, thus enhancing the stability of stored foods. Phosphatidylcholine is oxidised with formation of trimethylamine oxide, which reacts with another lipid hydroperoxide to form a keto derivative. Both phosphatidylcholine and phosphatidylethanolamine decompose lipid hydroperoxides in a non-radical way.
Under such conditions, natural antioxidants are rapidly destroyed unless metal chelators are present. In vacuum or inert gas packaging, oxidation processes, and thus destruction of antioxidants is minimised so that their content may be very low. The presence of spices is usually quite sufficient to keep oxidative processes under control. Lipid–protein complexes, formed on storage of meat products, possess moderate antioxidant activity.

Mincing, cooking and maturing expose meat products to oxidative stress for a long time so that larger additions of antioxidants are desirable. In a study on this subject, antioxidant properties of some compounds from vegetable sources, such as catechin, phytic acid, and sesamol were evaluated in two samples of minced, cured high-fat pork products, and compared with antioxidant properties of sodium ascorbate. The results indicated that the natural plant antioxidants slowed down lipid oxidation while sodium ascorbate seemed to have a rather pro-oxidative effect under experimental conditions. Its activity could be influenced by concentration. The conclusion was that the antioxidants tested acted in different ways on lipid and colour stability.

Onion and garlic juices were added to ground lamb and after cooking the mixture was stored. Onion juice was found more efficient than garlic juice in inhibiting the warmed-over flavour, due to slight oxidation and rancidity of the product.

The effects of addition of antioxidants (α-tocopherol acetate and sodium ascorbate) on physical, sensory and microbial properties of buffalo meat nuggets were examined. With increasing duration of storage, the quality of control products decreased, but that of products containing added antioxidants was stable and the stabilised nuggets were acceptable during 30 days of refrigerated storage. This is surprising as α-tocopherol acetate has no antioxidant activity. Therefore, it may be assumed that its cleavage with formation of active free α-tocopherol takes place. The additional use of vacuum packaging further improves the quality of refrigerated storage. In vacuum packaging the oxygen level is very low so that even the low addition of an antioxidant or the presence of weak antioxidants gives satisfactory results. Even carotenes may become active under such conditions.

Another important field of application is the stabilisation of fried products on storage. In fried materials a thin film of frying oil covers the fried product which is exposed to free access of oxygen on storage. The content of antioxidants is usually very low in frying oil as antioxidants have been consumed during frying. Even when fried products have been packed under nitrogen or in vacuum, they are still not sufficiently stable on storage, being attacked by residual oxygen in the atmosphere or dissolved in the fried material. The oxidation is much faster after the packaging has been opened. It is advisable to add antioxidants to the surface of fried food, for instance, to spike fried snacks with ground spices possessing antioxidant activity.
Vegetable products contain natural antioxidants so that further additions of antioxidants are mainly unnecessary. Other examples of antioxidant application in products of vegetable origin are given in Chapter 15. The effects of packaging in a modified atmosphere and of cooking were evaluated in fresh-cut spinach, which contains both flavonoids and vitamin C. The content of total flavonoids remained fairly constant during storage both in air and in a modified atmosphere, while vitamin C was better preserved in a modified atmosphere. A decrease in total antioxidant activity was observed during storage, particularly in spinach stored under modified atmosphere, which proves that antioxidants were gradually consumed by free radical scavenging. Boiling extracted 50% total flavonoids and 60% vitamin C in the cooking water, which could be used for further processing. Both ascorbic acid and total polyphenols were significantly destroyed during cold storage at +5°C for 6 months.

Wine flavonoids and anthocyanins are relatively stable in closed bottles but are easily oxidised once the bottle has been opened. The oxidation products easily polymerise. The flavour of wine, especially of red wine, is very much influenced by these processes so that wine should be consumed on the same day as the bottle is opened. Ascorbic acid added to extracts of elderberries and red grape skin caused higher degradation of anthocyanins during their storage for 6 months due to their reduction to colourless leucoanthocyanidins.

Changes in antioxidant properties of green and black tea infusions as a result of processing and storage were evaluated by measuring their chain-breaking activity, oxygen-scavenging activity and redox potential. The results showed that pasteurisation, storage and forced oxygenation caused increased browning in both green and black tea extracts. These changes were accompanied by increasing colour intensity. Green tea catechins and residual catechins of fermented black tea were obviously oxidised into the respective quinones, which were then dimerised. The dimers possessed similar chemical structures as black tea pigments – theaflavin and thearubigin.

Not only lipids, but also other lipophilic components are oxidised on storage, such as essential oils. The composition of products of their oxidation, including the ratio of cis and trans isomers, is influenced by natural antioxidants in food material, such as rosemary extracts. Similarly to oxidised lipids, oxidation products of essential oils also react with food proteins, and special sensory active reaction products are formed. The relative activity of natural antioxidants (expressed as protection factors or antioxidant indices) is only moderate in mixtures of lipids with proteins, and similarly in mixtures of essential oils with proteins, being much lower than in systems containing no proteins.

Although the loss of nutrients as a consequence of food processing and storing has been widely documented, relatively few data have been available up until now on the potential interactions of natural (and of course,
synthetic) antioxidants with other food components during industrial food processing, household cooking, and storage both in distribution and in households.

14.8 Future trends

Changes in synthetic antioxidants during food processing and storage are relatively well known, as are the interactions of their oxidation products with other food components. However, modern consumers ask for natural products, free of synthetic additives. Therefore, the application of natural antioxidants will probably continue even in future, and it will be necessary to study their changes and interactions in more detail. The best known and most widely used compounds with antioxidant activity will remain tocopherols, carotenoids and ascorbic acid. They have been the object of numerous studies, but usually only their changes in edible fats and oils, and in model systems were investigated. Their behaviour in such complex mixtures as foods and ready meals is far less well understood. Plants such as rosemary, sage, thyme, savory, marjoram, nutmeg and ginger contain different phenolic compounds possessing antioxidant activities. Their structures and antioxidant activities were studied in detail, but the course of their oxidation and their interactions with other food components should also be studied in future.

Vitamin E and carotenoids belong to natural antioxidants, which are deposited in adipose and other tissues of farm animals so that their fat and meat become more stable. The economics of this process should be evaluated from complex aspects, as until now it seems that the application of antioxidants after slaughtering and during processing of food materials is cheaper and more efficient.

Consumers believe that foods rich in antioxidants may afford a degree of protection against free radical damage not only in foods, but also in the human body, protecting against cardiovascular diseases, damage of nucleic acids, and other deteriorative processes. The absorption of tocopherols and carotenoids into the blood stream is well known, but much less has been published on the fate of other antioxidants and their reaction products. Some antioxidants may not be resorbed in the intestinal tract at all, even when they are active in foods.

A concerted effort should be made to eat a well-balanced diet, which includes a range of foods and beverages rich in natural antioxidants, such as fruit, vegetables, cereals, nuts, tea or wine. It is a very serious challenge for the food industry to produce a new generation of food products with enriched content of natural antioxidants. The question is open, however, as to how much such food would make people really healthier and happier. But this is probably not the task of food scientists and technologists.