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Reactive Oxygen Species, Aging, and Antioxidative Nutraceuticals

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ABSTRACT: The important roles of reactive oxygen species in diseases related to aging and the necessity and benefits of antioxidative nutraceuticals in the prevention of diseases and promotion of healthy aging have been extensively reported in recent years. Oxygen is an essential component of living organisms. The generation of reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and singlet oxygen is inevitable in aerobic metabolism of the body. Reactive oxygen species cause lipid oxidation, protein oxidation, DNA strand break and base modification, and modulation of gene expression. In the past several years, unprecedented progress has been made in the recognition and understanding of roles of reactive oxygen species in many diseases. These include atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury, and periodontis, which are age-related. The body protects itself from the potential damages of reactive oxygen species. Its first line of defense is superoxide dismutases, glutathione peroxidases, and catalase. Scientists have indicated that antioxidant nutraceuticals supplied from daily diets quench the reactive oxygen species or are required as cofactors for antioxidant enzymes. Nutraceuticals play significant roles in the prevention of a number of age-related diseases and are essential for healthy aging. Epidemiological studies also reported the relevance of antioxidative nutraceuticals to health issues and the prevention of age-related diseases. Health-conscious consumers have made antioxidative nutraceuticals the leading trend in the food industry worldwide in recent years.

Introduction

Aging is the accumulation process of diverse detrimental changes in the cells and tissues with advancing age, resulting in an increase in the risks of disease and death (Harman 2000). Aging is influenced by many factors, including lifestyle, environmental conditions, and genetic disposal (Spiteller 2001). With increasing age, the oxidation products from lipids, nucleic acids, proteins, sugars, and sterols are found to increase (Ashok and Ali 1999). The main causes of the aging process seem to be related to reactive oxygen species and free radicals, such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. Mitochondria, which consume more than 90% of the oxygen in aerobic living organisms, are the main reactive oxygen species and free radical source. Oxygen in mitochondria is reduced to water by 4 sequential steps (Ames and others 1993). Perhydroxyl radical ($HO_2\cdot$) or its ionized form, superoxide anion ($\cdot O_2^-$), is the first reduced intermediate of oxygen. Hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot$OH) are inevitable intermediates from oxygen to water reduction steps in body. Approximately 1% to 5% of the oxygen consumed by mitochondria is reduced and converted to these reactive oxygen species (Ames and others 1993).

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Harman (2000) suggested that initially generated superoxide anion and hydrogen peroxide are the main reactive oxygen species causing the oxidation of cells and tissues. Superoxide anion itself is not a strong oxidant, but it reacts with protons in water solution to form hydrogen peroxide, which can serve as a substrate for the generation of hydroxyl radicals and singlet oxygen (Stief 2003). Hydroxyl radicals are strong oxidants and can abstract a hydrogen atom from any carbon–hydrogen bond and oxidize the compound. For example, linoleic acids are mainly located in glycerolipids and phopholipids of cell membranes; therefore, cell membranes are easily oxidized and lose their functionality during the aging process.

Prooxidative enzymes such as lipoxygenase can generate free radicals (Spiteller 2001). Lipoxygenase can react with free forms of fatty acids, which can be released from glycerides by membrane-bound phopholipase A_2. Environmental sources, such as ultraviolet (UV) irradiation, ionizing irradiation, and pollutants, also produce reactive oxygen species (Halliwell 1997). Injured cells and tissues can stimulate the generation of free radicals (Spiteller 2001). Reactive oxygen species can be formed in foods through lipid oxidation and photosensitizers exposed to light (Boff and Min 2002). Nonenzymatic lipid oxidation requires the presence of free forms of bivalent metal ions such as copper and iron, which are not common for healthy adults (Gutteridge and Halliwell 1993). It has been assumed that free forms of irons are generated by the decompositions of ion-containing natural sources such as hemoglobin and ferritin (Halliwell 1997).

