Antioxidants in food systems. Mechanism of action

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ABSTRACT

The mechanisms of action of natural and synthetic antioxidants in food systems including lipids, proteins and carbohydrates have been discussed. It is essentially important and very useful in prediction of the antioxidants effectiveness in the processes of food storage. The main proposed mechanisms through which the antioxidants may play their protective role, including free radicals inactivating, the hydrogen atom transfer, prooxidative metals chelating, the single electron transfer, quenching of singlet oxygen as well as photosensitizers and lipoxygenase inactivation, have been analyzed and discussed in details. The majority attention was given to the antioxidants mixtures and most effective synergists.

Introduction

The main adverse effect of food oxidizing is a change in sensory quality, particularly development of off-flavors and toxic compounds, rancidity, vitamins destruction, color and food quality loss [1-3]. It is well known that lipid-containing food oxidizing mediated by free radical driven chain reactions, which involve alkyl $R^{\bullet}$, alkoxyl $RO^{\bullet}$, peroxyl $ROO^{\bullet}$ radicals and active forms of oxygen – singlet oxygen and superoxide anion radical [1-4]. The mechanism of reaction can be divided into the three stages: initiation, propagation and termination. On the first stage of oxidation reaction from biological systems XH are formed radicals $X^{\bullet}$ as a result of abstraction of a hydrogen atom $H^{\bullet}$:

$$XH \rightarrow X^{\bullet} + H^{\bullet}$$

After initiation, propagation of free radical chain occur, in which molecule of oxygen from environment react with reactive radical species, resulting in formation of peroxides and peroxyl radical $XOO^{\bullet}$. These intermediates may further propagate free radical reactions:

$$X^{\bullet} + {}^3O_2 \rightarrow XOO^{\bullet}$$

$$XOO^{\bullet} + XH \rightarrow XOOH + X^{\bullet}$$

$$XOOH \rightarrow XO^{\bullet} + HO^{\bullet}$$

On the last stage interact two radicals which may lead to formation of nonradical adduct and termination of free radical chain:

$$X^{\bullet} + X^{\bullet} \rightarrow X - X$$

Thus termination result in interrupting the sequence of chain reactions and lead to a significant decrease of the total reaction rate.

Results and discussions

Oxidizing factors. Oxygen activation

The molecules of oxygen are the main source of oxidizing in the food systems [1]. The other strong oxidants include hydrogen peroxide, benzoyl peroxide, potassium bromate, which consist atoms of Oxygen. These compounds either contained in food or accumulated during food processing.

The reactions between molecules of oxygen, which normally are in the ground state ($^3O_2$), and organic compounds proceeded very slowly because of their high energy of activation, although they are thermodynamically favorable. In the ground state the molecule of oxygen consists two electrons on the outer shell and gave triplet signal in the magnetic field*. The chemical bonds in the molecules of organic compounds are formed by means of pair of electrons with opposite spines on one orbital (singlet state). Therefore, a direct reaction between molecules of organic compounds and oxygen is highly improbable because of incompatibility or conflict with spine states. Since the values of energy of activation of organic compounds oxidation by triplet oxygen are within the range 146–273 кJ/mol, these reactions are hardly probable during food processing.

An activation of triplet oxygen, containing two unpaired electrons on the outer orbitals $2p_y$ та $2p_z$, as an oxidant in redox reactions, consume too much energy. A one approach of oxygen activation is to transfer molecule of oxygen from ground ($^3O_2$) to excited singlet state [2,3]. Another form of singlet oxygen (fig. 1) has a lesser lifetime and doesn`t play an active role in oxidation processes [2]. The others active species of oxygen, which formed in result of reduction of oxygen triplet state, included superoxide anion-radical ($O_2^{\bullet-}$), its conjugated acid ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical $OH^{\bullet}$.

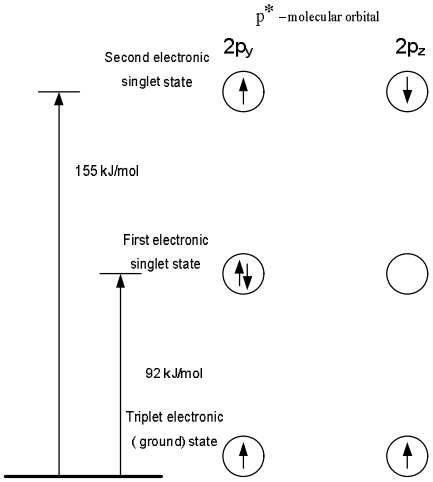


Figure 1. The scheme of formation of oxygen active forms

* Generally, each level of non-zero spin shifted into 2I+1 sublevels, where I is a summary spin.

The strongest electrophilic agents and active forms of oxygen are $^1O_2$ and $OH^{\bullet}$. [4].
The singlet oxygen often reacts with fatty acids by the cycloaddition mechanism (fig.2).

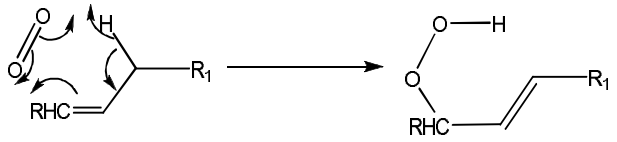


Figure 2. The mechanism of cycloaddition of singlet oxygen to a molecule of fatty acid

Some of these active species of oxygen can convert into others in the presence of specific catalysts. They also formed as a result of γ-radiation, light absorption by photosensitive pigments which contain food systems e.t.c. [4].

Catalysts of oxidation in food systems

The catalysts of the reactions of oxidation divided into the two groups — enzymatical and nonenzymatical. Enzymatical catalysts usually cause oxidation in particular biological objects [2,3]. For example, enzymes lipoxygenase, polyphenoloxidase, sulphhydrileoxidase and xantinoxidase, which generally can be found in food products, cause oxidation of unsaturated fatty acids, mono- and diphenyl- containing acids, fragments of cystein and xantine respectively. Glucoseoxydase converts glucose into gluconic acid as well as produces H_2O_2. Xanthinoxydase and peroxydase are able to produce H_2O_2, $O_2^{\bullet-}$ та $^1O_2$ respectively [2,3].

Cations of transitional metals are easily interacted with oxygen with formation of $O_2^{\bullet-}$ /$HO_2^{\bullet}$:

$$M^{n+} + {}^3O_2 \rightarrow M^{(n+1)+} + O_2^{\bullet-}$$

The superoxide radical $O_2^{\bullet-}$ can initiate an oxidation reactions. Transitional metals ion simulated oxidation of lipids by the cleavage of hydroperoxides (LOOH):

$$M^{n+} + LOOH \rightarrow M^{(n+1)+} + LO^{\bullet} + OH^{-}$$

Alkoxyl radicals $LO^{\bullet}$, formed by reaction described above, accelerate reactions of oxidation. The former process is very slow because of low concentration of LOOH in food products. Cations of transitional metals are also contributors in active oxygen species interactions by Huber-Weiss reaction:

$$O_2^{\bullet-} + H_2O_2 \rightarrow {}^3O_2 + OH^{-} + OH^{\bullet}$$

The interaction is accelerated by means of three intermediate reactions [4,5]. On the first stage metals cations subtract electron from $O_2^{\bullet-}$, which act as a reducing agent. The former compound simultaneously playing a role both oxidizing and reducing agent on the second stage, promoting oxygen and oxygen peroxide formation.

On the third stage cations of transitional metals, primarily Fe^{2+}, induce lipid oxidation, favoring reactive radical $OH^{\bullet}$ formation [5] through the Fenton reaction (reaction 3). The formation of oxidized cations leads to reinitiating the lipids oxidation by reaction with superoxide anion radical:

$$O_2^{\bullet-} + M^{(n+1)+} \rightarrow {}^3O_2 + M^{n+}$$

$$2O_2^{\bullet-} + 2H^{+} \rightarrow {}^3O_2 + H_2O_2$$

$$H_2O_2 + M^{n+} \rightarrow M^{(n+1)+} + OH^{\bullet} + OH^{-}$$

Ascorbic acid and thiols, which are consisted in several food systems, can act as a reducing agents instead of $O_2^{\bullet-}$.

The others nonenzymatical catalizators included photosensitive pigments of food products. The absorption of light in visible or UV by photosensitizers leads to the transfer of these compounds to its electronically excited triplet state. Subsequently the formers are able to transfer their energy excess on the molecules of oxygen and others biological components. The energy transfer to organic compounds favoring by some pigments lead to the oxygen and hydrogen peroxide formation from $O_2^{\bullet-}$. The photosensitizers are able to convert triplet oxygen to the singlet form. Several of these compounds, such as riboflavin and chlorophyll are containing in food products [2,4,5].

Lipids oxidation

It is well known that fats (oils) stability during storage has rapidly decreased in the presence of light. It caused the lipids autooxidation. Certain compounds, are so-called sensitizers, favoring this process.

