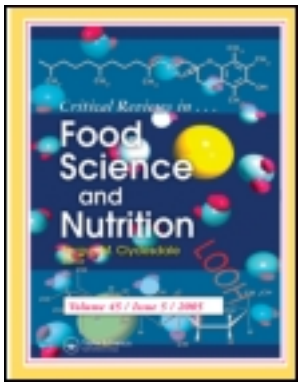

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Chemistry and Reactions of Reactive Oxygen Species in Foods

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Reactive oxygen species (ROS) are formed enzymatically, chemically, photochemically, and by irradiation of food. They are also formed by the decomposition and the inter-reactions of ROS. Hydroxy radical is the most reactive ROS, followed by singlet oxygen. Reactions of ROS with food components produce undesirable volatile compounds and carcinogens, destroy essential nutrients, and change the functionalities of proteins, lipids, and carbohydrates. Lipid oxidation by ROS produces low molecular volatile aldehydes, alcohols, and hydrocarbons. ROS causes crosslink or cleavage of proteins and produces low molecular carbonyls from carbohydrates. Vitamins are easily oxidized by ROS, especially singlet oxygen. The singlet oxygen reaction rate was the highest in β-carotene, followed by tocopherol, riboflavin, vitamin D, and ascorbic acid.

Keywords chemistry, reaction, reactive oxygen species

INTRODUCTION

Reactive oxygen species (ROS) is a collective term that includes oxygen radicals and nonradical derivatives of oxygen. The oxygen radicals are superoxide anion ($O_2 \cdot^-$), hydroxy (HO·), peroxy (ROO·), alkoxy (RO·), and hydroperoxy (HOO·) radicals. Nonradical derivatives are hydrogen peroxide (H_2O_2), ozone (O_3), and singlet oxygen ($^1O_2$). The ROS covered in this review are superoxide anion, hydroperoxy radical, peroxy radical, hydroxy radical, alkoxy radical, hydrogen peroxide, and singlet oxygen. Superoxide anion, hydrogen peroxide, and hydroxy radical are formed by a sequential univalent reduction of molecular triplet oxygen. Singlet oxygen is commonly formed by the excitation of triplet oxygen in the presence of sensitizer and light. Wettasinghe and Shahidi (2000) reported that ROS play a very important role in our health. They also reported that the occurrence of ROS in foods is inevitable due to the biological nature of foods. Research on the chemistry of ROS and their effects on foods has progressed greatly recently. ROS are mainly responsible for initiation of oxidation reaction of foods. ROS react with lipids, proteins, sugars, and vitamins, producing undesirable volatile compounds, destroying essential fatty acids, amino acids and vitamins, and producing carcinogens. ROS changes the functionalities of proteins, lipids, and carbohydrates by forming oxidized dimers and trimers. ROS make food products less acceptable or unacceptable to consumers (Min and Choe, 2002; Lee et al., 2003). Reversion flavor in soybean oil and sunlight flavor in milk are formed from the reaction by singlet oxygen (Min et al., 2003; Jung et al., 1998). The ROS in foods lower the overall nutritional, chemical, and physical qualities of foods during storage and marketing. To improve the food quality, it is very important to understand the formation of ROS and their reactions with food components during food processing and storage. The objective of this paper is to review the formation, chemistry, and reactions of ROS in foods as related to food quality.

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REACTIVE OXYGEN SPECIES FORMATION IN FOODS

Superoxide Anion

Superoxide anion is formed enzymatically and chemically from triplet oxygen. Triplet oxygen has two unpaired electrons, each is in a different antibonding π^* orbital at parallel spins (Figure 1). Triplet oxygen, the most stable and abundant form of oxygen, is the common oxygen that we breathe. Since triplet oxygen has a separate parallel spin in each of its antibonding π^* orbitals, it is a diradical. Diradical triplet oxygen can not react with food components that are not radical compounds unless the food compounds become radical compounds. Triplet oxygen can react mostly with radicals. When a single electron is added to one of the antibonding π^* orbitals of the triplet oxygen,

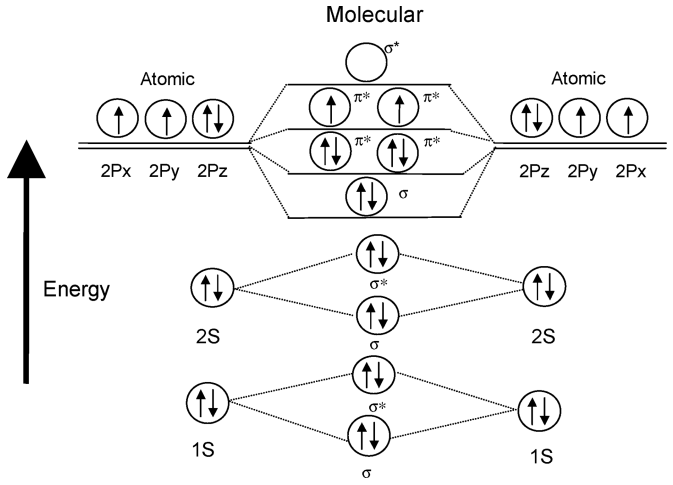

Figure 1 Electronic configuration of triplet oxygen.

superoxide anion is produced. Superoxide anion has only one unpaired electron and is a radical compound (Figure 2).

One of the enzymes that produces superoxide anion in foods is xanthine oxidase. Xanthine oxidase acts on xanthine or hypoxanthine in the presence of molecular triplet oxygen to produce superoxide anion (Das and Das, 2002).

$$\text{Xanthine} + H_2O + 2O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Uric acid} + 2O_2^{-\cdot} + 2H^+$$

NADPH oxidase produces superoxide anion although the exact mechanism is not fully understood (Martinez and Moreno, 1996). NADPH oxidase controls the development of plant cells by producing ROS (Schopfer et al., 2001; Foreman et al., 2003).

$$\text{NADPH} + 2O_2 \xrightarrow{\text{NADPH oxidase}} \text{NADP}^+ + 2O_2^{-\cdot} + H^+$$

Ingold et al. (1997) reported that molecular oxygen reacts with the decomposition products of some azo compounds ($X-N=N-Y$) such as azo dyes in food colorants and produces superoxide anion.

$$X-N=N-Y \rightarrow X\cdot + N_2 + Y$$

$$X\cdot + O_2 \rightarrow X^+ + O_2^{-\cdot}$$

$$Y\cdot + O_2 \rightarrow Y^+ + O_2^{-\cdot}$$

Photoactivation of hematoporphyrin or tetrapyrrols (TPR) produces superoxide anion (Buettner and Oberley, 1980). Light energy at a specific wavelength converts the ground singlet state of TPR to the excited singlet state of TPR (^{1}TPR*). The

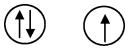

Figure 2 Electronic configuration of $2p\pi^*$ orbital of superoxide anion.

^{1}TPR* becomes an excited state of triplet TPR (^{3}TPR*) via intersystem crossing by emitting some of its energy. ^{3}TPR* reacts with molecular triplet oxygen to produce superoxide via radical reactions including formation of sensitizer radical (Haseloff and Ebert, 1989). Superoxide anion formation by photoexcitation of tetrapyrroles in pheophorbide is ethanol concentration-dependent (Athar et al., 1988; Haseloff and Ebert, 1989).

$$^1\text{TPR} + \text{light} \rightarrow {}^1\text{TPR}^*$$

$$^1\text{TPR}^* \xrightarrow{\text{Intersystem Crossing}} {}^3\text{TPR}^*$$

$$^3\text{TPR}^* + O_2 \rightarrow \text{TPR}^{\cdot+} + O_2^{-\cdot}$$

Superoxide anion can be formed by gamma irradiation, pulsed electric field, microwave, and ohmic processing of foods. During radiolysis of dilute aqueous solutions, most of the energy is absorbed by water and produces ionized water ($H_2O^{+\cdot}$) and excited water (H_2O^*) within 10^{-16} s (Halliwell and Gutteridge, 2001). The electrons are surrounded by water molecules within $10^{-12}\sim10^{-11}$ s and these hydrated electrons (e_{aq}^-), whose standard reduction potential is -2.84 V, are powerful reducing agents. In the presence of a high concentration of triplet oxygen, hydrated electrons reduce oxygen to superoxide anion.

$$2H_2O \rightarrow H_2O^{+\cdot} + H_2O^* + e_{aq}^-$$

$$^3O_2 + e_{aq} \rightarrow O_2^{-\cdot}$$

Hydroperoxy and Peroxy Radicals

Hydroperoxy radical ($HOO^\cdot$) is a protonated form of superoxide anion and produced by the reaction of hydrogen peroxide and hydroxy radicals ($HO^\cdot$).

$$HO^\cdot + H_2O_2 \rightarrow HOO^\cdot(H^+ + O_2^{-\cdot}) + H_2O$$

Hydroperoxy radical is also produced by the reaction of triplet oxygen and hydrogen atom produced from water during pulsed electric field processing. The excited state of water molecules by the absorbed energy undergoes homolysis in $10^{-14}\sim10^{-13}$ s and produces hydrogen atom ($H^\cdot$) and hydroxy radicals (Halliwell and Gutteridge, 2001).

$$2H_2O \rightarrow H_2O^{+\cdot} + H_2O^* + e_{aq}^-$$

$$H_2O^* \rightarrow H^\cdot + HO^\cdot$$

$$H^\cdot + O_2 \rightarrow HOO^\cdot \rightarrow H^+ + O_2^{-\cdot}$$

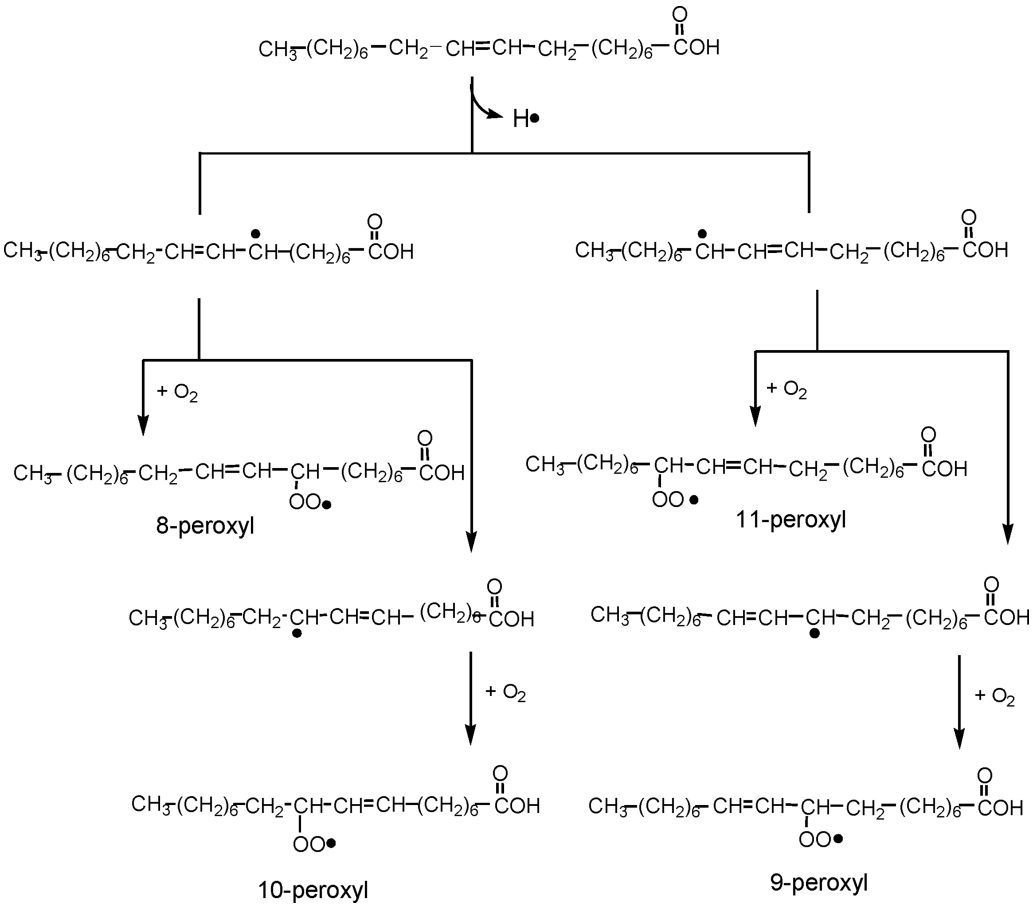

Figure 3 Peroxyl radical Formation from the reaction between oleic acid and oxygen.

Peroxy radical is formed by a direct reaction of triplet oxygen with alkyl radical in fatty acid oxidation (Figure 3).