Enzymatic and nonenzymatic antioxidant systems in the body,

including superoxide dismutase, catalase, glutathione peroxidase, lipid-soluble vitamin E, carotenes, and water-soluble vitamin C, regulate the balance of reactive oxygen species with antioxidants (Thomas 1995; Wickens 2001). As aging proceeds, the efficiency of antioxidant defense systems lowers, and the ability to remove deleterious reactive oxygen species and free radicals decreases. The prevalent free radical states, or so-called oxidative stress, initiate the oxidation of polyunsaturated fatty acids (PUFA), proteins, DNA, and sterols. The age-associated increases in oxidized proteins, oxidized DNA, sterol oxidation products, and lipid oxidation products support the fact that reactive oxygen species and free radicals are involved in the aging process (Halliwell 1997; Rikans and Hornbrook 1997). Consumption of fruits and vegetables containing high amounts of antioxidative nutraceuticals has been associated with the balance of the free radicals/antioxidants status, which helps to minimize the oxidative stress in the body and to reduce the risks of cancers and cardiovascular diseases (Kaur and Kapoor 2001).

Reactive Oxygen Species

Reactive oxygen species can be classified into oxygen-centered radicals and oxygen-centered nonradicals. Oxygen-centered radicals are superoxide anion ($\cdot O_2$–), hydroxyl radical ($\cdot$OH), alkoxyl radical (RO$\cdot$), and peroxyl radical (ROO$\cdot$). Oxygen-centered nonradicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Other reactive species are nitrogen species such as nitric oxide (NO$\cdot$), nitric dioxide ($NO_2\cdot$), and peroxynitrite ($OONO^-$) (Halliwell and others 1995; Simon and others 2000). Reactive oxygen species in biological systems are related to free radicals, even though there are nonradical compounds in reactive oxygen species such as singlet oxygen and hydrogen peroxide. A free radical exists with one or more unpaired electron in atomic or molecular orbital. Free radicals are generally unstable, highly reactive, and energized molecules. Reactive oxygen species or free radicals in biological systems can be formed by prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants, and glycoxidation (Halliwell 1997; Stief 2003). Clinical studies reported that reactive oxygen species are associated with many age-related degenerative diseases, including atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury, and periodontis (Figure 1) (Cohen and others 2000; Packer and Weber 2001). Reactive oxygen species also have been known to induce apoptosis of cells (Simon and others 2000).

Benign functions of free radicals have been reported, including the activation of nuclear transcription factors, gene expression, and a defense mechanism to target tumor cells and microbial infections (Simon and others 2000). Superoxide anion may serve as a cell growth regulator (Halliwell 1997). Singlet oxygen can attack various pathogens and induce physiological inflammatory response (Stief 2003). Nitric oxide is one of the most widespread signaling molecules and participates in every cellular and organ function in the body. Nitric oxide acts as a neurotransmitter and an important mediator of the immune response (Fang and others 2002).

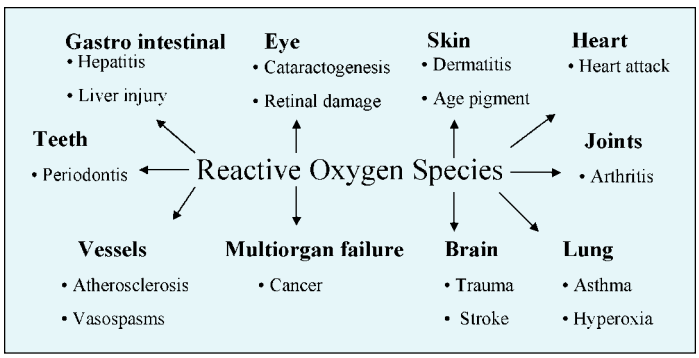


Figure 1—Clinical conditions involving reactive oxygen species

Superoxide anion ($\cdot O_2^-$)

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron (Figure 2). Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using 4 electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anions (Harman 2000).

$$O_2 \xrightarrow[H^+]{e^-} HO_2\cdot\ (\cdot O_2^-) \xrightarrow[H^+]{e^-} H_2O_2 \xrightarrow[H^+]{e^-} \cdot OH \xrightarrow[H^+]{e^-} H_2O$$

The superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen ($2\ \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$) in living systems (Stief 2003). The superoxide anion can react with nitric oxide (NO$\cdot$) and form peroxynitrite ($ONOO^-$), which can generate toxic compounds such as hydroxyl radical and nitric dioxide ($ONOO^- + H^+ \rightarrow \cdot OH + \cdot NO_2$) (Halliwell 1997).