Sensitizers are divided into the two groups — so called type I and type II. The activated by light sensitizers of the type I are directly react with substrate, generating free radicals. The sensitizers of type II are transfer molecule of oxygen from ground to the excited (singlet) state $^1O_2$. There is a competition between these two processes in the photoxidation reactions, which depend on nature of sensitizer and substrate, and the formers concentration.

Fats rancidity is a common effect and one of the most known cases of the food deterioration, caused by autooxidation.

Polyunsaturated fatty acids, which containing 1,4-pentadienic functional fragments are particularly sensitive to oxidation*. For example, linoleic acid oxidation, which consisted in several foods, realizes by two main mechanisms — abstraction of the atom of hydrogen and singlet oxygen addition (fig.3).

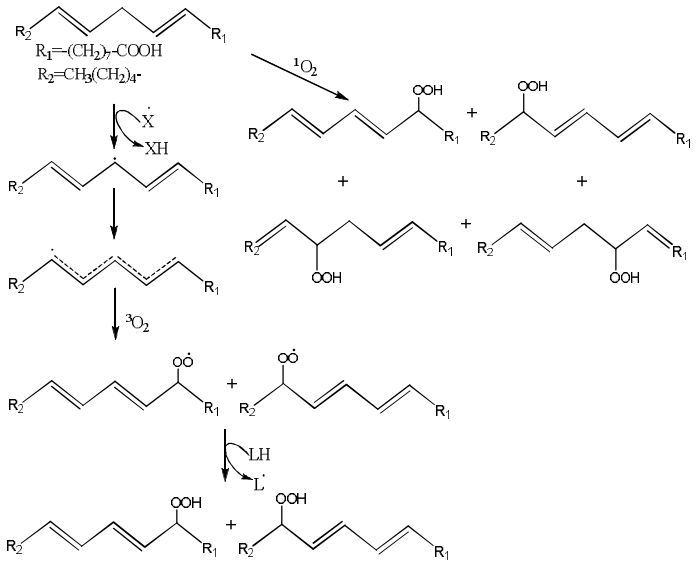


Figure 3. Initial stages of linoleic acid oxidation. Adapted from [4]

* Lipids peroxidation in human organism is an important factor, which is necessary for the prostaglandins, leukotriens, biologically active compounds biosynthesis e.t.c.

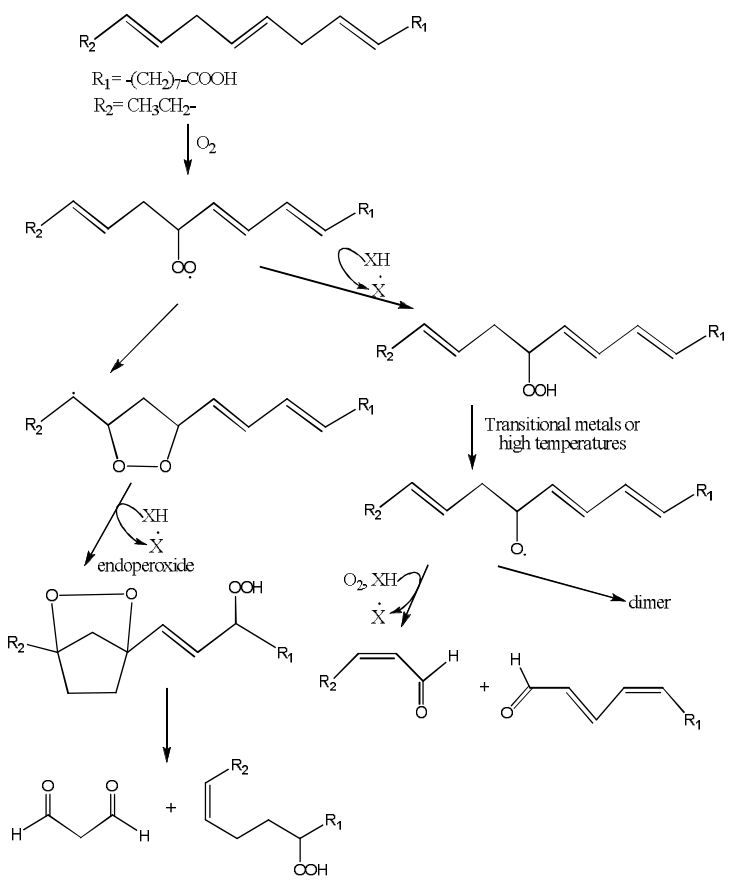


Figure. 4. Mechanism of linolenic acid oxidation. Adapted from [4]
* Lipids peroxidation in human organism is an important factor, which is necessary for the prostaglandins, leukotriens, biologically active compounds biosynthesis e.t.c.

The processes of secondary oxidation have arisen as a result of the further oxidation of double bonds or formation of oxidized polymers. The cleavage of oxidized fragments of fatty acids leads to the formation of low molecular aldehydes and ketones [4,5]. These volatile products of secondary oxidation are responsible for appearance of undesirable off-flavors, which are the indicators of oxidation of foods or oxidative rancidity. In some cases, when specific secondary products were formed by enzymatical reactions, as an example at the hydroperoxydliases action in sliced fresh tomatoes and cucumbers, an aroma of volatile compounds appears to be a very pleasant [2,4].

Thermally induced reactions of oxidation may occur either with saturated and unsaturated lipids at the temperatures of frying process. Therefore, an oxidation has mainly occurred due to the initial formation of hydroperoxides. The high temperatures give rises to reactions of isomerisation and decomposition, resulted in the formation of products of secondary oxidation, including epoxides, dihydroperoxides, aldehydes and ketones e.t.c. [4,5].

Malonic dialdehyde is a one of the most widespread end product of fats oxidation. It is often used is an indicator of oxidation degree of food lipids. It is accumulated in human organism due to the polyunsaturated fats decomposition, which has induced by action of active species of oxygen and serves as a marker of oxidative stress. It is well known that malonic dialdehyde is able to interact with DNA, forming mutagenic adducts.

Proteins oxidation

The food peptides, aminoacides and proteins are oxidized during food processing [1-5]. It is considered that methionin, cystein (cystin), hystidin, tryptophan and in certain conditions tyrosin, serin and treonin are the most the sensitive aminoacids, which susceptible to oxidative decomposition. Proteins and aminoacids oxidation induced by action of light, γ radiation, peroxidized lipids, metals, products of enzymatic and nonenzymatic reactions and food ingredients, such as hydrogen peroxide, benzoyl peroxide, bromates and azodicarbonamide. The action of hydrogen peroxide on proteins resulted in the methioninsulfoxide oxidation (reversible) and further oxidation with methioninsulfone production (irreversible).

Cystein is an aminoacid, containing in foods of animal origin. This aminoacid favor food digestion, participated in reactions of interamination, removing several toxic compounds from human organism and protecting from deleterious radiation. It is one of the strongest natural antioxidants, which action increased in the presence of vitamin C and selenium. Cystein is a component of glutathione, which protect liver and brain cells from injuries, caused by alcohol, certain medicines, and tobacco toxic compounds.

Certain functional groups of cysteine could be oxidized by peroxides and active oxygen species with formation sulphenic (Cy-SOH), sulphinic (Cy-SO_2H) and sulphonic (Cy-SO_3H) acids. The cystein oxidation resulted in the formation of mono-, di-, three- and tetrasulphoxides (fig. 5) [4].

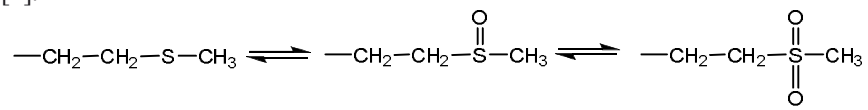


Figure 5. Scheme of oxidation of cysteine functional groups

Free thiol groups of proteins either rapidly oxidized by environmental oxygen with formation of cross-linked disulphidic bonds or catalize thiol-disulphidic interaction resulted in the proteins polymerization. Oxidizing agents, as an example $KBrO_3$, or azodicarbonamide often utilized as the additives to wheat flour with purpose to improve properties of dough. It is considered that these compounds oxidized and blocked thiol groups of proteins and nonprotein components and thus prevented thiol-disulphidic interactions in dough. Redox reactions, occurring in dough, have been modulated by addition of ascorbic acid, dehydroascorbinic acid and glutathion.

The presence of photosensitizers, such as riboflavin and chlorophyll, may cause the oxidation of certain aminoacids, including hystydin, cysteine, methionin, tryptophan and tyrozin by active oxygen species $O_2^{\bullet-}$, H_2O_2 та $^1O_2$ due to the absorption of light.

Hydrogen peroxide has formed due to the water radiolysis caused by γ radiation of food products resulted in the oxidative changes of proteins. Tryptophan fragments of proteins may oxidize in case of presence of acid [2,4,5].