Peroxy radical produces hydroperoxide (ROOH) by abstracting hydrogen from other molecule (Halliwell and Gutteridge, 2001). Most hydroperoxides are stable at room temperature, however, heat, UV light or transition metals accelerate homolysis of hydroperoxides and produce peroxy radicals.

$$R\cdot + O_2 \rightarrow ROO^{\cdot}$$

$$ROO^{\cdot} + R'H \rightarrow ROOH + R'^{\cdot}$$

$$ROOH \xrightarrow{\text{Heat, UV}} ROO^{\cdot} + H^{\cdot}$$

$$ROOH + Fe^{3+} \rightarrow ROO^{\cdot} + Fe^{2+} + H^{+}$$

Reaction of hydroperoxide with hydroperoxy radical produces peroxy radical (Aikens and Dix, 1991).

$$ROOH + HOO^{\cdot} \rightarrow ROO^{\cdot} + H_2O_2$$

Hydroxy and Alkoxy Radicals

Hydroxy radicals are produced from water or hydrogen peroxide. The high-energy radiation of γ-rays on water produces hydroxy radical (Jacobien et al., 1996).

$$2H_2O \rightarrow H_2O^{+\cdot} + H_2O^{*} + e^{-}_{aq}$$

$$H_2O^{*} \rightarrow H^{\cdot} + HO^{\cdot}$$

$$H_2O^{+\cdot} + H_2O \rightarrow H_3O^{+} + HO^{\cdot}$$

Hydroxy radical is also formed by UV-induced homolytic fission of oxygen-oxygen bond of hydrogen peroxide or decomposition of hydrogen peroxide in the presence of transition metal complexes (Salem et al., 2000). Production of hydroxy radicals from hydrogen peroxide in the presence of iron is known as Harber-Weiss reaction.

$$H_2O_2 \xrightarrow{hv} 2HO^{\cdot}$$

$$Fe^{2+} + H_2O_2 \xrightarrow{\text{Harber-Weiss}} Fe^{3+} + OH^{-} + HO^{\cdot}$$

The reductive half cycle of iron catalyst ($Fe^{3+} \rightarrow Fe^{2+}$) is the rate-determining step in Harber-Weiss reaction (Watanabe et al., 2002). Formate anion radical ($HCOO^{\cdot -}$), hydroquinone, or cysteine serves as a Fe^{3+} reductant to promote hydroxy radical production. The reduction of Fe^{3+} is catalyzed by superoxide anion (Watanabe et al., 2002). Superoxide anion and hydrogen peroxide can produce hydroxy radical by iron catalyzed Haber-Weiss reaction. Haber-Weiss reaction rarely occurs in aqueous solution in the absence of transition metal ions which catalyzes decomposition of hydrogen peroxide to hydroxy radical (Hu and Jiang, 1996).

$$O_2^{\cdot -} + Fe^{3+} \rightarrow {}^1O_2 + Fe^{2+}$$

$$H_2O_2 + Fe^{2+} \xrightarrow{\text{Haber-Weiss reaction}} Fe^{3+} + OH^- + HO^{\cdot}$$

Heat, UV light or transition metals accelerate homolysis of hydroperoxides (Heaton and Uri, 1961; Schaich, 1992; Jadhav et al., 1996) to produce alkoxy radicals ($RO^{\cdot}$).

$$ROOH \xrightarrow{\text{Heat, UV}} RO^{\cdot} + {}^{\cdot}OH$$

$$ROOH + Fe^{2+} \rightarrow RO^{\cdot} + Fe^{3+} + OH^-$$

Hydrogen Peroxide

Hydrogen peroxide, the protonated form of peroxide ion (O_2^{2-}), is formed by oxidase of xanthine, urate, and amino acid.

Dismutation of superoxide anion can produce hydrogen peroxide although the reaction rate is very low in aqueous or alcoholic solution (Bielski et al., 1983).

$$O_2^{\cdot -} + O_2^{\cdot -} \xrightarrow{\text{Dismutation}} H_2O_2 + O_2$$

Rate constants for the uncatalyzed dismutation of superoxide anion depend strongly on the pH of the solution. Rates for dismutation of superoxide were 10^2 and 5×10^5 $M^{-1}s^{-1}$ at pH 11 and pH 7.0, respectively (Haliwell and Gutteridge, 2001). Superoxide dismutase greatly accelerates the reaction, with the rate of 5×10^5 $M^{-1}s^{-1}$ (Paller and Eaton, 1995; Henderson and Chappell, 1996; Halliwell and Gutteridge, 2001). Hematoporphyrin derivative (HP) can produce hydrogen peroxide in the presence of light and reducing compounds at a pH of less than 6.5 via superoxide anion (Buettner and Hall, 1987). The formation of hydrogen peroxide from superoxide is faster at low pH where superoxide radical is present in its protonated form (pKa = 4.88), hydroperoxy radical.

$$HP + O_2 \xrightarrow{hv} HP^{\cdot +} + O_2^{\cdot -}$$

$$O_2^{\cdot -} + H^+ \rightarrow \quad HOO^{\cdot}$$

$$HOO^{\cdot} + HOO^{\cdot} \rightarrow H_2O_2 + O_2$$

Hydrogen peroxide can be produced by radiolysis of water.

$$2H_2O \rightarrow H_2O^{+\cdot} + H_2O^* + e_{aq}^-$$

$$H_2O^* \rightarrow H^{\cdot} + HO^{\cdot}$$

$$HO^{\cdot} + HO^{\cdot} \rightarrow H_2O_2$$

The reaction rate between hydroxy radicals to form hydrogen peroxide is 5×10^9 $M^{-1}s^{-1}$ (Halliwell and Gutteridge, 2001). Hydrogen peroxide is also formed from hydroperoxide and hydroperoxy radical.

$$ROOH + HOO^{\cdot} \rightarrow ROO^{\cdot} + H_2O_2$$

Singlet Oxygen

Singlet oxygen is not a radical compound because it has no unpaired electrons (Figure 4). High energy singlet oxygen, 93.6 kJ above the ground state (Korycka-Dahl and Richardson, 1978; Girotti, 1998), in solution is deactivated to molecular triplet oxygen by transferring its energy to the solvent. The lifetime of singlet oxygen depends on the chemical properties of the solvent. The lifetime of singlet oxygen in water is 2 μs (Merkel and Kearns, 1972) and 700 μs in carbon tetrachloride (Long and Kearns, 1975).

Singlet oxygen formation in the presence of sensitizer and triplet oxygen is shown in Figure 5 (Foote and Denny, 1968). If a ground singlet state photosensitizer (^{1}Sen) is exposed to light of a specific wavelength, it becomes an excited singlet state (^{1}Sen*). Sensitizers are dyes such as methylene blue, eosin, and curcumin (Das and Das, 2002), pigments such as chlorophylls and hematoporphyrin (Lee et al., 2003; Whang and Peng, 1988), and aromatic hydrocarbons such as rubrene and anthracene (Afonso et al., 1999). Riboflavin also acts as a sensitizer (Min and Choe, 2002). Excited singlet state sensitizers return to ground state via emission of light, internal conversion, or intersystem crossing. Fluorescence or heat is produced from the excited singlet sensitizer by light emission or internal conversion, respectively. The intersystem crossing (ISC) of singlet sensitizer produces an excited triplet sensitizer.

The reaction types of excited triplet sensitizer are shown in Figure 6. The exited triplet riboflavin has a redox potential of 1700 mV (Lu and others 1999). The excited triplet sensitizer can abstract electron or hydrogen atom from food compounds and form free radicals of food compounds (Type I). The excitation energy of triplet sensitizer can be transferred to triplet oxygen to produce singlet oxygen and the excited triplet sensitizer returns to its ground state (Type II). Kochevar and Redmond

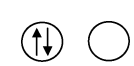

Figure 4 Electronic configuration of $2p\pi^*$ orbital of singlet oxygen.

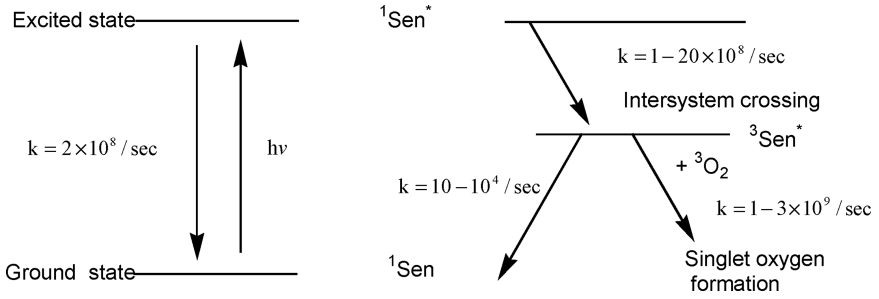

Figure 5 Excitation and deactivation of sensitizer.

(2000) reported that a sensitizer molecule may generate 10^3 to 10^5 molecules of singlet oxygen before the sensitizer is inactive.

The rate of Type I or II process depends on the types of sensitizers (Chen et al., 2001) and substrates, and concentrations of substrate and oxygen in the reaction environments (Foote, 1978). Easily oxidizable phenol or amine compounds or readily reducible quinones favor the Type I. Olefins, dienes, and aromatic compounds that are not readily oxidized or reduced favor Type II. Anthraquinone derivatives such as quinizarine produce singlet oxygen by Type II mechanism (Mothilal et al., 2003).

Singlet oxygen is also produced from hydrogen peroxide and superoxide anion by metal-catalyzed Haber-Weiss reaction (Kellogg and Fridovich,1975).

$$H_2O_2 + O_2^{-\cdot} \rightarrow HO^{\cdot} + OH^{-} + {}^1O_2$$

Singlet oxygen is produced by the Russell mechanism from peroxy radicals (Halliwell and Gutteridge, 2001).

$$2\text{R-CHOO}^{\cdot}\text{-R}' \xrightarrow{\text{Russell Mechanism}} \text{R-CHOH-R}' + \text{R-CO-R}' + {}^1O_2$$

Takayama et al. (2001) reported that metastable phosphatidylcholine hydroperoxides produced singlet oxygen during the hydroperoxides breakdown in the presence of Cu^{2+} in the dark. The major pathway for singlet oxygen formation in food is photosensitization. The chemical and physical quenching mechanisms of singlet oxygen are shown in Figure 7. Electrophilic singlet oxygen can directly react with electron rich compounds containing double bonds. Singlet oxygen reaction with hydrocarbons with one or more double bonds produces endoperoxides, allyl hydroperoxides, or dioxetanes (Gollnick, 1978). Endoperoxides are formed by the reaction of singlet oxygen with conjugated double bonds. Tocopherol, carotenoids, phenolics, urate, and ascorbate can quench singlet oxygen. Curcumin, a major component of food flavoring turmeric, was also reported to be a good singlet oxygen quencher at physiologically low concentrations of 2.75∼3.12 μM (Das and Das, 2002). Lipids can also quench singlet oxygen physically. Saturated fatty acids quench singlet oxygen by energy transfer from singlet oxygen to the vibrational sublevels of CH- and COOH groups of fatty acids (Krasnovsky et al., 1983).

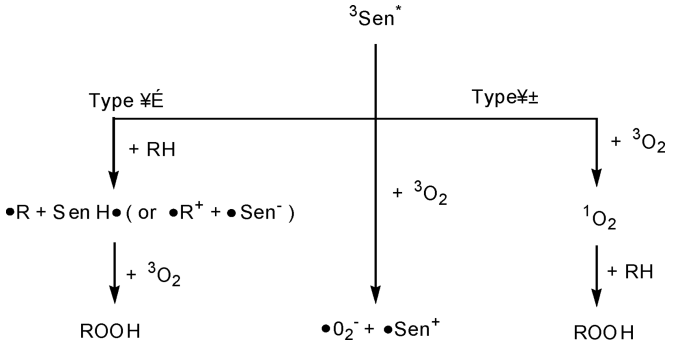

Figure 6 Reaction of triplet sensitizer with substrates.

Summary of Reactive Oxygen Species Formation

Formation of ROS in foods during storage and processing is closely interrelated among ROS as shown in Figure 8. The most important ROS are hydroxy radical and singlet oxygen. Hydrogen peroxide and superoxide anion are important precursors for hydroxy radical and singlet oxygen formation. It is extremely important to control the formation of ROS in foods to improve the food quality.

Superoxide anion is very important in the reduction of oxygen to generate other ROS such as hydrogen peroxide, hydroxy radical, and singlet oxygen. Dismutation of superoxide anion

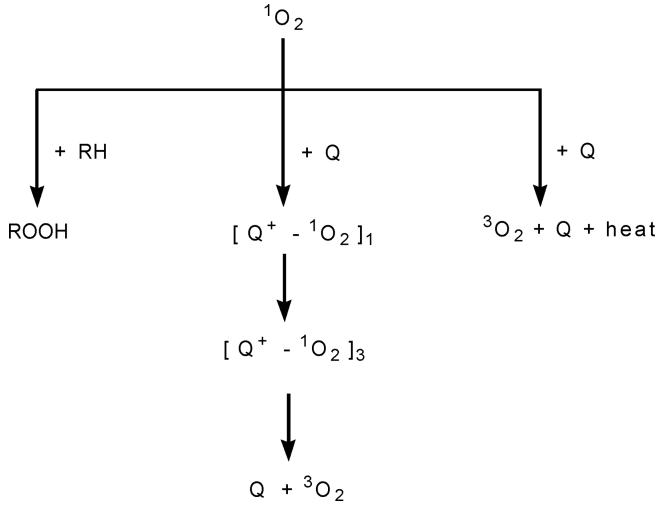

Figure 7 Quenching of singlet oxygen.