Hydroxyl radical (OH)

Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron ($\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$). Hydroxyl radicals have the highest 1-electron reduction potential (2310 mV) and can react with everything in living organisms at the 2nd-order rate constants of 10^9 to 10^{10}/M/s (Korycka-Dahl and Richardson 1978). In general, aromatic compounds or compounds with carbon–carbon multiple bonds undergo addition reactions with hydroxyl radicals, resulting in the hydroxylated free radicals. In saturated compounds, a hydroxyl radical abstracts a hydrogen atom from the weakest C–H bond to yield a free radical (Korycka-Dahl and Richardson 1978). The resulting radicals can react with oxygen and generate other free radicals.

Hydroxyl radicals react with lipid, polypeptides, proteins, and DNA, especially thiamine and guanosine (Ashok and Ali 1999). Hydroxyl radicals also add readily to double bonds. The barrier to the addition of hydroxyl radicals to double bonds is less than that of hydrogen abstraction, so that in competition addition is often favored. When a hydroxyl radical reacts with aromatic compounds, it can add on across a double bond, resulting in hydroxycyclohexadienyl radical (Padmaja and Madison 1999). The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxyl radical, or decompose to phenoxyl-type radicals by water elimination.

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily.

Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a

weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion ($\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$) (Halliwell 1997). Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems (Stief 2000, 2003). Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions.

Singlet oxygen

Singlet oxygen is a nonradical and excited status. The molecular orbital of singlet oxygen is shown in Figure 2. The electrons in the π antibonding orbitals of singlet oxygen are paired. Takayama and others (2001) reported that metastable phosphatidylcholine hydroperoxides present in the living organism produced singlet oxygen during their breakdown in the presence of Cu^{2+} in the dark. Singlet oxygen can be formed from hydrogen peroxide, which reacts with superoxide anion, or with HOCl or chloroamines in cells and tissues (Stief 2003).

Compared with other reactive oxygen species, singlet oxygen is rather mild and nontoxic for mammalian tissue (Stief 2003). However, singlet oxygen has been known to be involved in cholesterol oxidation (Girotti and Korytowski 2000). Oxidation of cholesterol by singlet oxygen results in formation of 5α–OOH (3β–hydroxy–5α–cholest–6–ene–5–hydroperoxide), 5β–OOH (3β–hydroxy–5β–cholest–6–ene–5–hydroperoxide), 6α–OOH, and 6β–OOH (Foote 1991; Yamazaki and others 1999). Oxidation and degradation of cholesterol by singlet oxygen was observed to be accelerated by the co-presence of fatty acid methyl ester. In the human organism, singlet oxygen is both a signal and a weapon, with therapeutic potency against various pathogens such as microbes, viruses, and cancer cells (Stief 2003).