Peroxidized lipids play a major role in the oxidation of free aminoacids and fragments of proteins. Methionin, cystein, hystidin and lysin are the most sensitive aminoacids. The proteins and peroxidized lipids interact by two main mechanisms —first included participation of alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals, and another occur due to the action of malonic dialdehyde and other carbonilic compounds. By the first mechanism free radicals of lipids react with proteins (P) resulted in the formation of protein radicals ($P^{\bullet}$), that cause polymerization of proteins molecules. Free radicals of lipids favor oxidation of methionin, cystein and tryptophan as well. Active dialdehyde of malonic acid, formed from peroxidized lipids, react with aminogroups of lysine fragments, which leads to crosslinking [2-4].

$$RO^{\bullet} + P \rightarrow ROH + P^{\bullet}$$

$$ROO^{\bullet} + P \rightarrow ROOH + P^{\bullet}$$

$$P^{\bullet} + P \rightarrow P\text{-}P^{\bullet}$$

$$P\text{-}P^{\bullet} + P \rightarrow P\text{-}P\text{-}P^{\bullet}$$

Thermal treatment of certain proteinious food products can cause oxidative changes of proteins. While moderate thermal treatment give rise to proteins denaturation, higher temperatures resulted in undesired chemical changes of aminoacids and complex reactions between proteins and other compounds, containing in foods, particularly with carbohydrates and lipids.

The thermal treatment at the temperatures more than 300 °C, which is usual in the frying and grilling processes, resulted in the thermal decomposition and pyrolysis of certain fragments of aminoacids. Several of these compounds are extremely mutagenic. Most of mutagenic (carcinogenic) compounds formed due to the decomposition of fragments of tryptophan, glutamate and lysine.

Oxidation of carbohydrates

Carbohydrates are not as sensitive in reactions of oxidation as proteins and lipids, and the end products are not volatile. Oxidation of food carbohydrates, particularly in Maillard and caramelization reactions, generally occur at high temperatures [2,4,6].

Carbohydrates oxidation may occur in food products due to the enzymatical reactions. The enzyme glucose oxidase catalyze glucose oxidation yielding gluconic acid and simultaneously reduces oxygen resulted in formation hydrogen peroxide. Commercial enzyme glucose oxidase is effective at removing glucose concentration (in order to prevent nonenzymatical browning in dry egg-white manufacture) as well as to decrease oxygen pressure decrease (favoring salad dressing stabilization against oxidative deterioration).

The mechanisms of carbohydrates free radical oxidation are similar to those of lipids. It is well known that low molecular carbohydrates, including glucose, mannitol, deoxyribose interacted with $HO^{\bullet}$, producing oxidized intermediates, which doesn`t influenced on the foods quality.

Caramel formation is an example of useful oxidation, occur due to the carbohydrates treatment at high temperatures. These transformations as well as Maillard reaction resulted to the brown pigments and volatile compounds formation [6]. Caramel, which has been formed during sucrose heating with sodium hydrosulfite is utilize in nonalcoholic beverages, such as Coca Cola, Diet Cola and others, and also in bakery and confectionery products as an ingredient contributing in color and aroma.

Mechanisms of action of natural antioxidants

The main method of foods protection against oxidation is an overall utilization of special additives, inhibiting this process. Antioxidants delayed the rate of food oxidation by several mechanisms: playing a role of free radical scavenger (favor entrapment of radicals $R^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$, $HO^{\bullet}$ e.t.c.), formation of chelate complexes with prooxidant metals, singlet oxygen and photosensitizers quenching, suppression of radical $NO^{\bullet}$ accumulation, peroxydinitrite and lipoxygenases deactivation. Certain compounds, which are called synergists are not the true antioxidants, but can increase an activity of other antioxidants [2,4-7].

Antioxidant activity depends on many factors in particular on lipids nature, antioxidants concentration, temperature, oxygen pressure, the presence of other antioxidants, water and nature of compounds of food products, mainly proteins. The antioxidants were first used after Second World War in order to increase storage stability of foods [8]. These compounds were natural compounds, which were gradually displaced on synthetic. Most of natural antioxidants usually contained in food products, which were consuming thousands years. Thus, it is considered that human organism has adapted to them.

Free radical scavengers

Antioxidants entrapped free radicals containing in foods by hydrogen binding, as well as producing relatively stable radicals with antioxidant properties, which characterized low reducing potential (less than 0,5 V) [7]. The increased stability of these antioxidant radical compared to those contained in foods has been linked to the resonance delecolisation of structures containing phenolic ring or sterical hindering of active sites with bulk substitutes [7]. The examples tocopherols, butylated hydroxytoluen (BHT), butylated hydroxyanisol (BHA), tret-butylhydroquinone (TBHQ), propylgallat (PG), lignines, flavonoids, phenolic acids, ubiquinone (coenzyme Q), carothenoids, ascorbic acid and aminoacids related to phenolic compounds, which serve as the effective free radical scavengers [7].

Polyphenols being primary antioxidants inactivated free radicals by mechanisms of atom of hydrogen transfer (AHT) and single electron transfer (SET). On the first mechanism an antioxidant ArOH interact with free radical $R^{\bullet}$ as a result of transfer of atom of hydrogen due to dissociation O-H bond (fig. 6.) [9].

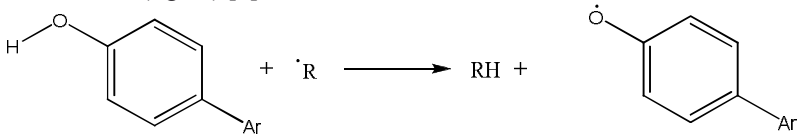


Figure 6. Mechanism of action of free radical scavenger by hydrogen transfer

The end products of this reaction are compounds with total formula RH, which more safe, than corresponding primary radical and oxidized $ArO^{\bullet}$ radical as well. Even if reaction leads to the formation other radicals, they were less reactive compare to $R^{\bullet}$, due to the different effects of stabilization [9].

On the SET mechanism single electron transferred to a free radical or from this radical on the acceptor as follows (fig. 7):

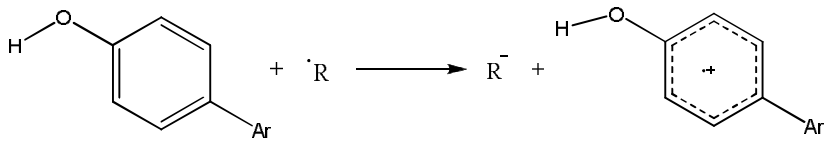


Figure 7. Mechanism of single electron transfer

Anion R^- is an energetically stable intermediate with even quantity of electrons, and the activity of cation-radical, formed by above mentioned reaction is also reduced [2,7,9]. The aromatic structures $Ar^{\bullet}$ та $ArOH^{\bullet+}$, formed during free-radical reactions contain unpaired electron, delocalized by aromatic structure, which contributes to stabilization of these compounds [9,10].

The enthalpy of O-H bond dissociation is an important factor in the above mentioned mechanism. The lesser ΔH value the easier dissociation and thus the greater reaction rate with antioxidants. The alternative mechanism of interaction is SET, in which radical can act both as an acceptor and donor of electrons as follows [1,2]:

$$R^{\bullet} + XH \rightarrow R^{+} + XH^{\bullet-}$$

$$R^{\bullet} + XH \rightarrow R^{-} + XH^{\bullet+}$$

The bond O-H dissociation energy of phenolic antioxidants affected on the stability of corresponding antioxidant radicals: the lower it value the more stable antioxidant radical [7]. Therefore, the better antioxidants are those, which characterize by low energy dissociation of O-H bond. The strength of this bond of phenolic antioxidants depends on nature of substituents of benzolic ring. The antioxidant activity of phenol-type antioxidants depends on balance between electron donor effect of substituents and possible steric hindrance around O-H group caused by them [7]. The functional groups, which destabilize ground state of antioxidants or/and stabilize phenoxyl radical form, reduced strength of O-H bond. The intramolecular hydrogen bonds formation between phenolic hydrogen and oxygen-containing substitutents, such as methoxy- groups in ortho position stabilize phenolic ring, and thus prevented the reducing of O-H bond strength. The presence of alkyl substituents or OH groups enhances the stability of the antioxidant radicals leading to increased phenols activity as free radical scavengers. Substitution with one methyl, tret-butyl or methoxy group at the ortho position decreases the energy of O-H bond on 1,75; 1,75 and 0,2 kcal/mol, whereas substitution with these functional groups of hydrogen atom in metha position of phenolic ring reduced bond strength on 0,5 kcal/mol.