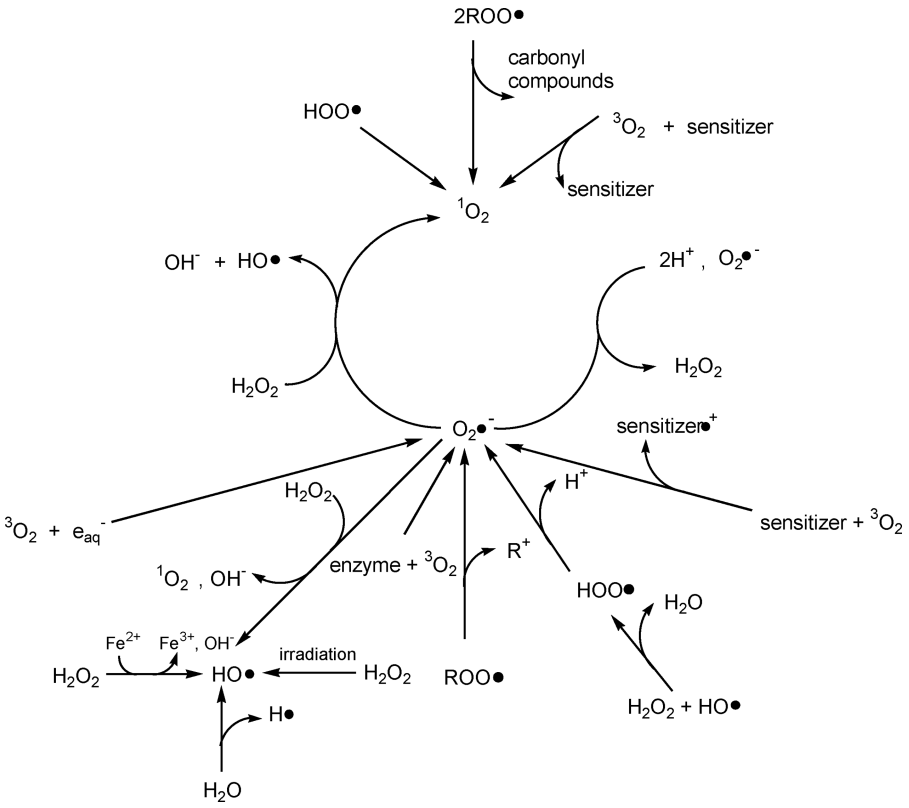

Figure 8 Interrelationships among the formations of reactive oxygen species in foods.

produces hydrogen peroxide, which is a less reactive oxidant than the superoxide. Superoxide dismutase catalyzes the dismutation of superoxide anions to hydrogen peroxide and oxygen. Hydroxy radical is formed by radiolysis of water or by decomposition of hydrogen peroxide by UV, catalases or peroxidases (Symons and Gutteridge, 1998). Reaction of superoxide anion and hydrogen peroxide in the presence of transition metals produces very reactive hydroxy radical and singlet oxygen by Haber-Weiss reaction. Singlet oxygen is most often produced by photosensitization reactions. Photosensitizers such as chlorophyll absorb energy from light and transfer it to triplet oxygen to form singlet oxygen. Peroxy radicals react with other peroxy radicals and produce singlet oxygen by the Russell mechanism (Halliwell and Gutteridge, 2001).

STANDARD ELECTRON REDUCTION POTENTIALS OF REACTIVITY OF REACTIVE OXYGEN SPECIES

The standard electron reduction potentials of reactive oxygen species are shown in Table 1. Hydroxy radical, which has very high standard reduction potential, is one of the most reactive species known. It is an extremely strong oxidizing agent and a powerful electrophilic radical. The electron accepting rate of hydroxy radical is $10^9 \sim 10^{10}$ $M^{-1}s^{-1}$ (Halliwell and Gutteridge, 2001). The electrophilic hydroxy radical reacts with aromatic compounds and double bonds by addition reaction. Superoxide anion reacts rapidly with other compounds. The superoxide anion has a standard reduction potential of −330 mV, but the reduction potential of hydropeoxy radical which is the protonated suproxide anion is 1060 mV. It is well known that the higher the reduction potential of reactive oxygen species, the greater the oxidizing capability of the compound. The reaction rates of hydroperoxy radical with amino acids are 10 to 100 times greater than those of superoxide anion.

REACTION MECHANISMS OF REACTIVE OXYGEN SPECIES WITH FOOD COMPONENTS

Lipids and Cholesterol

Unsaturated lipids are easily oxidized by ROS. Hydrogen peroxide is not directly involved in the initiation of lipid oxidation since its reduction potential of 320 mV is lower than the 600 mV

Table 1 Standard reduction potential of reactive oxygen species

Half-cell	Standard reduction potential (mV)
O_2, $H^+/HO_2\cdot$	−460
$O_2/O_2^{\cdot -}$	−330
H_2O_2, H^+/H_2O, $HO^\cdot$	320
$O_2^{\cdot -}$, $2H^+/H_2O_2$	940
$ROO^\cdot$, $H^+/ROOH$	1000
$HO_2^\cdot$, H^+/H_2O_2	1060
$RO^\cdot$, H^+/ROH	1600
$HO^\cdot$, H^+/H_2O	2310

From Min and Boff, 2002.

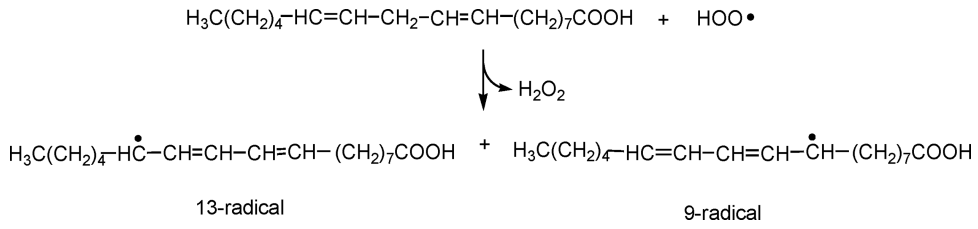

Figure 9 Linoleic acid and hydrogen peroxide reaction for the alkyl radical formation.

of unsaturated fatty acids. However, hydrogen peroxide can be implicated indirectly in lipid oxidation. Hydrogen peroxide is the precursor for the generation of hydroxy radical which are strong initiators of lipid oxidation. The reduction potential of superoxide anion is 940 mV, which is not strong enough to abstract hydrogen from unsaturated fatty acids (Bielski et al., 1983). ROS that have a reduction potential of greater than 1000 mV are thermodynamically capable of oxidizing PUFA which has 600 mV (Koppenol, 1990). The hydroperoxy radical with the reduction potential of 1060 mV is more reactive than the superoxide anion.

Hydroperoxy radical can abstract hydrogen from the lipids and produce lipid radicals ($R^{\cdot}$) as shown Figure 9. It was suggested that double allylic hydrogens are required for the reaction between unsaturated fatty acids and hydroperoxy radical (Bielski et al., 1983). Reaction rates of hydroperoxy radical with linoleic, linolenic, and arachidonic acids are $1.2 \times 10^3\ M^{-1}s^{-1}$, $1.7 \times 10^3\ M^{-1}s^{-1}$, and $3.1 \times 10^3\ M^{-1}s^{-1}$, respectively (Aiken and Dix, 1991; Bielski et al., 1983). Oleic acid did not react with hydroperoxy radical (Bielski et al., 1983). Hydroperoxy radical can also abstract hydrogen from lipid hydroperoxides and produces peroxy radicals as shown in Figure 10.

Lipid peroxy radicals are good oxidizing agents with 1000 mV of standard reduction potential and accelerate the lipid oxidation by abstracting hydrogen from other lipid molecules which have standard reduction potential of about 600 mV at neutral pH (Koppenol, 1990). C-13 peroxy radical of methyl linoleate reacts with linoleic acid at a rate of $1.1 \times 10^6\ M^{-1}s^{-1}$ (Iulaino et al., 1995). The lipid radical formed from this reaction reacts with triplet oxygen to form another lipid peroxy radical. Therefore, lipid oxidation is a catalytic free radical chain reaction. Aromatic peroxy radicals are less reactive than the straight chain peroxy radicals due to the delocalization of electrons in the aromatic ring.

$$ROO^{\cdot} + R'H \rightarrow ROOH + R'^{\cdot}$$

$$R'^{\cdot} + O_2 \rightarrow R'OO^{\cdot}$$

Hydroxy radical is a strong electrophilic compound and is added to the double bond of unsaturated lipids or abstracts hydrogen from lipids (Korycka-Dahl and Richardson, 1978; Lee et al., 2004). Hydroxy radical addition to the unsaturated fatty acids to form hydroxylated fatty acid radical is shown in Figure 11. The hydroxylated fatty acid radical reacts with triplet oxygen and forms hydroxylated lipid peroxy radicals. These peroxy radicals propagate the chain reaction of lipid autoxidation.

Hydroxy radical, which is the strongest oxidant with 2300 mV reduction potential, is mainly responsible for the initiation of lipid oxidation. Hydroxy radical usually abstracts allylic hydrogen from unsaturated lipids because of the relatively low bond dissociation energy. Ninety-five percent of hydrogen is abstracted from secondary carbon to form a free radical. The free radical can react with triplet oxygen for free radical chain reaction.

$$RH + HO^{\cdot} \rightarrow R^{\cdot} + H_2O$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$$

$$R^{\cdot} + R'H \rightarrow RH + R'^{\cdot}$$

Hydroxy radical abstracts hydrogen from sn-2 fatty acyl group of phospholipids, whereas the C-7 hydrogen of cholesterol is the most reactive and abstracted by hydroxy radical (Smith,

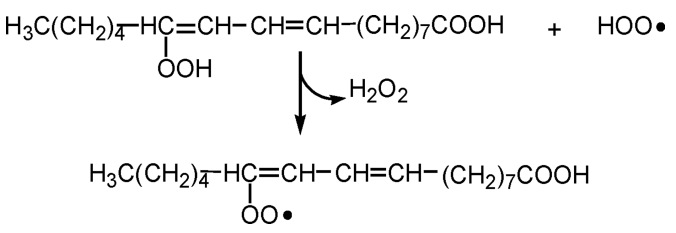

Figure 10 Reaction of linoleic acid hydroperoxide and hydroperoxyl radical for peroxy radical formation.

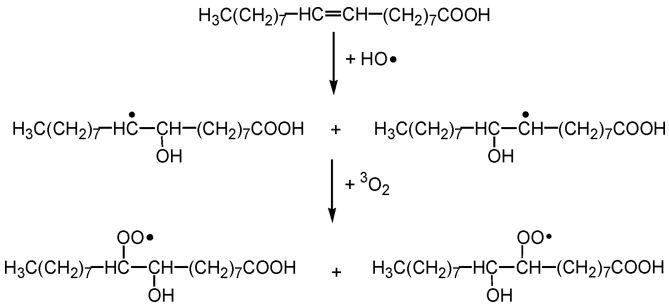

Figure 11 Addition reaction of electrophilic hydroxy radical to oleic acid.

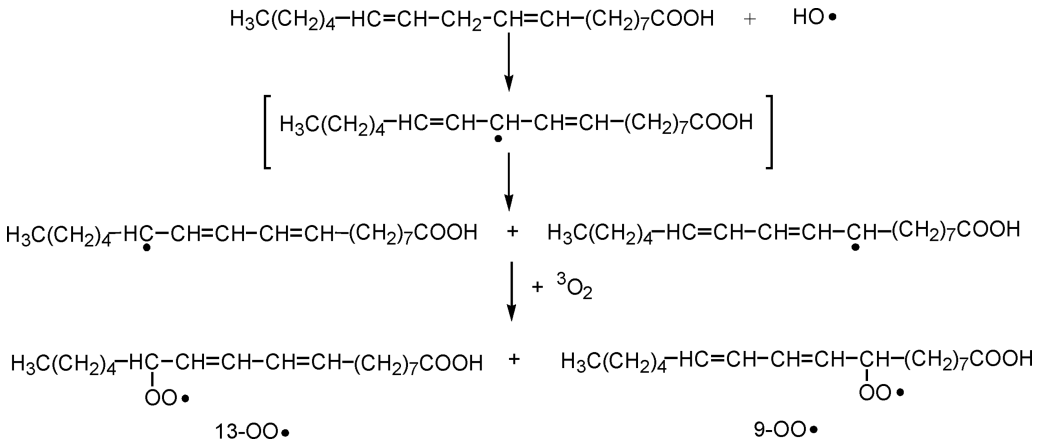

Figure 12 Hydroxy radical initiated oxidation of linoleic acid by triplet oxygen.

1981). The lipid radical abstracts hydrogen from other neighboring molecules or reacts with triplet oxygen and the lipid oxidation chain reaction takes place (Figure 12).

Alkoxy radical whose standard reduction potential is 1600 mV can abstract hydrogen from lipids having 600 mV of reduction potentials and continue the chain reaction of lipid oxidation. Singlet oxygen can directly attack the double bond of unsaturated lipid via ene reaction and produce allyl hydroperoxide. The double bond position is shifted during the ene reaction and the type of double bond is also changed from cis to trans (Min and Boff, 2002). Hydroperoxide formation from linoleic acid by ene reaction of singlet oxygen is shown in Figure 13. The ene reaction is commonly found in edible oils containing chlorophyll during storage under light. Min and Boff (2002) suggested that singlet oxygen is involved in the initiation of lipid oxidation.