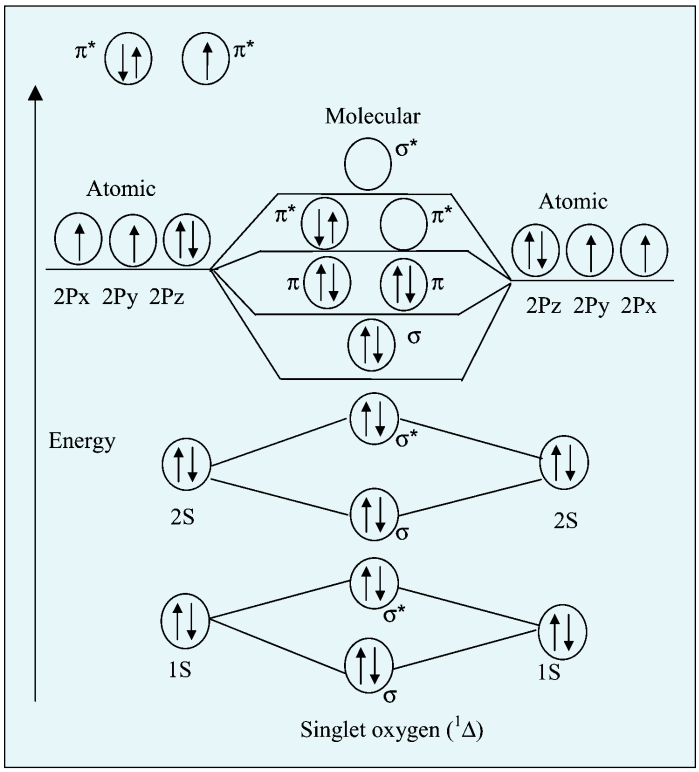


Figure 2—Molecular orbitals of singlet oxygen and superoxide anion

Peroxyl and alkoxyl radicals

Peroxyl radicals (ROO·) are formed by a direct reaction of oxygen with alkyl radicals (R·), for example, the reaction between lipid radicals and oxygen. Decomposition of alkyl peroxides (ROOH) also results in peroxyl (ROO·) and alkoxyl (RO·) radicals. Irradiation of UV light or the presence of transition metal ions can cause homolysis of peroxides to produce peroxyl and alkoxyl radicals ($ROOH \rightarrow ROO\cdot + H\cdot$, $ROOH + Fe^{3+} \rightarrow ROO\cdot + Fe^{2+} + H^+$).

Peroxyl and alkoxyl radicals are good oxidizing agents, having more than 1000 mV of standard reduction potential (Decker 1998). They can abstract hydrogen from other molecules with lower standard reduction potential. This reaction is frequently observed in the propagation stage of lipid peroxidation. Very often the alkyl radical formed from this reaction can react with oxygen to form another peroxyl radical, resulting in chain reaction. Some peroxyl radicals break down to liberate superoxide anion or can react with each other to generate singlet oxygen (Halliwell and Gutteridge 1985). Aromatic alkoxyl and peroxyl radicals are less reactive than respective open chain radicals because of the delocalization of electrons in the ring.

Nitric oxide and nitric dioxide

Nitric oxide (NO·) is a free radical with a single unpaired electron. Nitric oxide is formed from L-arginine by NO synthase (Fang and others 2002). Nitric oxide itself is not a very reactive free radical, but the overproduction of NO is involved in ischemia reperfusion, and neurodegenerative and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Nitric oxide, exposed in human blood plasma, can deplete the concentration of ascorbic acid and uric acid, and initiate lipid peroxidation (Halliwell 1996).

Nitric dioxide ($NO_2\cdot$) is formed from the reaction of peroxyl radical and NO, polluted air and smoking (Noguchi and Niki 1999). Nitric dioxide adds to double bonds and abstract labile hydrogen atoms initiating lipid peroxidation and production of free radicals. It also oxidizes ascorbic acid (Papas 1999a).

Peroxynitrite

Reaction of NO and superoxide anion can generate peroxynitrite ($O_2^- + NO\cdot \rightarrow OONO^-$). Peroxynitrite is a cytotoxic species and causes tissue injury and oxidizes low-density lipoprotein (LDL) (Halliwell 1997). Peroxynitrite appears to be an important tissue-damaging species generated at the sites of inflammation (Papas 1999a) and has been shown to be involved in various neurodegenerative disorders and several kidney diseases (Knight 1999).

Peroxynitrite ($OONO^-$) can cause direct protein oxidation and DNA base oxidation and modification acting as a "hydroxyl radical-like" oxidant (McVean and others 1999). The significance of peroxynitrite as a biological oxidant comes from its high diffusibility across cell membranes (Knight 1999). Nitrotyrosine, which can be formed from peroxynitrite-mediated reactions with amino acids, has been found in age-associated tissues (Knight 1999).

Enzymatic formation

Prooxidative enzymes, including NADPH–oxidase (Babior 1999), NO–synthase (Stuehr and others 1990), or the cytochrome P–450 chain (Stief 2000), can generate reactive oxygen species. Lipoxygenase generates free radicals. Lipoxygenase needs free PUFA, which are not present in healthy tissue. Membrane-bound phospholipase produces PUFA and lysolecithins. Lysolecithins change the cell membrane structures, and free PUFA are oxidized to form lipid hydroperoxides. Lipoxygenase with Fe^{2+} ion is inactivated status. Once Fe^{2+} oxidized to Fe^{3+}, lipoxygenase can convert PUFA into hydroperoxides (Spiteller 2001).