The bond O-H energy dissociation of phenolic antioxidants lies in the range of 70-80 kcal/mol and for tocopherols decreases from δ > γ > β > α-tocopherol [9,10]. It depends on solute nature – higher for polar solutes, such as acetonitryl (ε = 37) and tret-butyl alcohol (ε = 12,3), than for nonpolar, like benzene (ε = 2,2) [11]. Therefore an antioxidant activity as free radical scavengers being diminished by polar solutes due to formation of intramolecular hydrogen bonds between atom of oxygen or nitrogen and OH- group of phenolic antioxidants [7,12]. The OH-bond strength additionally is affected by double substitution of hydrogens at ortho position. Electron acceptor groups, like -COOH and -COOR in para position, enhance phenolic antioxidants stability and destabilize phenoxyl radical forms, increasing OH bond strength and thus reducing antioxidant activity [13]. The phenoxylic radicals are significantly stabilized if the substitute in para position is an unsaturated hydrocarbon with highly

delocalized electron. The hydrogen-donor ability of natural antioxidants in olive oil decreases as follows: hydroxytirosol, oleiropein, caffeic, chlorogenic, ferulic acid [14].

In the SET mechanism the most important factor of the reaction rate is an ionization potential or electrode potential: the lower it value the easier electron detachment and correspondingly the faster reaction rate with free radicals.

Antioxidant activity of phenolic acids, particularly caffeic, procatechinic and chlorogenic depends on pH; in acidic environment their effectiveness of free radical scavengers is very little, whereas in pH 7-8 their activity significantly increased [7,15]. In alkaline media phenolic acids ionized with phenolate formation. The antioxidant capacity of the above compounds greater than common phenolic compounds, that enhanced their antioxidant activity [13]. The last has been caused by fast electron transfer from anions of phenolic acids to the peroxyl radicals of lipids [7,14,15].

One of most strong phenolic antioxidant is a chlorogenic acid, contained in coffee beans. Consumption of the above compound may prove beneficial in diabetes type 2, certain types of cancer and cardiovascular disease prevention [16,17]. The bulk quantity of coffee is harmful for pregnant women and those, suffering from hypertension, ischemic heart disease, gastritis and others. Furthermore it may cause dependence on instant coffee lovers.

Tirosol and hydroxytirosol, contained in the olive oil as well as sesamol and sesaminol, which consisted in sesame oil scavenge free radicals according to the mechanism similar to that of tocopherol due to the presence of phenolic ring.

Reduction potential of antioxidant radicals is very useful to predict the ease of atom hydrogen transfer from certain compound to free radical; the lower reduction potential the higher ability of antioxidant to act as a donor of hydrogen atom [7]. Each compound having a lower reduction potential than food radicals may act as a hydrogen donor and thus it gain properties of antioxidant. The reduction potential of hydroxyl, alkyl, alkoxyl, alkperoxyl and superoxide anion-radical is approximately 2,3; 0,6; 1,6; 1,0 and 0,94 V, respectively [7]. The reduction potential values of tocopherol, ascorbic acid and quercetin radicals are 0,5; 0,33 and 0,33 V respectively, which are lower than peroxyl, alkoxyl and alkyl radicals [18]. Thus, food radicals readily abstract hydrogen atom from molecules of ascorbic acid and tocopherol, leading to the inhibition of free radicals formation. Phenolic compounds may play a role of the donor of hydrogen atom for alkperoxyl radicals, and the new radical, which were formed doesn`t catalize an oxidation of other molecules, due to the low reduction potential [6]. Phenolic radicals react with other phenolic radicals with hydroquinone formation and phenolic antioxidants regeneration or resulted in the phenolic dimmers formation. Phenolic radicals can also interact with lipid peroxyl radicals, which lead to phenolperoxides formation, which further being decomposed [7].

Hydrogen atoms of phenolic rings of tirosol and hydroxytirosol molecules being coupled to food radicals give rise to the semiquinon radical formation. These intermediates can react with other radicals resulted in the quinon formation, while disproportionation between two radicals lead to the quinon and corresponding compound (tirosol and hydroxytirosol) formation, or being interacted with molecule of oxygen producing quinon and hydroxyperoxyl radical (fig.8). The phenol-semiquinon-quinon system acts as a ascorbic acid synergetic and play a significant role in redox equilibrium maintenance [19].

Flavonoids, particularly quercetin and luteolin are potential inhibitors of xanthinoxidase, which incorporated into the processes of oxidative injuries, especially after ischemic reperfusion since superoxide radical being produced as a result of interaction with molecular oxygen [9,20].

The property of flavonoids to depress growth of cancer cells is also related to their free radical scavenger function [21]. The growth of cancer cell being inhibited by flavonoids, which also can cause apoptosis of these cells [9,22].

Flavonoids must have characteristic structural features, particularly orthohydroxy or catechol groups in B-ring in order to scavenge free radicals (fig. 8) [7,23]. Quercetin, rhutin and luteolin are completely fulfilled with these requirements and being known as the most effective free radical scavengers. Catechol is an effective scavenger of free radicals, which doesn`t consist 2,3-double bond and 4-carbonyl group, but due to the number of hydroxyl groups, which are the source of hydrogen atom, also served as an acceptor of free radicals [23]. Flavonoids, having structures similar to that of catechol, capture lipid peroxyl radicals, which, in turn can abstract hydrogen from flavonoid, yielding more stable phenoxyl radicals. The latter undergo reaction of disproportionation, producing phenolic quinone and dihydroxyphenolic compound (fig. 8.) [7].

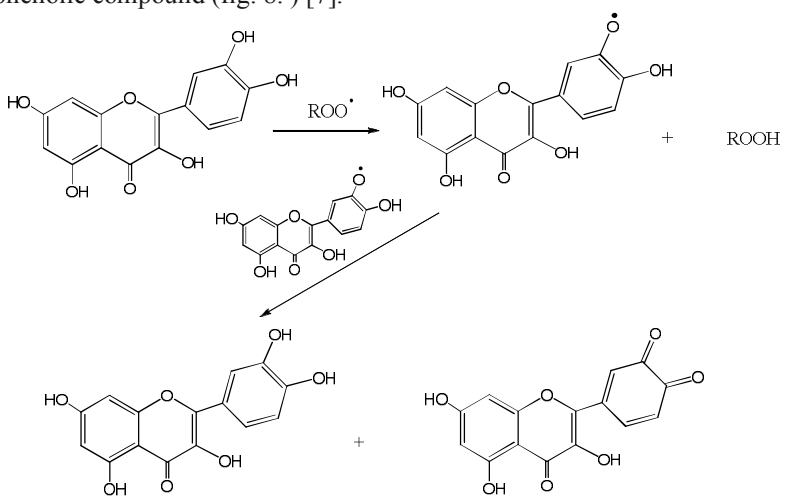


Figure 8. Reaction between flavonoid catechol and peroxyl radicals. Adapted from [20]

α-Tocopherol react with alkylperoxyl radicals much faster than with alkyl, because of the difference between reduction potential of tocopherol radical and alkylperoxyl radical (0,5 V), which is a bigger than reduction potential value between tocopherol radical and alkyl radical (0,1 V). Tocopherol act as a donor substrate of hydrogen atom of 6-hydroxy from of chromanolic ring alkperoxyl radical, resulted in the alkylperoxide formation and give rise to the realatively stable tocopherol radical due to the resonance structure of its molecules. Further it may being dimerised or interact with lipid peroxyl radicals to obtain tocopherol semiquinon, which is not as active as vitamin E (fig. 9) [7]. Tocopherols can slowly and irreversibly react with superoxide anion radicals, but this process is not significant in aqueous solutions.

At high concentrations lipid peroxyl radicals react with tocopherols give rise to the tocopherolperoxide, which produced two isomers of epoxy-8α-hydroperoxytocopherons as a result of atom hydrogen abstraction by alkoxyl radical. These isomers undergo hydrolysis to form epoxyquinones, giving rise to the alkoxyl radicals instead of peroxyl with a loss of tocopherol. There was no significant decrease in quantity of the radicals, which lead to gradual

loss of a tocopherol activity. The tocopherol can be regenerated from tocopheryl quinone by the reducing agents addition, for example ascorbic acid [7].

Hydrogen atom being bounded with tocopherol radicals at their high concentration and low quantity of peroxyl radicals, but the reaction rate is slow and as a result of reaction tocopherol and lipid radicals are accumulated [7,25]. Lipid peroxyl radicals by this reaction can accelerate lipids peroxidation by interaction with triplet oxygen, whereas tocopherol act as a pro- rather than an antioxidant in this case. This type of lipid peroxidation, caused by tocopherol species would be inhibited by addition of ascorbic acid, which act as a reducing agent [26].