The reaction rate between lipid and singlet oxygen is much higher than that of triplet oxygen. The reaction rates of triplet oxygen and singlet oxygen with linoleic acid are 8.9×10^1 $M^{-1}s^{-1}$ and 1.3×10^5 $M^{-1}s^{-1}$, respectively (Min and Boff, 2002). This high oxidation rate of singlet oxygen explains the importance of singlet oxygen in lipid oxidation. Singlet oxygen oxidation rates with oleic, linoleic, and linolenic acids are not greatly different whereas the double bond numbers

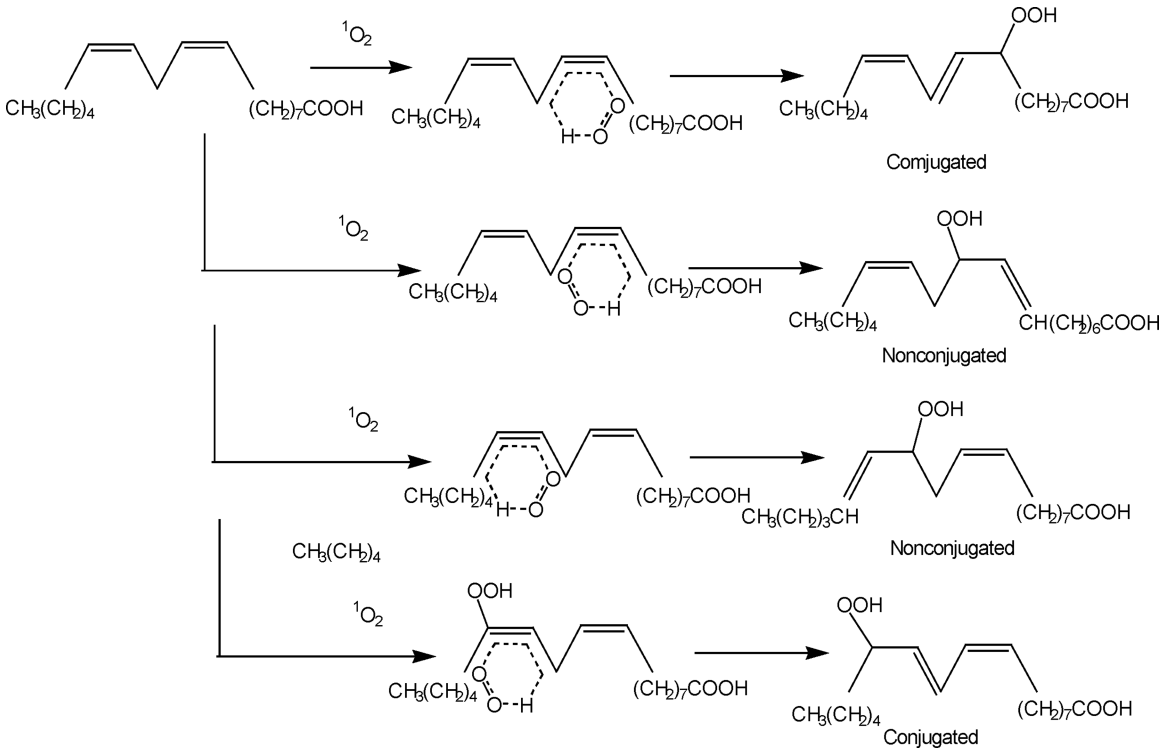

Figure 13 Linoleic acid hydroperoxide formation by ene reaction of singlet oxygen.

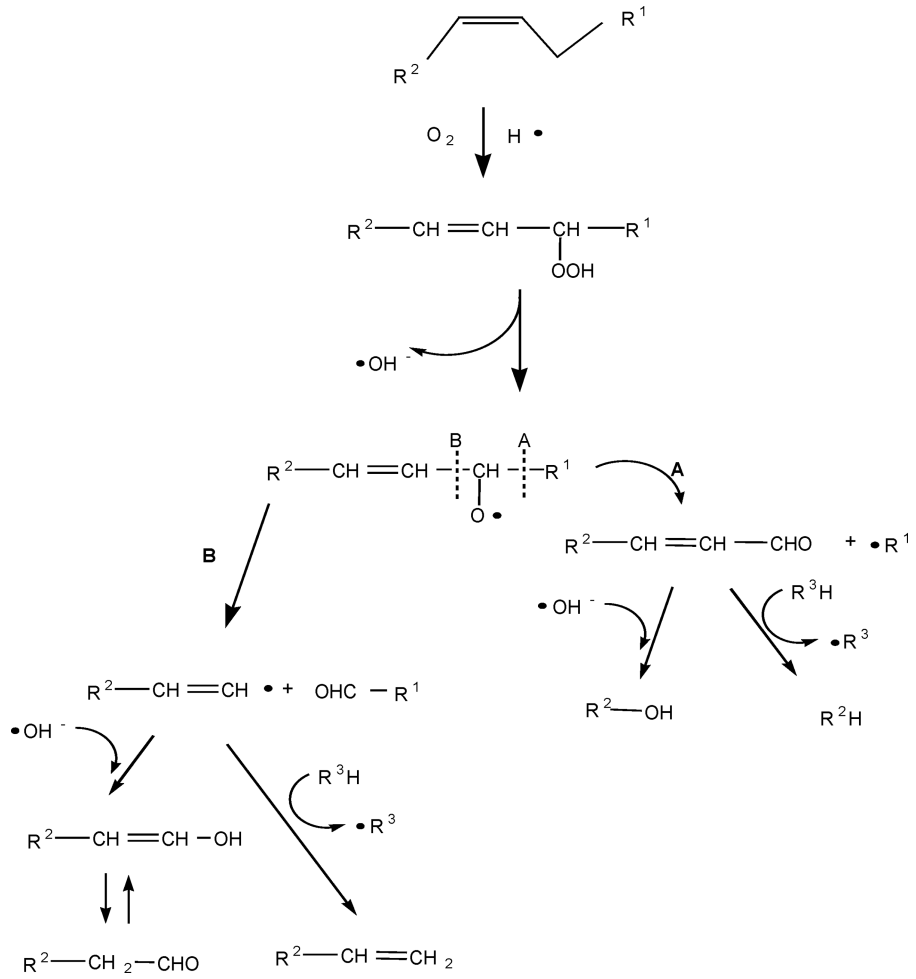

Figure 14 Decomposition of lipid hydroperoxides.

have noticeable effects on triplet oxygen oxidation. The reaction rates of stearic, oleic, linoleic, and linolenic acids with singlet oxygen are 1.2×10^4 $M^{-1}s^{-1}$, 5.3×10^4 $M^{-1}s^{-1}$, 7.3×10^4 $M^{-1}s^{-1}$, and 1.0×10^5 $M^{-1}s^{-1}$ (Vever-Bizet et al., 1989), respectively. Soybean oil reacts with singlet oxygen at a rate of 1.4×10^5 $M^{-1}s^{-1}$ in methylene chloride at 20°C (Lee and Min, 1991). Soybean oil oxidation by singlet oxygen can be minimized by the removal of chlorophyll from oil during refining.

Many factors such as inhibitors or catalysts and reaction environment affect the reaction rate between lipids and singlet oxygen. However, the reaction temperature and the type of polyunsaturated fatty acids, non-conjugated or conjugated dienes or trienes, have little effect on singlet oxygen oxidation compared to triplet oxygen oxidation due to its low activation energy of 0 to 6 kcal/ mole (Yang and Min, 1994).

Lipid hydroperoxides are decomposed to alkoxy radical by the homolytic cleavage between the oxygen and oxygen which has lower bond energy of 44 kcal/mole than the 90 kcal/mole between oxygen and hydrogen of hydroperoxide (Hiatt et al., 1968). The alkoxy radical is cleaved by homolytic β-scission of the C—C bond and produces oxo compound, aldehydes, acids, alcohols, and short chain hydrocarbons (Lee et al., 2003) as shown in Figure 14.

Cholesterol is oxidized by ROS (Doleiden et al., 1974; Girotti and Korytowski, 2000) although the oxidation rate is relatively low compared to polyunsaturated fatty acid-containing phospholipids (Girotti, 1998). Reaction between singlet oxygen and cholesterol produces 5α-OOH (3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide), 5β-OOH (3β-hydroxy-5β-cholest-6-ene-5-hydroperoxide), 6α-OOH (3β-hydroxy-6α-cholest-6-ene-5-hydroperoxide), and 6β-OOH (3β-hydroxy-6β-cholest-6-ene-5-hydroperoxide; Foote, 1991; Yamazaki et al., 1999) and does not produce 7α- or 7β-OOH (Beckwith et al., 1989). The cholesterol hydroperoxides are further degraded to 7-keto (5-cholesten-3β-ol-7-one), 5,6β-EP (cholestan-5β,6β-epoxy-3β-ol), 3,5-cholestadien-7-one, 5,6α-EP (cholestan-5α,6α-epoxy-3β-ol), 7α-OH (5-cholesten-3β,7α-diol), and 7β-OH (5-cholesten-3β,7β-diol) in decreasing order (Chien et al., 2003). Hydroxy radical abstracts hydrogen from cholesterol, mainly C-7 hydrogen (Girotti, 1998), producing radicals. In free radical mediated reactions, the epimeric pair 7α- and

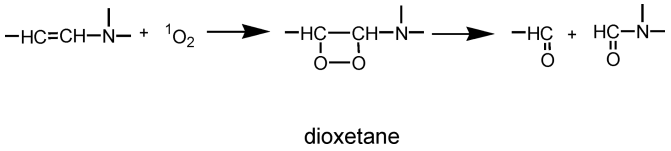

Figure 15 Formation of dioxetane by singlet oxygen.

7β-OOH are generally the most prominent hydroperoxides, with lesser amounts of 7α- and 7β-OH, 7-ketone, and epimeric 5,6-epoxides (Sevanian and McLeod, 1987; Ozawa et al., 1991). The reaction rate of cholesterol with singlet oxygen is 2.5×10^8 $M^{-1}s^{-1}$ (Kruk et al., 1992). The cholesterol oxidation products are one of the important classes of toxic compounds (Hu and Chen, 2002) and are carcinogens and mutagens (Chien et al., 2003).

Proteins and Amino Acids

Proteins and amino acids are oxidized by ROS, mostly hydroxy radical and singlet oxygen. Reaction rates of superoxide anion and hydroperoxy radical with amino acids are very low. Superoxide anion selectively oxidizes iron-sulfur containing proteins and inactivates their biological activities (Fridovich, 1986).

Singlet oxygen reaction with proteins containing an electron-donating double bond activated by amino or alkoxy groups to produce dioxetanes is shown in Figure 15. Dioxetanes are unstable at room temperature and are easily decomposed by C—C cleavage and produce carbonyl compounds (Bartlett and Schaap, 1970; Mazur and Foote, 1970; Jefford et al., 1978).

Protein oxidation by hydroxy radical produces crosslinked products and carbonyl compounds as shown in Figure 16 (Levine

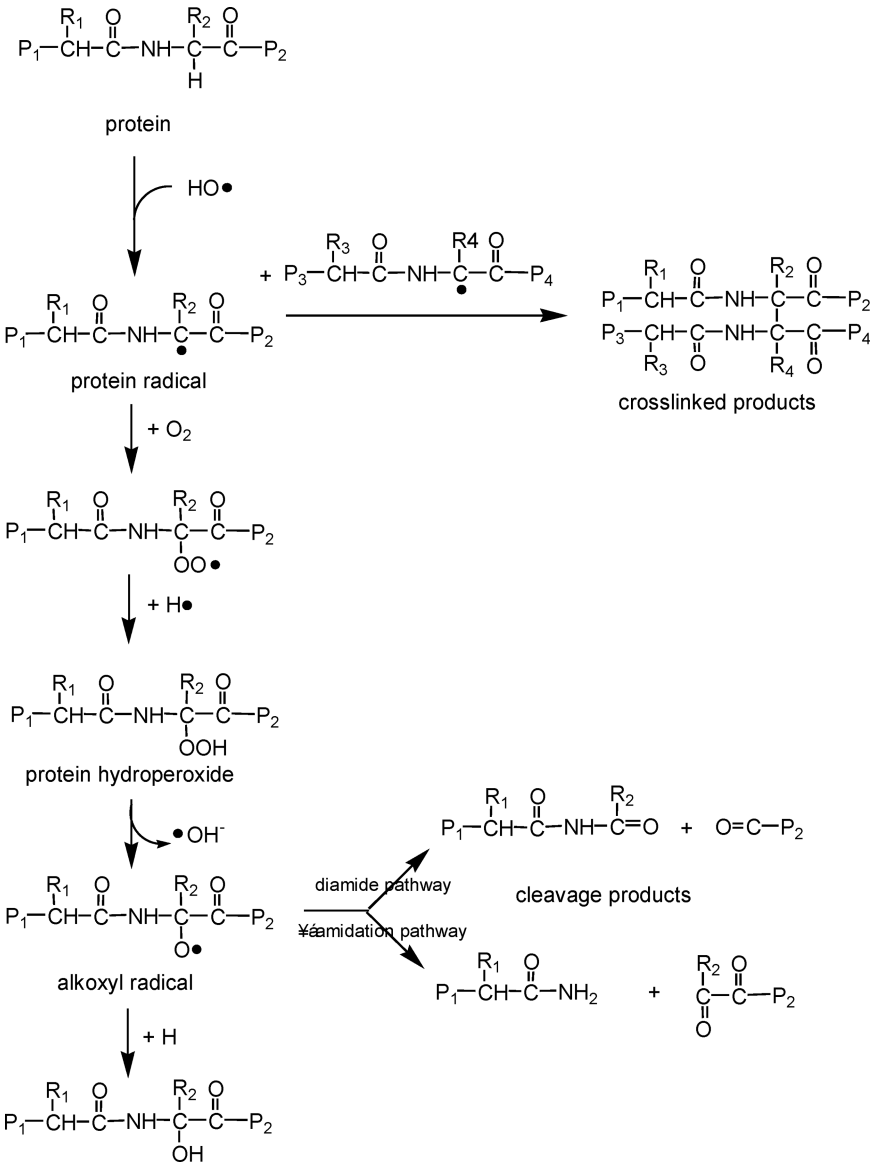

Figure 16 Oxidation of proteins by hydroxy radical.