There are 3 major mammalian lipoxygenases: 5–, 12–, and 15–

lipoxygenases (Hari and others 2000). These enzymes can oxidize arachidonic acid, a PUFA rich in the central nervous system, into hydroperoxyeicosatetraenoic acid. The primary localizations of 5–, 12–, and 15–lipoxygenases are in leukocytes and lymphocytes, platelets, and leukocytes and airway cells, respectively (Hari and others 2000). 15–Lipoxygenase has been identified within atherosclerotic lesions, which suggests that this enzyme may be involved in the in vivo formation of oxidized lipids (Knight 1999).

Reactive Oxygen Species and Aging

Theories explaining aging processes are diverse, including disease, environment, immune dysfunction, inborn processes, and free radicals. Generally accepted aging theories are based on either developmentally programmed aging or damage-accumulation aging, both involving generation of free radicals (Halliwell 1997). Free radical theories are based on the chemical characteristics of free radicals, which are ubiquitous in body systems. Free radicals or reactive oxygen species in the body can cause lipid oxidation, protein oxidation, DNA strand break and base modification, and modulation of gene expression. As age processing increases, oxidized DNA such as 8–hydroxydeoxyguanosine, and oxidative damaged proteins with carbonyl modifications and loss of protein –SH group, increase several times and the ratio of redox couples such as glutathione:oxidized glutathione, NADPH: $NADP^+$, and NADH:NAD^+ tend to shift to more prooxidant values (Halliwell and others 1995; Halliwell 1997; Spiteller 2001).

A serious imbalance between reactive oxygen species and antioxidants causes oxidative stress. Oxidative stress is caused by antioxidant-deficient diets or by increased production of reactive oxygen species by environmental toxins such as those caused by smoking or by inappropriate activation of phagocytes such as with chronic inflammatory disease (Halliwell 1997). Clinical studies reported that reactive oxygen species are associated with many degenerative diseases, which are associated with aging (Figure 1) (Packer and Weber 2001).

Lipid oxidation and aging

Lipid oxidation is a free-radical chain reaction, and reactive oxygen species can accelerate lipid oxidation (Boff and Min 2002). Cell membranes are phospholipid bilayers with extrinsic proteins and are the direct target of lipid oxidation (Girotti 1998). As lipid oxidation of cell membranes increases, the polarity of lipid-phase surface charge and formation of protein oligomers increase; and molecular mobility of lipids, number of SH groups, and resistance to thermodenaturation decrease.

Malonaldehyde, one of the lipid oxidation products, can react with the free amino group of proteins, phospholipid, and nucleic acids leading to structural modification, which induce dysfunction of immune systems. A high level of lipid oxidation products can be detected in cell degradation after cell injury or disease. The increases of lipid oxidation products are found in diabetes, atherosclerosis, liver disease, apoplexy, and inflammation. Low-density lipoproteins are complicated structures, and oxidative modification of LDLs has been reported to be involved with the development of atherosclerosis and cardiovascular disease (Frei 1995). Oxidized cholesterol or fatty acid moieties in the plasmatic LDL can develop atherosclerosis (Rikans and Hornbrook 1997; Girotti 1998; Nedeljkovic and others 2003).

Protein oxidation and aging

Reactive oxygen species can attack proteins and produce carbonyls and other amino acid modifications, including methionine sulfoxide, 2–oxohistidine, and protein peroxides.

Modification of protein is mainly initiated by hydroxyl radicals, leading to the oxidation of amino acid side chains, protein–protein cross linkage, and protein fragmentation (Figure 3a) (Berlett and Stadtman 1997; Stadtman 2000). The availability of oxygen, superoxide anion, and its protonated form (HO_2^-) determines the pathways of protein oxidation processes.

Induction of 3–chlorotyrosine from tyrosine by hypochlorous acid, the oxidization of histidine to 2–oxohistidine in the metal-binding site of proteins, the oxidation of thiol groups, and the generation of carbonyl derivatives of amino acids are some examples of protein modifications (Berlett and Stadtman 1997).