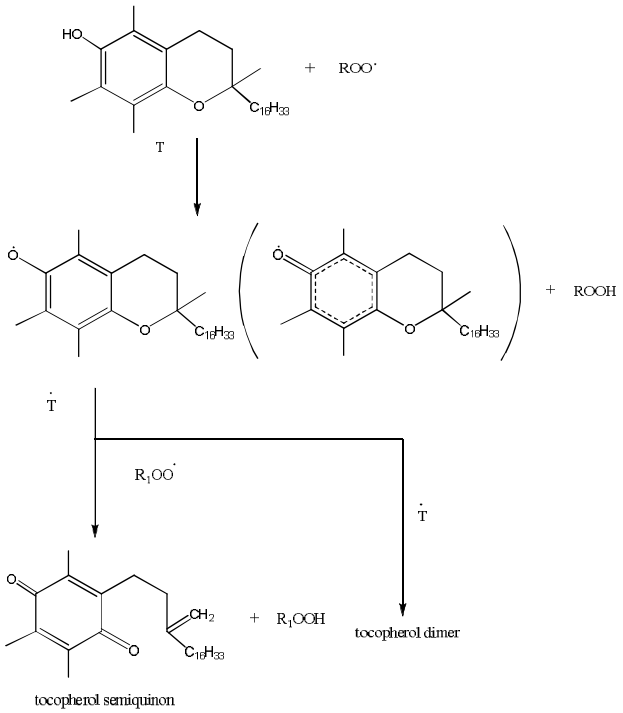


Figure 9. Reaction between α-tocopherol and lipid peroxyl radicals. Adapted from [24]

Carotenoids are the group of the most effective antioxidant, which are abundant in nature [2,7,27]. It has been known, that these compounds lose their color, than exposed to radicals or to oxidized species due to the interruption of conjugated double bonds system. The carotenoid crocin contained in the plant saffron. Lose of color of this water-soluble carotenoid serves as a measure for determining antioxidant capacity in serum plasma and plant extracts. One of the most potent product of carotenoid oxidation is retinoic acid, which participated in the processes of bones synthesis and embryo development, but it is considered a potent teratogen. There are at least three possible mechanisms for the reaction of carotenoids with radicals: 1) radical addition; 2) electron transfer to the radical; 3) allylic hydrogen abstraction [27].

1. Radical addition*: adduct formation.* Burton and Ingold first proposed the mechanism of addition reaction [28]. They supposed that lipid peroxyl radical $ROO^{\bullet}$ would added to carotenoid polyene chain (CAR) with radical $ROO\text{-}CAR^{\bullet}$ formation. Since this radical would

be resonance stabilized it would further interact with lipid radicals that would accounts for the antioxidant effect of carotenoids in solution:

$$ROO^{\bullet} + CAR \rightarrow ROO - CAR^{\bullet}$$

However, the subsequent reactions of ROO-CAR$^{\bullet}$ radical are not well understood. Antioxidant activity of carotenoids depends on oxygen tension. Therefore peroxyl radical-carotenoid adduct ROO-CAR$^{\bullet}$ could reversibly react with molecular O_2 to form a new peroxyl radical as follows [27]:

$$ROO - CAR^{\bullet} + O_2 \rightarrow ROO - CAR - OO^{\bullet}$$

At sufficiently high partial oxygen tension (≥ 150 mm Hg) this carotenoid peroxyl radical can generate new radicals due to the cleavage of the resulting peroxyl bond [27]. Thus, in this case carotenoids would act as a rather pro- than antioxidants since they could generate more radicals than capture. It is supposed that carotenoid peroxyl radical can subtract atom hydrogen from R'H by ROO-CAR$^{\bullet}$ giving rise to the new radicals [29]. They could further propagate lipids peroxidation and thus β-carotene act as a prooxidant:

$$ROO - CAR - OO^{\bullet} + R'H \rightarrow ROO - CAR - OOH + R'^{\bullet}$$

Human blood plasma contains approximately 1-2 μM carotenoids. At this concentrations and physiological oxygen pressure, a prooxidant ability of carotenoids is relatively low, whereas antioxidant activity have a big significance [27,30].

It has been reported by other authors that the carotenoid adduct does not react with molecular oxygen at certain conditions even at 100 % oxygen pressure [30]. The adduct, that being formed on the first stage due to the reaction between β-carotene and acylperoxyl radicals, further being converted into the end products by two different ways dependently on the solvent polarity (fig.10).

Retinol (the first form of vitamin A, which was characterized) is a fat-soluble antioxidant, being converted from β-carotene by human organism. It is essential in appropriate amount for vision, bones functionality, immune system as well as for skin and hair health. Retinol and β-carotene are strong antioxidants, which being utilized as the therapeutic agents in cancer prevention, particularly they prevent recurrent tumor cells growth after operation. Retinol and β-carotene protect brain tissues against deleterious effect of free radical active species, most dangerous of them have neutralized by β-carotene.

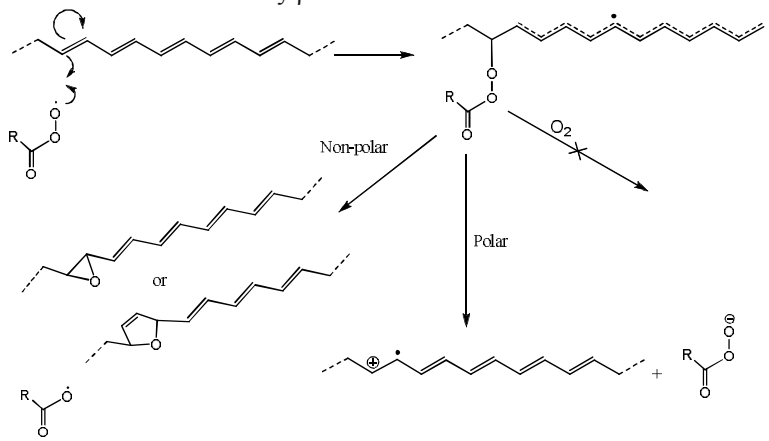


Figure 10. Scheme of interactions between carotenoids and acylperoxyl radicals in both polar and non-polar solvents. From [31]

2. Electron transfer. Reactions of this type give rise to the cation radical $CAR^{\bullet+}$, anion-radical $CAR^{\bullet-}$ or radical $CAR^{\bullet}$. Carotenoid cation radical can be detected by laser flash photolysis [32]. The carotenoids in electron transfer reactions being acted as electron donors, while in certain conditions they play a role of atom hydrogen donors (fig. 11).

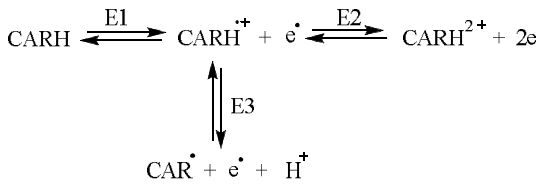


Figure 11. Electron transfer reactions of carotenoids. Adapted from [7]

The carotenoids are the donors of one or two electrons in electron reactions E1 and E2 respectively [7]. The easiness of electron elimination from carotenoid molecule depends on the nature of substituents [7]. The two carotenoids canthaxanthin and astaxanthin are distinguished by their reducing potential of transfer of two electrons ($E1 < E2$), whereas for lycopene, β-carotene and zeaxanthin values of the reducing potential are almost equal [33]. Electron elimination from molecule of carotenoid, which have electron acceptor end group is very complicated. The lower electron acceptor degree of substitutes the smaller ΔE (E1-E2) and cation radical would be reduced to carotenoid radical with reducing potential E3, which significantly lower than that E1 [7]. The values of standard reducing potential of cation radical of carotenoids (from 0,7 to 1,0 V) is not sufficiently low to serve as a hydrogen donor for alkyl ($E°' = 0,6$ V) or peroxyl radicals ($E°' = 0,77…1,44$ V) of polyunsaturated fatty acids [34].

β-Carotene can be a donor of electrons for free radicals resulted in β-carotene cation radical formation [7,35]. The carotenoid cation radical is resonance stable to such an extent that its reaction with molecular oxygen is negligible [36]. However, tocopherols, ubiquinones and also tyrosin and cysteine might be easily oxidized by β-carotene cation radical.

The hydroxyl radicals with high reducing potential (2,31 V) more easy abstracted atom hydrogen from carotenoids than alkylperoxyl radicals [7]. Lycopene cation radical has the lowest value of reducing potential (0,748 V), it farther increased from cation of the β-carotene (0,78 V), zeaxanthin (0,812 V) and xanthaxantine (0,93 V). Astaxanthin is a weaker antioxidant than zeaxanthin [7,37].

When lycopene reacts with superoxide anion radical $O_2^{\bullet-}$, the anion radicals $CAR^{\bullet-}$ were formed:

$$Lycopene + O_2^{\bullet-} \Leftrightarrow Lycopene^{\bullet-} + O_2$$

3. *Hydrogen abstraction.* β-Carotene with certain restrictions can play a role of donor of atom hydrogen for peroxyl radicals give rise to the cation radical [7,27,28]. It is supposed that latter is relatively stable due to delocalization of unpaired electron within conjugated polyene. It can react with lipid peroxyl radicals at low oxygen concentration resulted in the non-radical caroteneperoxide formation. The molecules of oxygen would be bounded to carotene-radical, and the formed adduct further interacted with other molecule of carotene producing, carotene epoxides and carbonyl compounds of carotene (fig. 12).