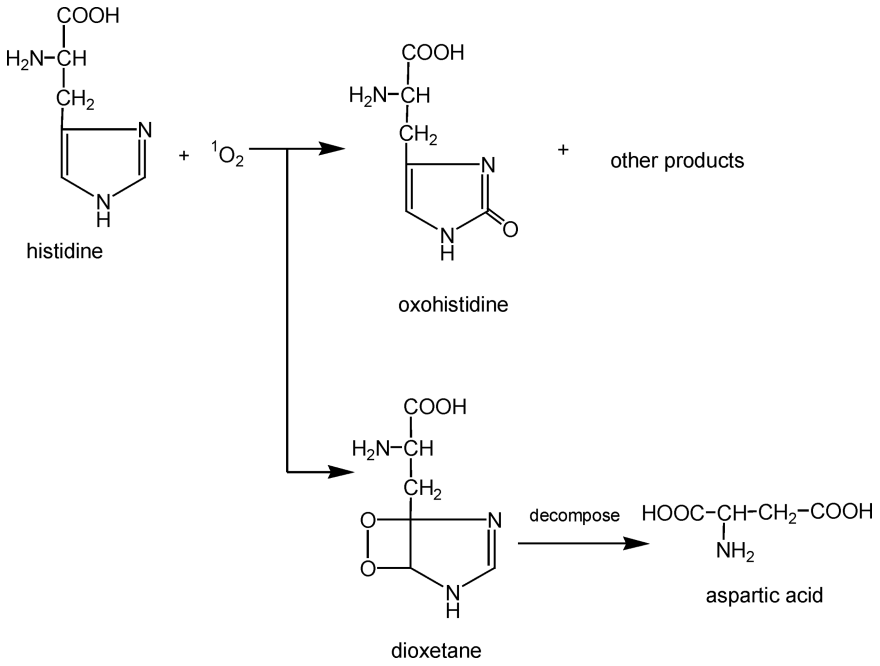

Figure 17 Oxidation of histidine by singlet oxygen.

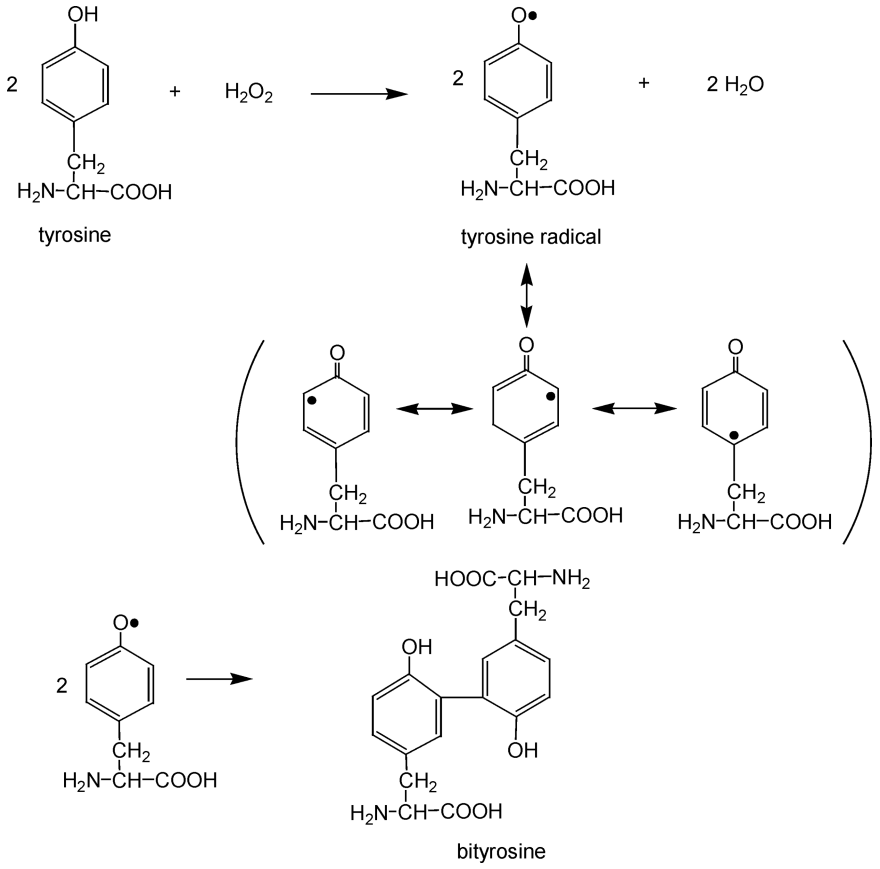

Figure 18 Oxidation of tyrosine by hydrogen peroxide.

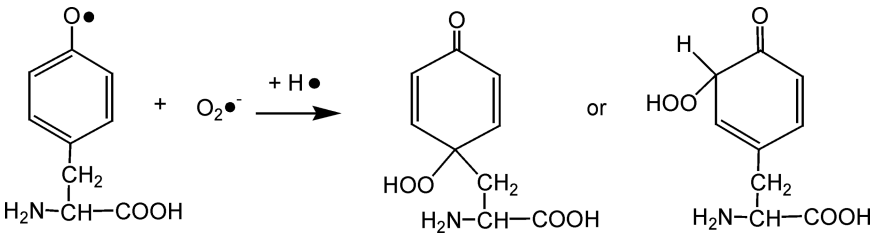

Figure 19 Formation of tyrosine peroxide from tyrosine radical by superoxide.

and Stadtman, 2001). Hydroxy radical which has a reduction potential of 2310 mV can abstract hydrogen from proteins with the reduction potential of less than 1000 mV (Ahmad and Armstrong, 1984; Pruetz et al., 1986; Harriman, 1987) and produce protein radicals. Protein radicals react with each other and produce crosslinked products. Protein radical reacts with oxygen and produces protein peroxy radical and then protein hydroperoxide by abstracting hydrogen from other molecules. Protein or amino acid radicals can abstract hydrogen from other amino acid with lower reduction potential and oxidize the other amino acid (Halliwell and Gutteridge, 2001). Tryptophane radical which has the reduction potential of 1020 mV can oxidize tyrosine with 930 mV (Harriman, 1987). Tyrosine radical can oxidize cysteine which has the reduction potential of 730 mV (Pruetz et al., 1986). Protein hydroperoxides can be easily decomposed to alkoxy radicals by heat or in the presence of transition metal ions. Alkoxy radicals are reduced to hydroxylated protein. Cleavage at the carbon bonded to the oxygen radical via diamide or α-amidation pathways to produce carbonyl compounds (Ashok and Ali, 1999; Lee et al., 2004) is also shown in Figure 16.

Histidine

The histidine reaction rate with singlet oxygen is 4.6×10^7 $M^{-1}s^{-1}$ (Michaeli and Feitelson, 1994) and its rate with hydroxy radical is 4.8×10^9 $M^{-1}s^{-1}$ (Motohashi and Saito, 1993). The reaction rates of histidine with superoxide anion and hydroperoxy radical are less than 1.0 $M^{-1}s^{-1}$ and less than 95 $M^{-1}s^{-1}$, respectively and are very low (Bielski and Shiue, 1979). Reaction of hydroxy radical upon histidine produces histidine radical. Histidine radicals react with each other to form crosslinked products. Histidine radical also reacts with triplet oxygen to produce hydroperoxides. By decomposition of hydroperoxides, carbonyl compounds are produced.

The reaction between histidine and singlet oxygen produces 2-oxohistidine (Halliwell and Gutteridge, 2001) or cleavage products such as aspartic acid via either a dioxetane (Figure 17) or endoperoxide formation.

Tryptophane

The reaction rate of tryptophanes with singlet oxygen is 1.3×10^7 $M^{-1}s^{-1}$ (Michaeli and Feitelson, 1994) and the reaction produces peroxy radicals, peroxides, and eventually N-formylkynurenine and kynurenine. Tryptophane reacts with hydroxy radical at a rate of 1.3×10^{10} $M^{-1}s^{-1}$ at pH 6.5∼8.5 (Solar et al., 1984).

Tyrosine

Tyrosine is hydroxylated to dihydroxyphenylalanine by the reaction with hydroxy radical. Tyrosine reacts with hydrogen peroxide and forms tyrosine radicals (Tyr·) in the presence of peroxidase, and finally gives off dityrosine by radical-radical coupling as shown in Figure 18 (Pichorner et al., 1995).

Superoxide radical decreases dityrosine formation from tyrosine radical by producing tyrosine peroxide (Pichorner et al., 1995) as shown in Figure 19.

Cysteine and Cystine

The reaction rate of cysteine (CysSH) with hydroxy radicals is 1.9×10^{10} $M^{-1}s^{-1}$ (Hoffman and Hayon, 1973). Thiol group

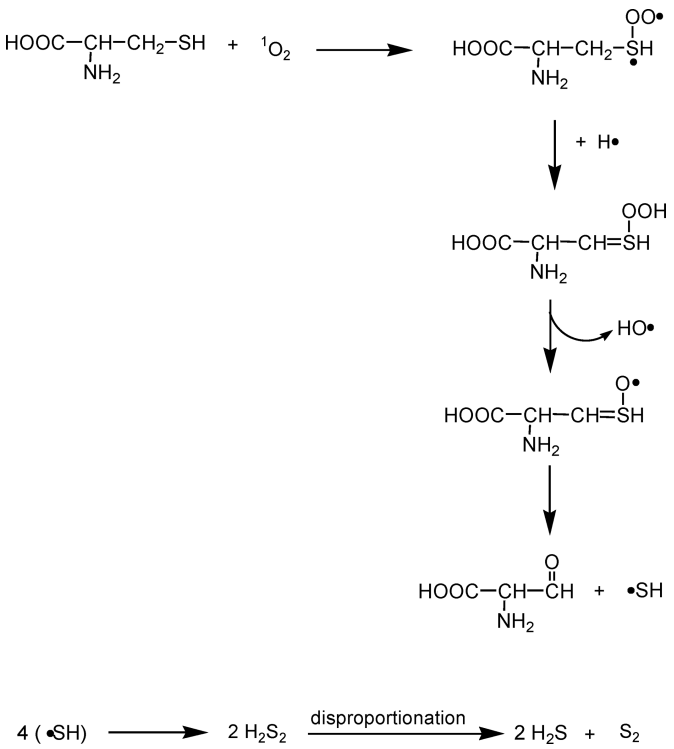

Figure 20 Hydrogen sulfide formation from singlet oxygen oxidation of cystein.

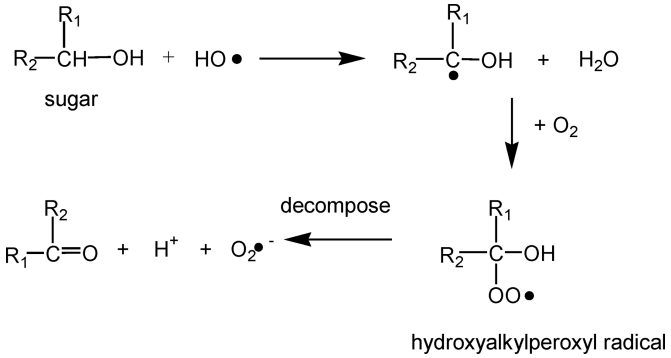

Figure 21 Oxidation of sugars by hydroxy radical.

in cystein becomes cysteine thiyl radicals ($CysS^{\cdot}$) by hydroxy radical. The thiyl radical reacts with another cysteine molecule to form cystine, CysSSCys (Adams and Wardman, 1976), or is further oxidized to sulfonic acid ($CysSO_3H$; Halliwell and Gutteridge, 2001). Sulfonic acid is also produced by the reaction of cysteine with hydrogen peroxide (Denu and Tanner, 1998).

$$CysSH + {}^{\cdot}OH \rightarrow CysS^{\cdot} + H_2O$$

$$CysS^{\cdot} + CysSH \rightarrow CysS - SCys + H^{\cdot}$$

$$CysS^{\cdot} + O_2 \rightarrow CysSOO^{\cdot}$$

$$CysSOO^{\cdot} + O_2 \rightarrow CysSO_2OO^{\cdot}$$

$$CysSO_2OO^{\cdot} \xrightarrow{\text{Reduction}} CysSO_2OOH \xrightarrow{\text{Reduction}} CysSO_3H + H_2O$$

The reaction rate of cysteine with singlet oxygen is $5.0 \times 10^7\ M^{-1}s^{-1}$ (Michaeli and Feitelson, 1994) and the reaction produces peroxy radical and then produces hydrogen sulfide as shown in Figure 20.