4–Hydroxy–2–nonenal from lipid oxidation can react with protein lysine, histidine, and cystein residues (Figure 3b). Malonaldehyde from lipid oxidation reacts with protein amino groups. NO·, which is an intracellular messenger in the nervous, immune, and cardiovascular systems, is synthesized from L–arginine by NO synthetase in mitochondria and causes oxidation of protein. Nitrotyrosines, products of reactive nitrogen species on tyrosine, have been detected in atherosclerotic lesions, human urine, and body fluids from patients with chronic inflammatory diseases. Peroxynitrite (ONOO–) oxidizes essential –SH groups of proteins. Peroxynitrite-induced protein modifications include protein oxidation on methionine, cysteine, tryptophane or tyrosine residues, and protein nitration of tyrosine or tryptophane residues (Virag and others 2003). Protein oxidation affects the alterations of signal transduction mechanisms, transport systems, enzyme activities, atherosclerosis, and ischemia reperfusion injury (Stadtman 1992; Berlett and Stadtman 1997).

Aging is associated at least partly with oxidative modification of proteins. For example, in vitro exposure of enzymes to reactive oxygen species induces changes in enzymatic activity, heat stability, and proteolysis susceptibility similar to those that occur during aging (Berlett and Stadtman 1997), and old animals are more susceptible to protein damage during oxidative stress such as hydro-

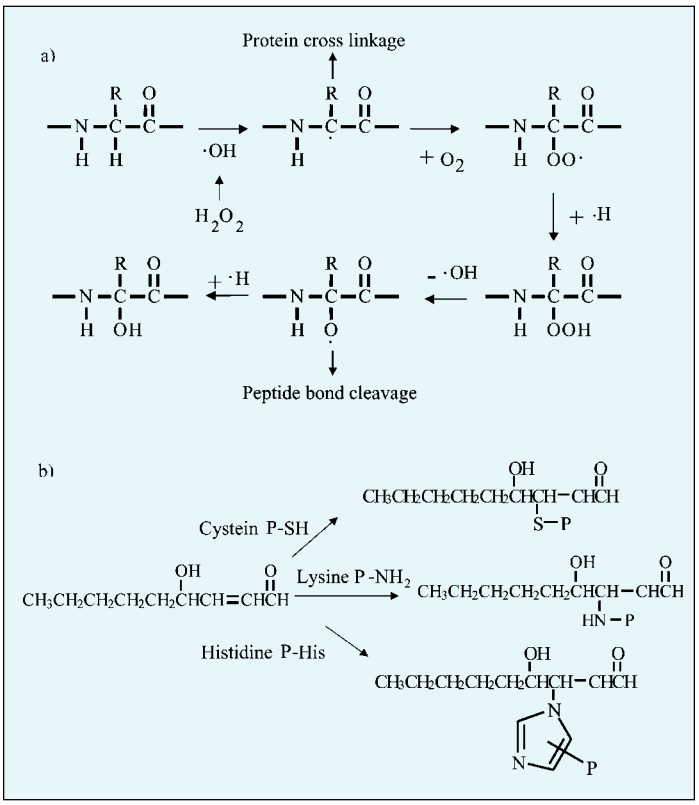


Figure 3—Oxidation mechanisms of proteins (a) and protein carbonyl formation from aldehydes (b)

gen peroxide than are younger animals (Agarawal and Sohal 1993).

DNA strand breaks and modification and aging

Mitochondria and nuclei have their own DNA. Mitochondrial DNA is susceptible to oxidative damages because of the lack of protective protein, histones, and close locations to the reactive oxygen species-producing systems. Hydroxyl radical oxidizes guanosine or thymine to 8–hydroxy–2–deoxyguanosine and thymine glycol, respectively, which changes DNA and leads to mutagenesis and carcinogenesis (Figure 4) (Ames and others 1993). 8–Hydroxy–2–deoxyguanosine has been used as a biological marker for oxidative stress (Kasai 1997). Altered DNA can be repaired by DNA glycosylase. A low level of oxidative base damage in DNA is found in the cells of a healthy person. However, concentration of oxidized DNA base increases in humans with chronic inflammatory diseases such as rheumatoid arthritis or under oxidative stresses such as smoking (Halliwell 1997). If oxidative stress is too great, the DNA repair system using glycosylase is not enough, and mutagenesis and/or carcinogenesis can be induced.