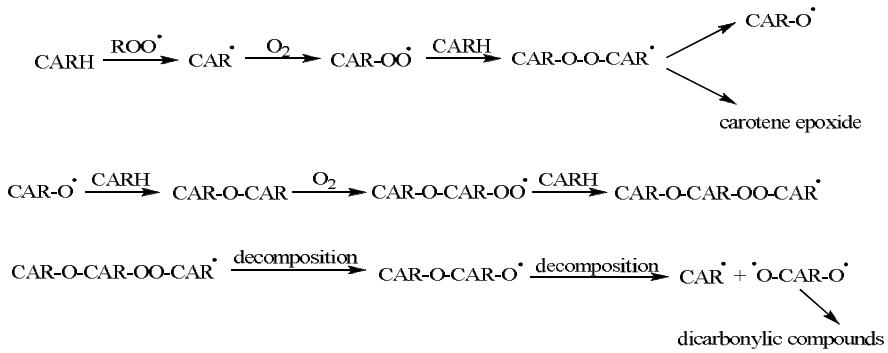


Figure. 12. Reactions of β-carotene with peroxyl radicals. Printed from [7]

An example of hydrogen abstraction is an interaction between β-carotene and nitrogen monoxide contained in cigarette smoke resulted in the formation of 4-nitro-β-carotene:

$$CAR + NO \rightarrow NO\text{-}CAR$$

Thus, the carotenoids are potentially useful for smokers because of decreasing of quantity of toxic oxidants contained in tobacco smoke.

Single electron or hydrogen atom transfer from carotenoids to food radicals depends on their reducing potential and chemical nature of carotenoids, especially on presence of hydroxyl groups. Single electron transfer reaction between free radicals and carotenoids can be relieved if alkylperoxyl radicals contain electron acceptor groups R.

Ascorbic acid, gluthation and cystein, which have properties of scavengers of free radicals, act as donors of atom hydrogen, producing more stable gluthathion and ascorbic acid radicals. Further, ascorbic acid radical are converted to dehydroascorbic acid. Food free radicals are also inactivated by aminoacids, containing sulfhydryl and hydroxyl groups, such as cystein, phenylalanine and prolin. The competition between proteins and lipids for food free radicals may occur [7].

The cystin and cystein aminoacids are playing an active role in redox reaction occurring in biochemical processes of breathing, metabolism, nervous system due to the reversible cystine-cystein interactions (fig. 13).

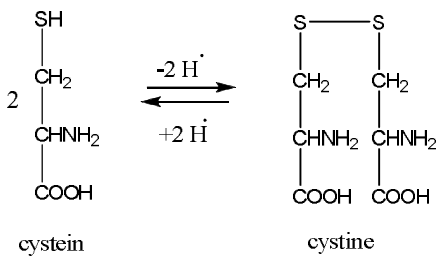


Figure. 13. Cystine-cystein interactions

Trehalose is a thermodynamically and kinetically the most stable nonreducing disaccharide, which can perform specific function of a free radical scavenger for superoxide anion radical $O_2^{\cdot -}$ and hydrogen peroxide [38].

Chelates formation

The cations of transitional metals are good promoters of peroxidation favoring decomposition of peroxides, which were formed on the early stages [2,9]. It is leads to the radicals formation, which being participated in the radical chain reactions of autooxidation. Fats, oils and other foods containing a traces of heavy metals, full removing of which is economically unsuitable. The most widely utilized metals in food industry are copper, cobalt and iron and in the lesser extent manganese, chromium and aluminium. They incorporated into the food products from raw materials and on food processing and packaging [2].

Metal ions are indispensable cofactors of many enzymes and metaloproteins. The proteins heme (contains ion Fe^{2+}) and hemin (contains ion Fe^{3+}) find widespread use in food products. Lipid peroxidation of animal foods can be accelerated by hemoglobin, myoglobin and cytochrome C. These reactions are responsible for rancidity development in meat and poultry food during storage. Peroxidase and catalase are the main sources of heme proteins in plant food [2].

Traces of transitional metals are solubilized during oils processing. These traces are passive physiologically, but are active prooxidants. Metal foils, cans and wrapping papers being served as a source of food contamination by metals, which diffuse into the oil phase [2]. Another source of transitional metals in food is the technological equipment. Metals can be incorporated into the oil phase during oilseed crushing.

The concentration of transitional metals depends on the nature of metal and fatty acids composition of fat. Edible oils, contained substantial quantities of linoleic acid, such as sunflower or corn oil, should contain no more than 0,03 ppm of Fe and 0,01 ppm of Cu, which is necessary to maintain oil stability. The concentration limit of Cu and Fe in fats with a high content of oleic or stearic acids is 0,2 and 2 ppm, respectively [2]. Raw oil contains transitional metals in a form of free cations or chelate comlexes. Unrefined oils, such as olive and sesame contain significant quantity of metal cations [7]. Refinery of the oils leads to substantial drop of metals concentration.

The decomposition rates of hydroperoxides emulsified in water depends on pH. The optimal Fe and Cu activity lies in the pH range between 5,5 and 6. The presence of ascorbic acid accelerates the rate of hydroperoxides decomposition due to its ability to partially reduce cations of metals [2].

The direct oxidation of the unsaturated fatty acids by transitional metals with acyl radicals formation proceeds at very slow rate and doesn`t affected on the initiation of autooxidation:

$$RH + M^{(n+1)+} \rightarrow M^{n+} + H^{+} + R^{\cdot}$$

The activation energy for lipid oxidation, particularly on the initial stages was reduced by traces of metals resulted in the development of oils oxidation [7,39]. The values of activation energy for autooxidation of the refined, clarified and deodorized soy, sunflower and olive oiles are 73,0; 79,5 та 52,3 kJ/mol, respectively [40]. Transitional metals are also catalyze food radicals formation by mechanism of atom hydrogen abstraction. Traces of Fe cations decrease oxidative stability of olive, favoring decomposition of phenolic antioxidants, such caffeic acid [41]. The metal cations, primarily Fe^{2+}, react with hydrogen peroxide by Fenton, producing reactive oxygen species, especially hydroxyl radicals [42]:

$$H_2O_2 + M^{n+} \rightarrow HO^- + HO^{\cdot} + M^{(n+1)+}$$

The $OH^{\bullet}$ radical is considered to be a one of the most reactive radical, its half-life in aqueous solution is approximately 10^{-9} s. Unlike the hydroperoxides, which were metabolized by superoxide dismutase, the hydroxyl radicals cannot be removed during enzymatic reactions. Therefore, they react with all compounds of a substrate [7,43]. Transitional metals including copper, manganese and cobalt catalyze these reactions. Fenton reactions may lead to accumulation of the active radicals and and so contribute to the initiation of biomolecules decomposition.

Chelate complexes formation inhibits the oxidation process due to: insoluble complexes formation, decreasing of the redox potential of metals, or providing sterical hindrance between metals and oxidized intermediates or components of food products. Citric acid and Ethylenediamine tetraacetic acid (EDTA) are the classical examples of chelate producers. The majority of the complexing agent are water soluble, while citric acid is a partially fat soluble. Phospholipids and flavonoids may also play a role of chelating agents [44]. Cations of transitional metals being bounded by flavonoids, activity of which depends on the structure features [45]. Presence of 3,4-dihydroxyl groups of B ring and 4-carbonyl and 3-hydroxy group of C ring, or 4-carbonyl group of C-ring and 5-hydroxyl group of A-ring can facilitate complex formation with metals at the certain available sites (fig. 14). Lignans, polyphenols, ascorbic acid and aminoacids, such as carnosine and histidine are bound to metals with chelate complexes formation.

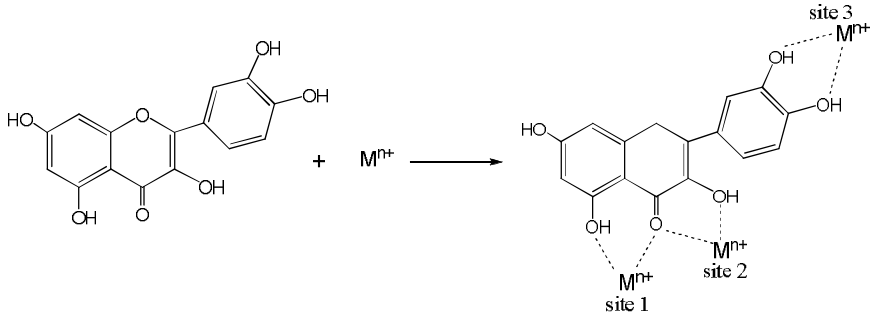


Figure 14. The mechanism of flavonoids chelate complexes formation. Adapted from [9]

The chelates formation with metals cations is an important process not only in food products. Fenton reaction occurs in the dophamine neurons of the nerve cells, where some quantity of hydrogenperoxide was formed by catabolism [9]. The formation of radicals is considered to be the main aetioligical factor of the Parkinson`s disease [42]. The significant accumulation of Fe cations in some brain tissues may be recognized as a marker of other neurodegenerative diseases, such as Alzheimer disease and Huntington`s chorea [46,47]. Basal ganglia Fe content is increased in patient suffering from Alzheimer disease [48].