Hydroxy radical can directly attack electron rich sulfur in cystine whose reduction potential is 700 mV (Ahmad and Armstrong, 1984) at a rate of $2.1 \times 10^9\ M^{-1}s^{-1}$ (Masuda et al., 1973) and produce hydroxylated products. Adams and Wardman (1976) reported unusual trisulfide CysSSSCys upon reaction of hydroxylated disulfide with other cysteine molecules in fairly high yield.

$$CysSSCys + \cdot OH \rightarrow CysSOH + CysS\cdot$$

$$CysSSCys + \cdot OH \rightarrow CysSSOH + Cys\cdot$$

$$CysSSOH + CysSH \rightarrow CysSSSCys + H_2O$$

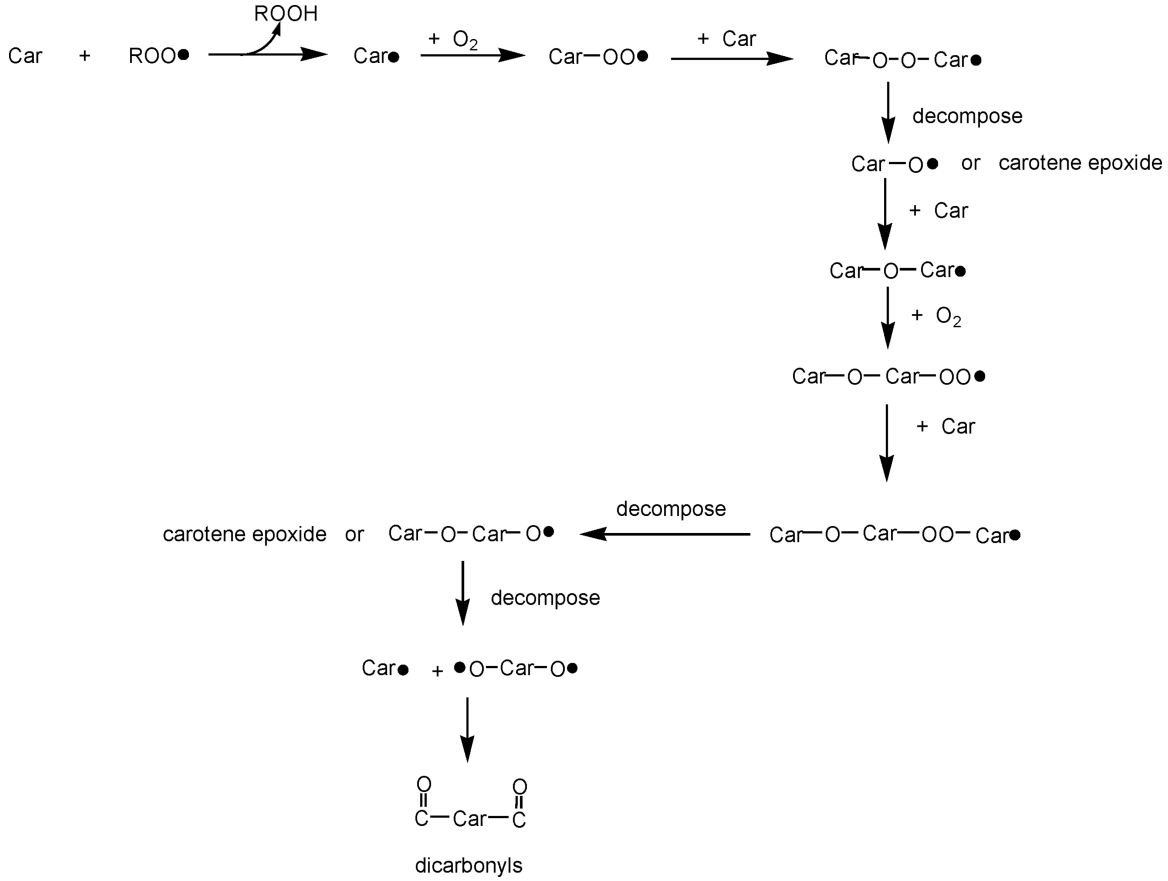

Figure 22 Oxidation of β-carotene by peroxyl radical through carotene radical.

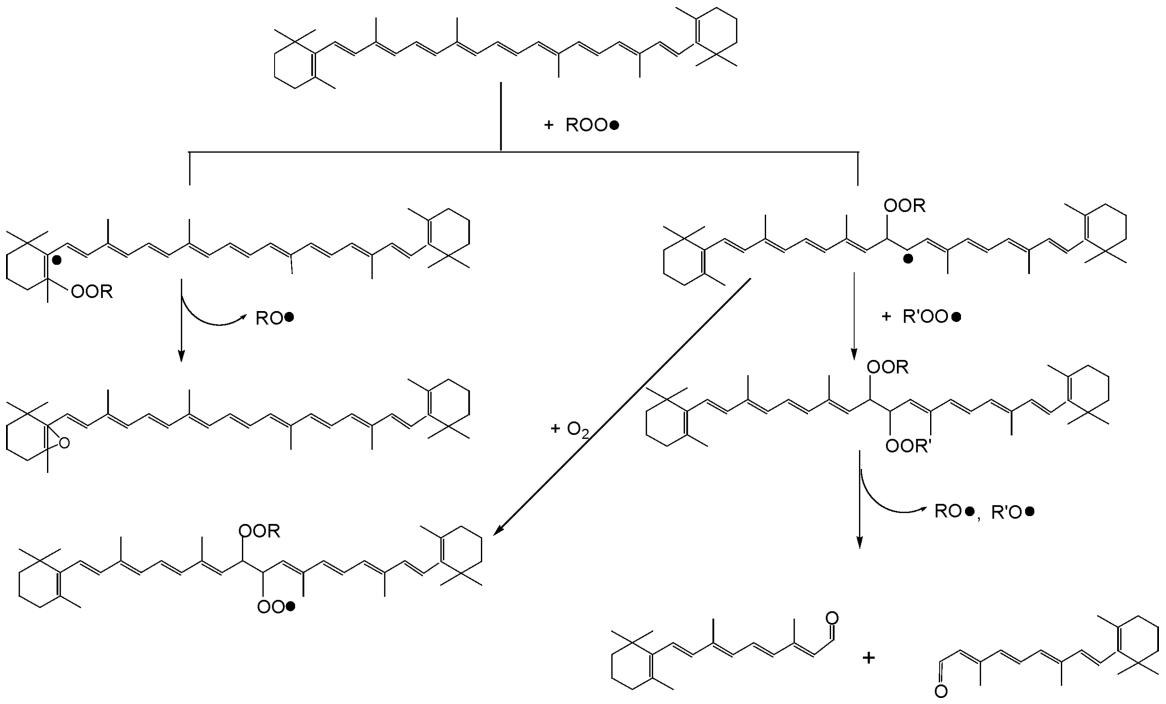

Figure 23 Addition reaction of peroxyl radical to β-carotene.

Methionine

Methionine is readily oxidized by hydroxy radical at the rate of 7.4×10^9 $M^{-1}s^{-1}$ (Zhao et al., 1994) and by singlet oxygen at 1.3×10^7 $M^{-1}s^{-1}$ (Michaeli and Feitelson, 1994). The singlet oxygen oxidation of methionine produces methionine sulfoxide. Methionine sulfoxide can undergo further oxidation to the sulfone (Foote, 1976).

Sugars

Hydroxy radical reacts readily with sugars such as glucose, fructose, maltose, and sucrose at the rate of 10^9 $M^{-1}s^{-1}$ (Bucknall et al., 1978; Moore et al., 1979; Motohashi and Saito, 1993). Hydroxy radical abstracts hydrogen from glucose whose reduction potential is less than 500 mV (Vassilyev et al., 1985) and forms glucosyl radical which will react with triplet oxygen to form peroxy radical of sugars. Peroxy radical of sugars is decomposed and produces low molecular weight carbonyl compounds and superoxide anion (Foote, 1978) as shown in Figure 21.

Oxidation of glucose and sucrose by singlet oxygen is much slower than that by hydroxy radicals. The reaction rates of glucose and sucrose with singlet oxygen are 1.4×10^4 $M^{-1}s^{-1}$ and 2.5×10^4 $M^{-1}s^{-1}$ (Egorov and Krasnovsky, 1986), respectively.

Vitamins

β-Carotene

β-Carotene, provitamin A, reacts with ROS such as peroxy and hydroxy radicals and singlet oxygen. Peroxy radical can abstract hydrogen from β-carotene or be added to β-carotene. The reduction potentials of β-carotene and peroxy radical are 542 mV and 1000 mV, respectively (Jeevarajan and Kispert, 1996). β-Carotene (Car) can donate hydrogen to peroxy radicals and form carotene radical (Car·). Carotene radical undergoes repeated radical abstraction and oxygen addition reactions and produces carotene epoxides and carbonyl compounds of carotene (Beutner et al., 2001) as shown in Figure 22.

Peroxy radical is added to β-carotene and produces carotene peroxy adducts as shown in Figure 23 (Decker, 2002). Peroxy radical is added to the cyclic end group of β-carotene, then alkoxy radical is lost, and produces 5,6-epoxides of carotene. A peroxy radical is also added to the polyene chain of the β-carotene specifically at 15 and 15′ position and peroxides of carotene radical (ROO-Car·) are produced (Iannone et al., 1998). The peroxides of carotene radical react with another peroxy radical and produce nonradical compound (R′OO-Car-OOR), or react with oxygen to produce peroxy radical which accelerates the chain reaction of lipid oxidation. Cleavage in the polyene chain in the nonradical compound (R′OO-Car-OOR) after

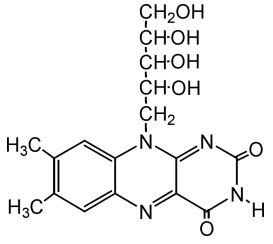

Figure 24 Structure of riboflavin.

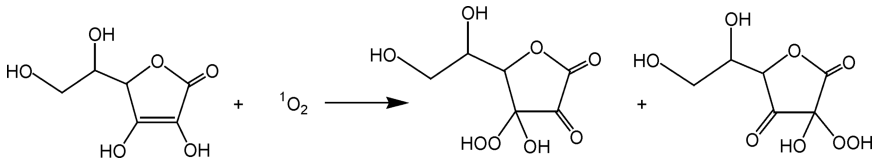

Figure 25 Hydroperoxide of ascorbic acid by singlet oxygen oxidation.

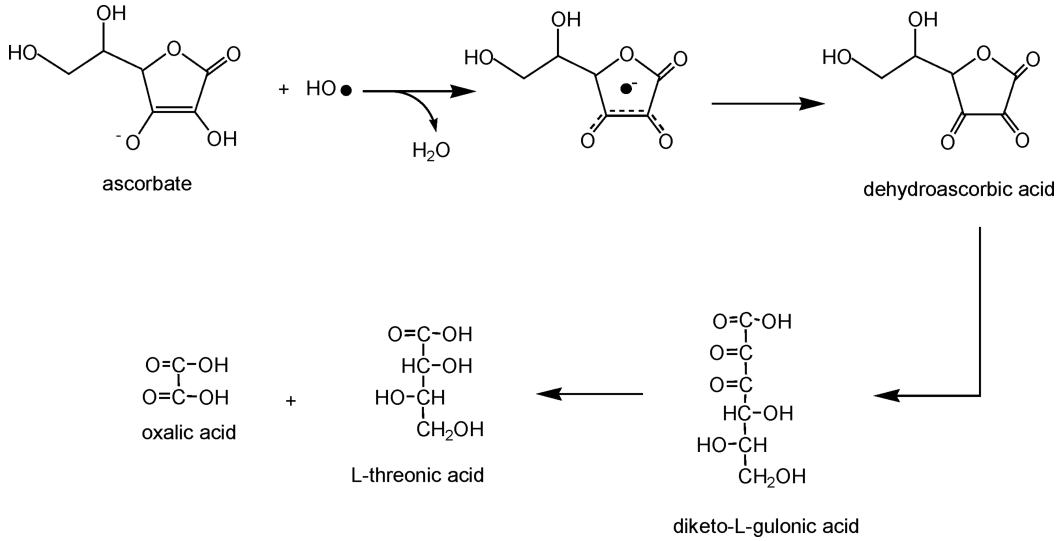

Figure 26 Oxidation of ascorbate by hydroxy radical.