Antioxidative Nutraceuticals

Nutraceuticals or functional foods are any food or food ingredients that may provide beneficial health effects beyond the traditional nutrients they contain (Wildman 2001a).

Nutraceuticals are also known as medical food, nutritional supplements, and dietary supplements. Nutraceuticals range from isolated nutrients, dietary supplements, genetically engineered "designer" foods, herbal products, and processed products such as cereals and soups. The functional food market has increased because of the fast growth of the older generation in the United States and their concerns about health-beneficial foods (Dillard and German 2000). Nutraceuticals can be grouped in different ways, depending on the food sources, mode of action, and chemical structures (Wildman 2001b).

As oxidative stress increases, the level of the prooxidants against antioxidants increases and the aging process accelerates. If reactive oxygen species and free radicals are the major causes of aging processes, antioxidative nutraceuticals can reduce the level of reactive oxygen species and free radicals, slow the aging process, and increase life span. It has been reported that levels and activities of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, are much higher in long-living species than in short-living ones. The concentration of vitamin E in elderly people (older than 65 years) is lower than that in younger adults. Consumption of optimal amounts of vitamin A and E increased the average life expectancy of animals (Duthie and others 1996; Teoh and Davies 2002).

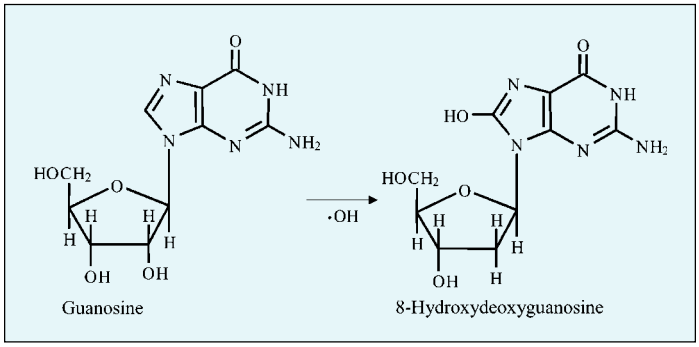


Figure 4—Formation of 8-hydroxydeoxyguaosine from the reaction of guanosine and hydroxyl radical

Table 1—Antioxidative enzymes

Proteins	Functions
Superoxide dismutase	Superoxide removal
Catalase	Hydroperoxide removal
Glutathione peroxidase	Hydroperoxide removal
Glutathione disulfide reductase	Oxidized glutathione reduction
Glutathione-S-transferase	Lipid hydroperoxide removal
Methionine sulfoxide reductase	Repair oxidized methionine residues
Peroxidase	Decomposition of hydrogen peroxide and lipid hydroperoxide

Antioxidative nutraceuticals can inhibit or slow the formation of free alkyl radicals in the initiation step and interrupt the free-radical chain reactions in the propagation step during lipid oxidation. Antioxidative nutraceuticals can be antioxidative enzymes, hydrogen donating compounds, metal chelators, and singlet oxygen quenchers.

Antioxidative enzymes

Antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase/reductase, convert reactive oxygen species into nonreactive oxygen molecules. Proteins showing antioxidant properties are listed in Table 1.

Superoxide dismutase (SOD) converts superoxide anion into hydrogen peroxide and oxygen. There are 2 types of SOD: a magnesium-containing SOD and a copper-zinc–dependent SOD. Catalase is involved in cellular detoxification and can convert hydrogen peroxide into water and oxygen (Figure 5). Glutathione peroxidase is the most important hydrogen peroxide–removing enzyme existing in the membrane. Glutathione disulfide reductase is a flavoprotein that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADH to NAD^+ (Figure 5) (Papas 1999c).