Singlet oxygen quenching

As it was mentioned above singlet oxygen is considerably more active than that of in triplet state. The cellular components (membrane lipids, enzymes, nucleic acids) e.t.c. may be imparted or destroyed by singlet oxygen. It can potentially transfer high energy to other molecules. Tocopherols, carotenoids, curcumin, phenols, urates and ascorbates are able to quench singlet oxygen [2,7,48]. Singlet oxygen quenching included both physical and chemical

components. Singlet oxygen deactivation and its transfer into the ground state is performed by physical quenching due to an energy loss or recharging. Quenching of singlet oxygen by energy transfer being occurred when energy level of a quencher (Q), is near or below that of a singlet oxygen:

$$^1O_2 + Q \rightarrow {}^3O_2 + {}^3Q$$

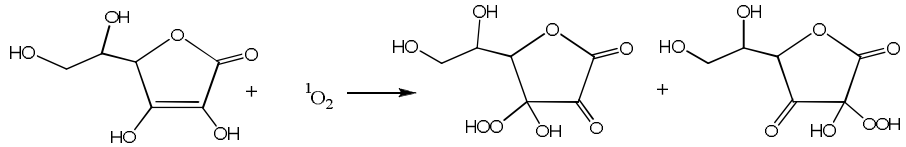
$$^3Q \rightarrow {}^1Q \text{ (no radiation)}$$

Carotenoids activity depends on number of conjugated double bonds in the molecule and the substituents in the β-ionone ring [7,50].Carotenoids with 9, 10 and 11 conjugated double bonds quench single oxygen activity by energy transfer [2,7,9]. They are the better quenchers than those with 8 or less conjugated bonds. β-Carotene and lycopene, which contained 11 conjugated double bonds are the more effective quenchers of singlet oxygen, than lutenin which has just 10 of these bonds [7,51]. These carotenoids can absorb the energy from the singlet oxygen, which further would be distributed over all the single and double bonds in the molecule. One molecule of β-carotene is estimated to quench up to 1000 molecules of singlet oxygen. The presence of conjugated keto groups or cyclohexaene ring favoring singlet oxygen quenching [7,52]. However, β-ionone ring substitution by hydroxyl, epoxy- and methoxy-groups resulted in the decrease of quenching activity of the caritenoids. The values of the rate constants of single oxygen quenching by canthaxanthin, β-apo-8´-carotenal, β-carotene and ethyl-apo-8´-carotenal are $1{,}45 \cdot 10^{10}$; $1{,}38 \cdot 10^{10}$; $1{,}25 \cdot 10^{10}$ and $1{,}2 \cdot 10^{10}$ l/mol·s, respectively [7].

The process is proceeded by charge transfer mechanism in the case of singlet oxygen quencher with high reducing potential and low triplet energy. These compounds included amines, phenols, sulfides, iodides, and azides and are the donors of electrons for singlet oxygen. They formed complex with singlet oxygen, which further would be transferred into the triplet state. In the last stage the triplet complex would be disrupted with quencher and triplet oxygen formation:

$$^1O_2 + Q \rightarrow [O_2^- \text{----} Q^+]^1 \rightarrow [O_2^- \text{----} Q^+]^3 \rightarrow {}^3O_2 + Q$$

Chemical quenching is a chemical reaction between singlet oxygen and quencher with oxidized products formation [2,7,9,24]. β-Carotene, aminoacids, tocopherols, ascorbic acid, peptides, and phenols can be oxidized by singlet oxygen, thus all of them are chemical quenchers [22]. β-Carotene react with singlet oxygen at a rate of the $5 \cdot 10^9$ l/mol s producing 5,8-endoperoxides [7]. The singlet oxygen and ascorbic acid react in an aqueous solution as follows:



Fiure. 15. Scheme of ascorbic acid hydroperoxides formation in the presence of singlet oxygen.
Printed from [7]

Tocopherol reversibly react with singlet oxygen, producing hydroxydienone, tocopherylquinone and quinonperoxide. The reaction rates for different isomers are: $2{,}1 \cdot 10^8$

l/mol·s for α-tocopherol; $1,5 \cdot 10^8$ l/mol·s for β-tocopherol; $1,4 \cdot 10^8$ l/mol·s for γ-tocopherol and $5,3 \cdot 10^7$ l/mol·s for γ-tocopherol [7].

Photosensitizers deactivation

Light radiation affected on the food quality [2,7,52,53]. Undesirable changes of food quality caused by milk oxidation resulted in deterioration, off-flavors development and profound reduction in the shelf life and nutritive value of food products [52]. Milk and dairy foods are the most sensitive food products to the light action due to the high concentration of riboflavin and vitamin B2, which are effective photosensitizers of oxidative processes.

The reducing of beer quality occurs by the same mechanism as a result of riboflavin oxidation [54].

Riboflavin is a water soluble vitamin, contained in meat and dairy food products, eggs, vegetables e.t.c. [52]. Flavines acted as photosensitizers due to their chemical interaction with substrate, components of which are in singlet or triplet state (I type mechanism), or physical interaction with triplet oxygen, producing singlet oxygen (II type mechanism) [7,53,55]. In the I type mechanism light radiation causes flavin excitation with further abstraction of atom hydrogen or electron transfer from corresponding compounds, such as aminoacids or flavonoids [56]. Its regeneration and superoxide anion $O_2^{\bullet-}$ formation occur in the presence of oxygen [52,57]. In the II type mechanism triplet oxygen provokes formation of highly reactive singlet oxygen (E ~ 1,7 V), which react with lipids and give rise to the hydroperoxides. It was reported earlier that milk and dairy products are oxidized by the type II mechanism [52]. Recently it has been found by some authors that the main mechanism is the second (type II) [52,58]. Aminoacids, purine bases, wheat proteins, phenols are certainly contained in foods in high amount supposed to be interacted with flavines in the excited state. The reaction rate between these compounds and flavines in the excited state is higher than that of flavines and oxygen [58]. Chlorophylles, contained in food products, are effective photosensitizers as well.

It is known that photosensitizers can be deactivated by vitamin C, carotenoids, flavonoids and uric acid [7,52]. Photosensitizers were deactivated mainly by carotenoids with less than 9 conjugated double bonds, while singlet oxygen was scavengered predominantely by carotenoids with more than 9 conjugated double bonds [51]. The energy transferred to the surroundings by phosphorescence due to the interaction between carotenoid and photosensitizers. The distance between chlorophyll and carotenoid must be lesser 0,36 nm in order to overlap two electron orbitals between these pigments [37].

Lipoxygenase deactivation

As it was mentioned above, the lipid peroxidation may be non-enzymatic and enzymatic. The latter is catalysed by lipoxygenase a lipid peroxidation enzyme that oxidize fatty acids giving rise to the hydroperoxides [2,7,59]. Lipoxygenase is widespread in food of animal origin and in common edible plants, particularly in the potato tubers and beans.

Linoleic and linolenic acids are the main polyunsaturated fatty acids, which have oxidized in the presence of oxygen with C9 and C13 hydroperoxides formation, respectively [2,59]. Certain isoformes of lipoxygenase are able to produce hydroperoxides, which are necessary for the jasmonic acid synthesis (fig. 16). The latter is playing an important role in the plants [58].

Lipoxygenases are responsible for quality loss of the juices, particularly melon juice, but the mechanism of lipoxygenase action is still not clear [60]. As it was mentioned above,

lipoxygenase causes oxidation of unsaturated fatty acids, 1,4-*cys*-*cys*-penthadienic system. This enzyme catalyzes the cooxidation of carotenoids, resulted in the color loss of food products. Furthermore, lipoxygenase causes formation of volatile aldehydes, and consequently the sensory properties of juices.