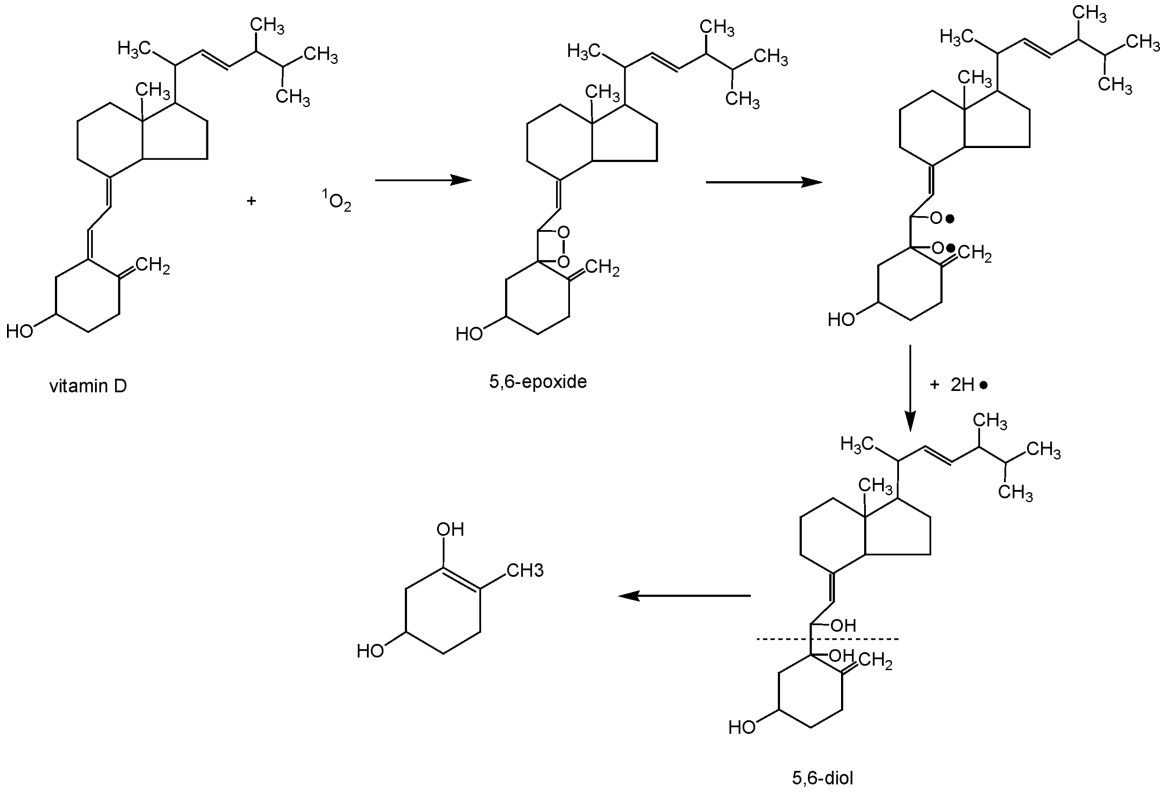

Figure 27 Singlet oxygen oxidation of vitamin D.

elimination of alkoxy radical produces aldehyde compounds containing cyclic group.

β-Carotene having many double bonds can donate an electron to peroxy radical and produce β-carotene cation radical and peroxy anion. β-Carotene cation radical is stable due to resonance and the rate of reaction with oxygen is very low (Decker, 2002).

β-Carotene reacts with hydroxy radical and produces carotene radical (Car$^{\cdot}$) which is fairly stable species due to electron delocalization in the structure. Carotene radical reacts with other radicals rather than molecular triplet oxygen and forms nonradical products.

$$\text{Car} +^{\cdot}\text{OH} \rightarrow \text{Car}^{\cdot} + \text{H}_2\text{O}$$

$$\text{Car}^{\cdot} + \text{ROO}^{\cdot} \rightarrow \text{Car-OOR}$$

β-Carotene reacts with singlet oxygen at the rate of 5.0×10^9 $\text{M}^{-1}\text{s}^{-1}$ (Devasagayam et al., 1992) and produces 5,8-endoperoxides of β-carotene (Stratton et al., 1993). β-Carotene does not react with alkoxy radicals. The oxidation of carotenoids by ROS causes the loss of their characteristic color (Halliwell and Gutteridge, 2001).

Riboflavin

Riboflavin, a photosensitizer, is oxidized by ROS. Riboflavin whose reduction potential is -318 mV (Anderson 1983) reacts with hydroxy radicals at 1.2×10^{10} $\text{M}^{-1}\text{s}^{-1}$ (Kishore et al., 1991). Riboflavin having many double bonds in the structure (Figure 24) can directly react with singlet oxygen (Allen and Parks, 1979) at the rate of 6.0×10^7 $\text{M}^{-1}\text{s}^{-1}$ (Chacon et al., 1988).

Maniere and Dimick (1976) reported that free-form riboflavin was more labile to light than riboflavin bound to protein. Sunlight was more detrimental to riboflavin than fluorescent light (Bradley and Min, 1992). Light wavelength of 450 nm, which corresponds to the maximum absorption of riboflavin, was the most detrimental. Riboflavin degradation by singlet oxygen was a first order reaction with the light exposure time (Allen and Parks, 1979). Degradation of riboflavin by singlet oxygen was affected by intensity and wavelength of light, light exposure time, packaging materials, and type of food in which riboflavin is present. King and Min (1998) reported that the degradation rate of riboflavin was lower in whole milk (1.5×10^5 s^{-1}) than in skim milk ($1.89 \times 10^5 \text{s}^{-1}$). When oxygen is not present under light, riboflavin was very stable (Gutierrez et al., 2001).

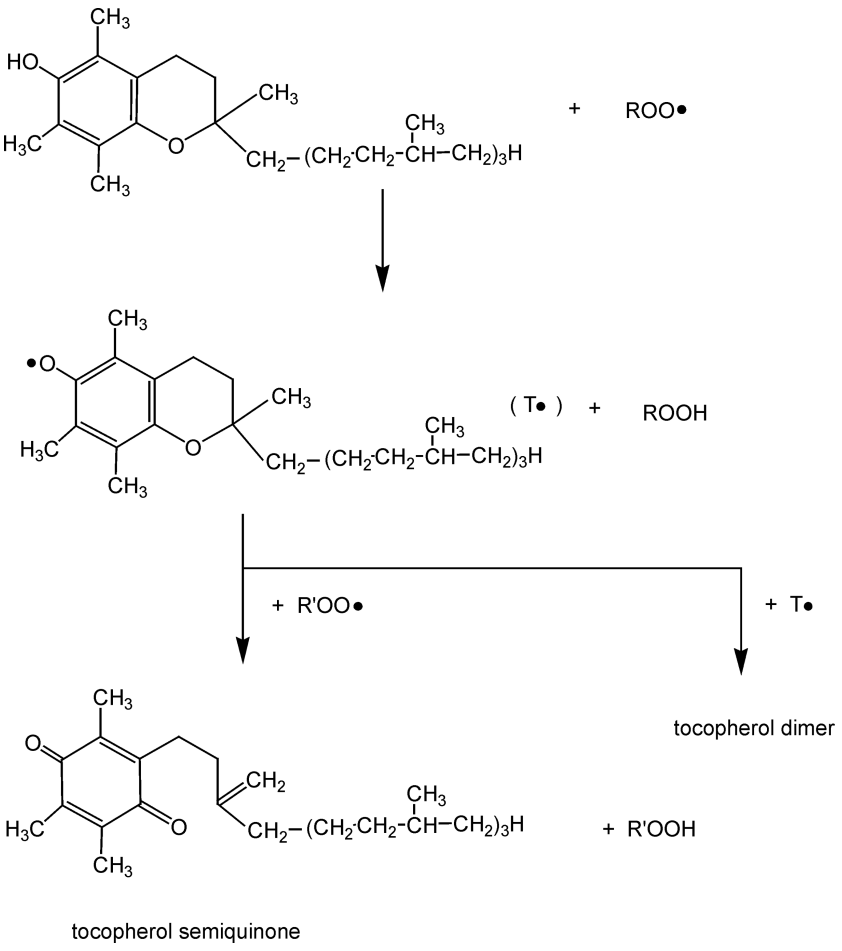

Figure 28 Reaction of tocopherol with peroxyl radical.

Ascorbic Acid

The reaction rates of ascorbic acid with singlet oxygen and hydroxy radical were 1.1×10^7 $M^{-1}s^{-1}$and 8.2×10^9 $M^{-1}s^{-1}$, respectively (Rooney, 1983; Schoeneshoefer, 1972). Ascorbic acid is oxidized by superoxide anion with the reduction potential of −330 mV (Steenken and Neta, 1982). Reaction of ascorbic acid with singlet oxygen produces unstable hydroperoxide of ascorbic acid as shown in Figure 25. The reaction rate of ascorbic acid with singlet oxygen decreased with the pH decrease of the solution (Jung et al., 1995). The ascorbate ion was more reactive with singlet oxygen than ascorbic acid (Bisby et al., 1999).

Superoxide anion reacts with ascorbic acid and produces dehydroascorbic acid by abstracting proton. The reaction rate of superoxide with ascorbic acid was reported as 2.7×10^5 $M^{-1}s^{-1}$ (Halliwell and Gutteridge, 2001). The reaction of superoxide and ascorbic acid produces hydrogen peroxide, too. Dehydroascorbic acid is unstable and breaks down rapidly to produce oxalic acid and L-threonic acid.

Hydroxy radical oxidizes ascorbic acid and produces dehydroascorbic acid by abstracting proton as shown in Figure 26. The reaction rate of ascorbic acid with hydroperoxy radical is 1.6×10^4 $M^{-1}s^{-1}$ (Cabelli and Bielski, 1983).

Vitamin D

Vitamin D has 650 mV of standard reduction potential and is oxidized by ROS (Hasegawa, 1992). King and Min (2002) reported that vitamin D was oxidized by singlet oxygen and produced 5,6-epoxide (Figure 27). The oxidation of vitamin D by singlet oxygen was temperature-independent (Li and Min, 1998) and the rate constant was 2.2×10^7 M^{-1} s^{-1} (Li et al., 2000).

Tocopherols

Tocopherol reacts with peroxy radicals. Hydrogen atom at 6-hydroxy group on chromanol ring of tocopherols is transferred to peroxy radical and produces alkyl hydroperoxide and tocopheryl radical (T·), which is relatively stable due to resonance structure. Reaction rates of peroxy radical of stearic and oleic acids with α-tocopherol are 2.8×10^6 $M^{-1}s^{-1}$ and 2.5×10^6 $M^{-1}s^{-1}$, respectively (Simic, 1980). Reaction of tocopherols with peroxy radicals slows down the lipid oxidation. Tocopherols compete with unsaturated lipids for lipid peroxy radicals. Lipid peroxy radical with the reduction potential of 1000 mV reacts with tocopherol much faster at $10^4 \sim 10^9$ $M^{-1}s^{-1}$ than with lipid at $10 \sim 60$ $M^{-1}s^{-1}$ due to lower reduction potential of tocopherols of 500 mV than that of unsaturated lipid of 600 mV. One tocopherol molecule can protect about $10^3 \sim 10^8$ polyunsaturated

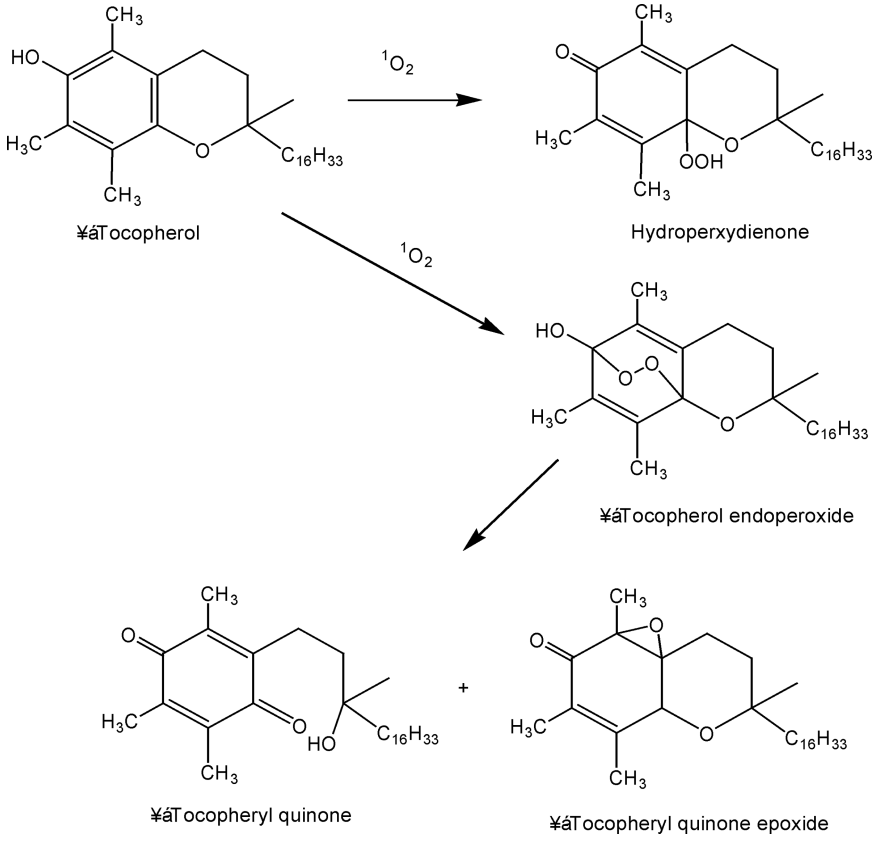

Figure 29 Singlet oxygen oxidation of α-tocopherol.

fatty acid molecules at low peroxide value (Kamal-Elden and Appelqvist, 1996).