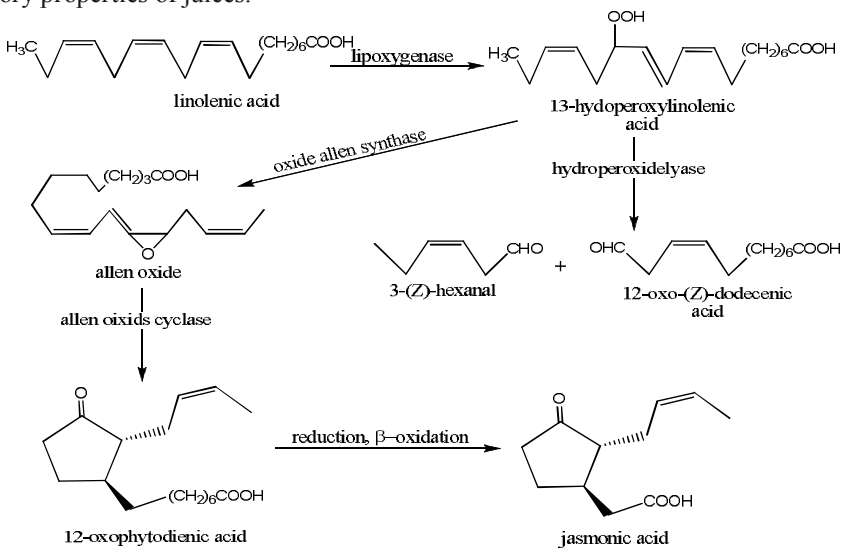


Figure. 16. Scheme of linolenic acid oxidation by lipoxygenases in plants.
Printed from [60]

Wheat grains have different oxidases, particularly lipoxygenases, which affected on the metabolism of the antioxidants and may cause changes of antioxidant potential of the end products [59,61]. Lipoxygenases action on the unsaturated fatty acids leads to quality loss of food products as well as to changes of color and sensory characteristics. For example, color loss, that taking place in the processes of the pasta manufacturing, mainly accounted for by the lipoxygenase action on linolenic acid, that caused and oxidative decomposition of carotenoid pigments [2,59,62]. Frozen tomato cubes have been covered by layer of the modified starch, in order to prevent color loss, cased by lipoxygenases activity [59].

Lipoxygenase is a catalytic oxidative enzyme, which lost activity by heating at the temperatures more than 60 °C [7]. This procedure improves shelf life of the food products. Furthermore, heating leads to increase of non-enzymatic oxidation degree as well. Lipoxygenases can be deactivated by steam treatment of the soy beans at the temperature 100 °C during 2 min resulted in the substantial drop of concentration of peroxides and finally improved quality of soy oil. Lipoxygenases activity rises during ripening of fruits. This enzyme affected on the strawberry ripening, it causes the color development.

Generally, oxidative stability of lipid containing food products would be achieved in case of low exposure to the light radiation, high temperatures and air oxygen.

Antioxidants interactions. Synergism and antagonism

Interactions between different antioxidants would be synergistic, antagonistic or additive. Synergism is a phenomenon which occurs if the total antioxidant effect is higher than the sum

of effect of each antioxidant. An example of synergistic antioxidant effect is the action of mixture of α-tocopherol and ascorbic acid in the processes of lipids autooxidation and photooxidation [7,62]. Antagonism is an opposite effect that would be observed when the summary antioxidant effect lower than that of each antioxidant. An example of antagonism of the antioxidants is the action of mixture catechine and caffeic acid. The additive effect has observed when the summary effect is equal to the effect of antioxidant in mixture. Polyphenolic compounds, such as epigallocatechin gallate, quercetin, epicatechin gallate, epicatechin andcyanidine have an additive effect with α-tocopherol, which play a role of free radical scavenger [7,63].

The synergism can be explained by different mechanisms of action of the antioxidants: combination of two or more free radical scavengers and thus primary protection of the certain antioxidant; combination of the two antioxidants with different antioxidant mechanism [7]. Regeneration of the most effective free radical scavenger (primary antioxidant) by less effective (coantioxidant, synergist) occurs at the large differences in reduction potentials of these two compounds. The free radical scavenger with bigger reduction potential serves as a primary antioxidant. The total antioxidant effect can be enhanced by regeneration of the primary antioxidant. The example of a such system is a mixture of tocopherol ($E° = 0,5$ V), which being acted as a primary antioxidant and ascorbic acid ($E° = 0,33$ V), playing a role of synergist [7]. The direct interaction of a tocopherol molecules (TH) with alkyl or alkylperoxyl radicals of food products would lead to the formation of tocopherol radicals, which does not have antioxidant properties [7]. Ascorbic acid (AsH) donates hydrogen atom to tocopherol radical, which favors tocopherol regeneration and giving rise to the semihydroascorbic acid radical ($As^{\bullet}$), which can be further oxidized to give dehydroascorbic acid (DHAs) [65]:

$$TH + R^{\bullet} \rightarrow T^{\bullet} + RH$$

$$TH + ROO^{\bullet} \rightarrow T^{\bullet} + ROOH$$

$$T^{\bullet} + AsH \rightarrow TH + As^{\bullet}$$

$$As^{\bullet} \rightarrow DHAs + H^{\bullet}$$

Interaction between tocopherols and carotenoids and their regeneration is another, more complicated example of synergism. In this case carotenoids can be regenerated by tocopherols and vice versa. Though, carotenoids are regenerated predominantly due to larger value of the standard reducing potential of carotenoid cation radical (0,7-1,0 V) compared with that of tocopherol radical (0,5 V) [7,66,67]. It is well known that β-carotene disappeared soon after oxidation of oleic acid. However, duration of antioxidant activity of carotenoids have been increased from 100 to 1500 hours by α-tocopherol addition [67]. Carotenoids can be regenerated from corresponding cation radicals by α-tocopherol action. It is interesting, that in certain systems the interaction between carotenoids and α-tocopherol may not to be occurred, for example at safflower oil oxidation [68].

Two antioxidants, which significantly differ by energy dissociation of O-H bond are considered to be synergists [7]. The bigger energy dissociation of O-H bond of synergist in compare with primary antioxidant the faster the regeneration rate [7,70]. The primary antioxidant can be regenerated as well in case of reaction rate constant at least 10^3 l/mol·s, whereas constant of reaction with peroxyl radical approximately equal to that of with antioxidant radicals [7,71]. Regeneration would be terminated by electron transfer from molecule of synergist to the antioxidant [72].

Synergistic antioxidant effect would observed when one antioxidant quickly oxidized and thus protected another [7]. The less active antioxidant scavengers alkyl and alkylperoxyl food

radicals, that resulted in the protection of the more effective antioxidant. In the other case antioxidant radical, that has been formed during oxidation of the less efficient antioxidant, being competed with more effective in reactions with alkylperoxyl radical, which reduce oxidation level of the more efficient antioxidant [7]. Interactions between tocopherols and carotenoids can partly proceed via the above mentioned mechanism [7,72].

The synergists may act as the hydrogen donors to the phenoxyl radical, thereby regenerating the primary antioxidants. The synergistic effect would be observed in case of two antioxidants with different mechanism of action [7]. It has been well established that the combination of metal chelators and free radical scavengers have synergistic antioxidant effect. Metal chelators, including phospholipids, citric acid, ethylenediamine tetraacetic acid are not truly antioxidants, but they are effective as the synergists. They inhibit metal catalyzed oxidation, and decrease total quantity of free radicals, which have captured by scavengers [43]. Metal chelators acted on the initiation stage of oxidation, while scavengers on the propagation stages [7]. Phosphatidylinositol act as a synergist in mixture with the tocopherols, reducing level of lipids oxidation due to inactive metal complexes formation [74]. Quercetin and α-tocopherol are synergists, which inhibited oxidation of lard due to α-tocopherol serves as free radical scavenger, while a quercetin acts as metal chelator [7]. Many synergists also provide an acidic media that improves the stability of primary antioxidants.

Antagonism has been observed between α-tocopherol and both rosmarinic and caffeic acid, between caffeic acid and catechine or quercetin [75,76]. Plant extracts, rich in polyphenols have the antagonistic effect to the α-tocopherol functionality in lard and safflower oil.

Antagonism between two antioxidants action would occur in case of: competition between formation of antioxidant radical adducts and regeneration of antioxidants; the less efficient antioxidant is regenerated by more efficient; predominant oxidation of the most efficient antioxidant by radicals formed from less efficient; interactions of two antioxidant in certain systems [75,76]. Antagonism of antioxidants occurring in oxidized food systems is still not clear [7].

The antioxidant properties depend on the environment in which they act [77]. As it has been shown by Becker and coauthors, α-tocopherol and quercetin in emulsion are strong synergists, in liposomes synergistic antioxidant effect weaker, whereas in a dry sunflower oil these compounds have antagonistic effect. The mechanism of action of antioxidant in multiphase systems distinguishes from that of in oils, which can be explained by solvation effects. The authors suggested that antioxidant antagonism occurring in dry can arise through the formation of intermediates at elevated temperatures besides those formed from quercetin, which are susceptible to oxidation [76].

Conclusions

Thus the mechanisms of action of natural and synthetic antioxidants has been analyzed. Understanding of mechanisms of action allows to select the most efficient antioxidant in a certain food system. Even a negligible (0,01-0,001 %) quantity of an antioxidant significantly inhibit processes of oxidation either in food systems and living organisms, in which strong intracellular antioxidant protection is complemented by extracellular. The main role in this system is playing vitamins A, C and E, antioxidant ferments: glutathione, glutathionperoxidase, superoxiddismutase, catalase e.t.c.

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