Tocopheryl radical can react with peroxy radical and produces tocopherol semiquinone (Reische et al., 2002), or it reacts with another tocopheryl radical and forms tocopherol dimer as shown in Figure 28.

At very high concentrations of lipid peroxy radical, tocopheryl radical can react with lipid peroxy radical to produce tocopherol peroxide (TOOR).

$$T + ROO^{\cdot} \rightarrow T\cdot + ROOH$$

$$T\cdot + ROO^{\cdot} \rightarrow TOOR$$

Even though it is very slow, tocopheryl radical sometimes abstracts hydrogen from lipids to give tocopherol and lipid radical at a very low concentration of lipid peroxy radical. Lipid radical promotes the lipid oxidation, so called tocopherol-mediated peroxidation (Bowry and Stocker, 1993; Yamamoto, 2001). Ascorbic acid (Asc) quickly reduces tocopheryl radical and prevents tocopherol-mediated peroxidation (Yamamoto, 2001).

$$T\cdot + RH \rightarrow T + R\cdot$$

$$R\cdot + O_2 \rightarrow ROO^{\cdot}$$

$$T\cdot + Asc \rightarrow T + \text{Dehydroascorbic acid}$$

Tocopherol is slowly oxidized by hydroperoxy radicals at the rate of 2.0×10^5 $M^{-1}s^{-1}$; (Arudi et al., 1983) and produces substituted tocopherones and epoxy or hydroperoxy tocopherone (Halliwell and Gutteridge, 2001). The tocopherone and epoxy tocopherone have no vitamin E activity and are hydrolyzed to tocopherylquinone and epoxyquinone, respectively.

Superoxide slowly and irreversibly oxidizes tocopherol in organic solvent and produces tocopheryl radical, but the reaction is insignificant in aqueous solution (Arudi et al., 1983; Halliwell and Gutteridge, 2001).

Tocopherol reacts irreversibly with singlet oxygen and produces tocopherol hydroperoxydienone, tocopherylquinone, and quinone epoxide as shown in Figure 29. The reaction rate of tocopherol with singlet oxygen is affected by the structure of tocopherol. The α-tocopherol showed the highest reaction rate of 2.1×10^8 $M^{-1}s^{-1}$, followed by β-tocopherol with 1.5×10^8 $M^{-1}s^{-1}$, γ-tocopherol with 1.4×10^8 $M^{-1}s^{-1}$ and δ-tocopherol with 5.3×10^7 $M^{-1}s^{-1}$ (Mukai et al., 1991).

Summary of ROS Reaction Rates with Food Components

Reaction rates of ROS with food components are summarized in Table 2. Among ROS, hydroxy radical is the most reactive oxygen species followed by singlet oxygen. Superoxide anion and hydroperoxy radicals are much less reactive oxidants of food components. Amino acids and vitamins are more labile to the singlet oxygen oxidation than lipids or sugars.

Singlet oxygen has high energy and is an electrophilic compound. It reacts easily with polyunsaturated fatty acids and aromatic amino acids which have electron-rich double bonds in the molecules. The higher the unsaturation of fatty acids, the greater the reactions with singlet oxygen as shown in Table 2. The reaction rate of singlet oxygen with linoleic acid is about 1450 times greater than that of triplet oxygen. The higher the reduction potential of reactive oxygen species, the greater the oxidation reaction rate with food compounds. Hydroxy radical, which is electrophilic and has high reduction potential, can react with any food components at the diffusion controlled rate of $10^9 \sim 10^{10}$ $M^{-1}s^{-1}$ order.

SOYBEAN OIL REVERSION RANCID FLAVOR AND MILK SUNLIGHT FLAVOR FORMATION BY SINGLET OXYGEN

Soybean oil accounts for 70% of the total edible oil in the United States and is the major food oil used worldwide. Soybean

Table 2 Reaction rates ($M^{-1}s^{-1}$) between reactive oxygen species and food commponents

	Reactive oxygen species			
Food components	$^1O_2$	$HO^{\cdot}$	$O_2^{\cdot -}$	$HOO^{\cdot}$
Lipid				
Oleic acid	5.3×10^4 [a]			No reaction[b]
Linoleic acid	7.3×10^4 [a]			1.2×10^3 [b]
Linolenic acid	1.0×10^5 [a]			1.7×10^3 [b]
Arachidonic acid				3.1×10^3 [b]
Soybean oil	1.4×10^5 [c]			
Cholesterol	2.5×10^8 [d]			
Amino acids				
Histidine	4.6×10^7 [e]	4.8×10^9 [f]	<1.0[g]	<95[g]
Tryptophane	1.3×10^7 [e]	1.3×10^{10} [h]	<24[g]	
Cysteine	5.0×10^7 [e]	1.9×10^{10} [i]	<15[g]	<600[g]
Cystine		2.1×10^9 [j]	$<4.0 \times 10^{-1}$ [g]	
Methionine	1.3×10^7 [e]	7.4×10^9 [k]	$<3.3 \times 10^{-1}$ [g]	<49[g]
Sugars				
Glucose	1.4×10^4 [l]	1.5×10^9 [f]		
Fructose		1.6×10^9 [m]		
Sucrose	2.5×10^4 [l]	2.3×10^9 [m]		
Maltose		2.3×10^9 [n]		
Vitamins				
β-Carotene	5.0×10^9 [o]			
Riboflavin	6.0×10^7 [p]	1.2×10^{10} [q]		
Ascorbic acid	1.1×10^7 [r]	8.2×10^9 [s]		1.6×10^4 [t]
Vitamin D	2.2×10^7 [u]			
Tocopherol	13.2×10^7 [v]		No reaction[w]	2.0×10^5 [w]

[a] Vever-Bizet et al., 1989; [b] Bielski et al., 1983; [c] Lee and Min, 1991; [d] Kruk et al., 1992; [e] Michaeli and Feitelson, 1994; [f] Motohashi and Saito, 1993; [g] Bielski and Shiue, 1979; [h] Solar et al., 1984; [i] Hoffman and Hayon, 1973; [j] Masuda et al., 1973; [k] Zhao et al., 1994; [l] Ergorov and Kransnovsky, 1986; [m] Moore et al., 1979; [n] Bucknall et al., 1978; [o] Devasagayam et al., 1992; [p] Allen and Parks, 1979; [q] Kishore et al., 1991; [r] Rooney, 1983; [s] Schoeneshoefer, 1972; [t] Cabelli and Bielski, 1983; [u] Li et al., 2000; [v] Mukai et al., 1991; [w] Arudi et al., 1983.

oil is inexpensive, nutritious and widely available compared to other edible oils. However, it has a problem of reversion flavor, described as beany or grassy, when its peroxide values are still very low (Ho et al., 1978). Other edible oils do not produce reversion (Ho et al., 1978). Due to the economic importance of soybean oil, the reversion flavor has been extensively studied since 1932 by scientists throughout the world. Reversion flavor in soybean oil is known to be mainly due to 2-pentyl furan (Smouse and Chang, 1967) and 2-pentenyl furan (Ho et al., 1978; Smagula et al., 1978). However, the mechanisms of its formation and how to prevent the reversion flavor have not been known for a long time. 2-Pentenylfuran was formed from linolenic acid by singlet oxygen oxidation as shown in Figure 30 (Min et al., 2003). Similarly 2-pentylfuran was formed from linoleic acid by singlet oxygen. Theses compounds were only formed in soybean oil containing chlorophyll under light. Chlorophyll can produce singlet oxygen under light in the presence of triplet oxygen. The reversion flavor is no longer a problem in soybean oil since it can be prevented by removing chlorophyll during the refining process. Sunlight flavor formation in milk has been recognized and extensively studied. The sunlight flavor is generally described as sulfur flavor. Methionine was proposed to be responsible for the formation of sunlight flavor of milk in the presence of riboflavin (Patton and Josephson, 1953). They reported that methional and dimethyl sulfide were the compounds responsible for sunlight flavor. The mechanisms for the formation of sunlight flavor have been very controversial. Singlet oxygen is formed from molecular triplet oxygen in the presence of riboflavin in milk under sunlight. Jung et al. (1998) reported that singlet oxygen reacts with electron-rich sulfur in methionine to form hydroperoxide. The hydroperoxide on sulfur is decomposed to methional and thiomethyl radicals shown in Figure 31. Reaction between thiomethyl radicals produces dimethyl disulfide. Contents of dimethyl disulfide were highly correlated with the sunlight flavor sensory score (Jung et al., 1998). The concentration of methional increases under the nonaqueous solvent (Foote, 1976). Addition of ascorbic acid, a good singlet oxygen quencher, reduced dimethyl disulfide formation in milk and improved the sensory quality of milk (Jung et al., 1998).

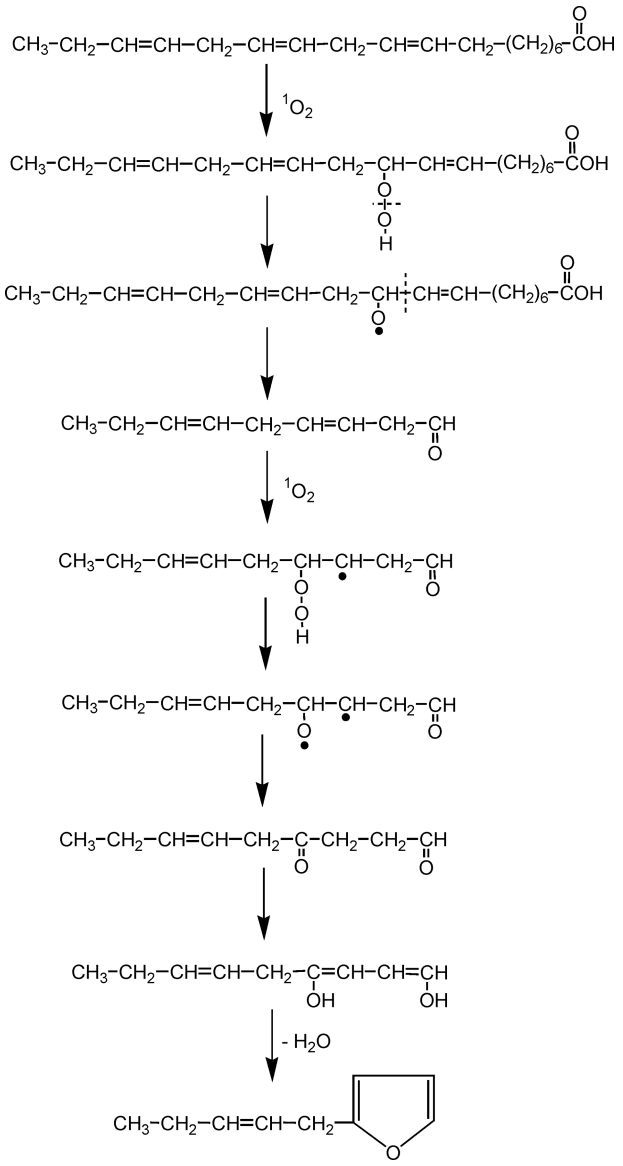

Figure 30 Formation of 2-pentenylfuran from linolenic acid by singlet oxygen.

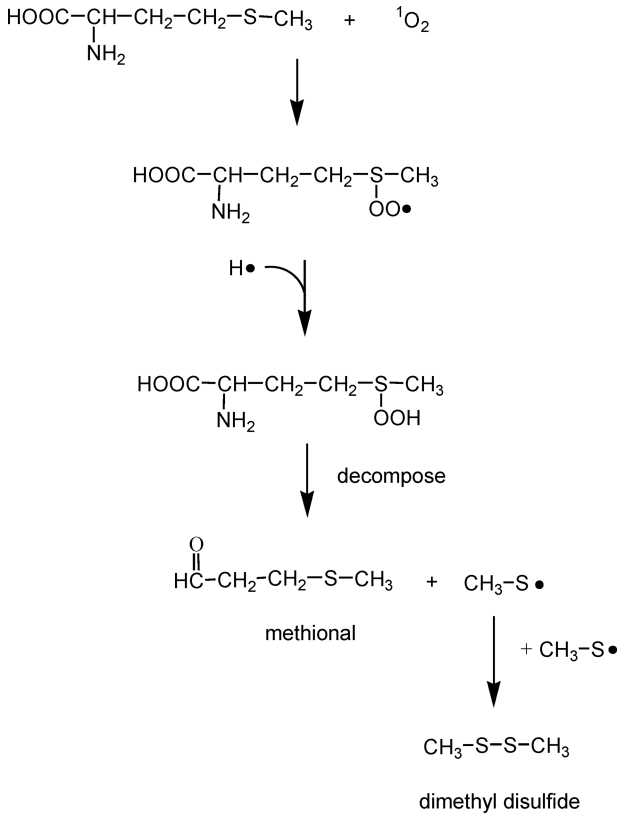

Figure 31 Formation of dimethyl disulfide from methionine by singlet oxygen.

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