Hydrogen-donating nutraceuticals

Antioxidative nutraceuticals, which can donate hydrogen atoms

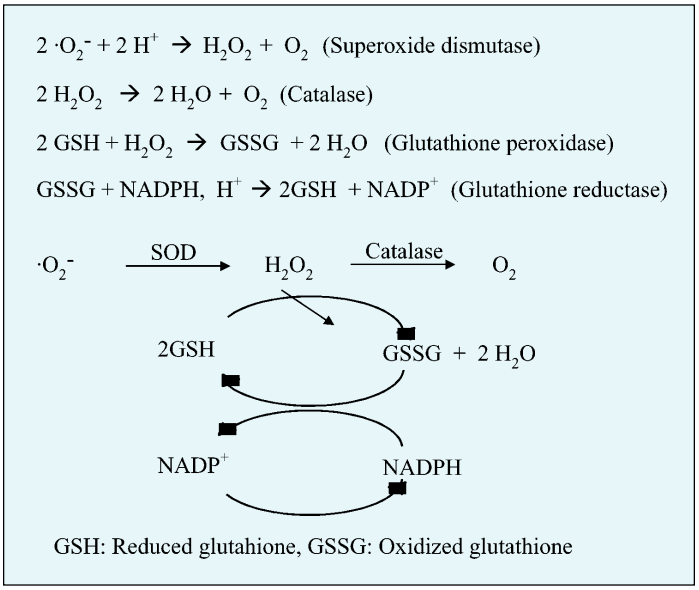


Figure 5—Antioxidant enzymes and their reaction mechanisms

to free radicals, can scavenge free radicals and prevent lipid oxidation. Lipid oxidation in foods and in biological systems is a typical free-radical chain reaction of unsaturated fatty acids with initiation, propagation, and termination steps. The propagation step is a slow step in lipid oxidation, and the concentration of peroxyl radicals is found to be the greatest of all fatty acid radicals (Frankel 1985). Free-radical scavengers, which react with peroxyl radicals before the PUFA react with peroxyl radicals, can prevent lipid oxidation. Chain-breaking antioxidants donate hydrogen atoms to peroxyl radicals and convert them to more stable and nonradical products (Table 2) (Decker 1998; Decker and others 1999). Antioxidant radicals formed from hydrogen-donating antioxidants can react with alkyl, alkoxyl, and peroxyl radicals of PUFA and generate nonradical stable compounds (Table 2).

Whether a compound acts as an antioxidant or a prooxidant can be determined by the standard 1-electron reduction potential (Table 3). Standard 1-electron reduction potentials of alkyl, peroxyl, and alkoxyl radicals of PUFA are 600, 1000, and 1600 mV, respectively (Table 3) (Buettner 1993). To work as an antioxidant and prevent lipid oxidation, the reduction potential of a free-radical scavenger should be lower than 600 mV, which is a reduction potential of PUFA. For example, ascorbic acid and tocopherol, which have lower standard 1-electron reduction potential (282 and 480 mV, respectively) than PUFA (600mV), can donate a hydrogen atom to peroxyl radicals of PUFA before PUFA do (Buettner 1993).

The newly generated free radicals from antioxidative nutraceuticals should be stable enough not to participate in other lipid oxidation chain reactions. Radicals from phenolic compounds can be stabilized through resonance formation (Figure 6).

Table 2—Reaction of hydrogen-donating antioxidants with radicals[a]

R·	+	AH		RH	+	A·
RO·	+	AH	→	ROH	+	A·
ROO·	+	AH	→	ROOH	+	A·
R·	+	A·	→	RA		
RO·	+	A·	→	ROA		
ROO·	+	A·	→	ROOA		
Antioxidant	+	O_2	→	Oxidized antioxidant		

[a]AH = antioxidant; R· = alkyl free radical; RO· = alkoxyl free radical; ROO· = peroxyl free radical.

Table 3—Standard 1-electron reduction potential (mV) at pH 7.0 for selected radical couples[a]

HO·, H+ / H_2O	2310
RO·, H+ / ROH	1600
ROO·, H+ / ROOH	1000
GS·/GS^- (glutathione)	920
PUFA·, H+ / PUFA	600
Catechol·, H+ / Catechol	530
α-Tocopheroxyl·, H+ / α-Tocopherol	480
H_2O_2, H^+ / H_2O, HO·	320
$Ascorbate^-$·, H^+ / Ascorbate	282
O_2/O_2^-·	–330
RSSR/ $RSSR^-$· (GSH)	–1500
H_2O / e^-_{aq}	–2870

[a]GSF = reduced glutathione; PUFA = polyunsaturated fatty acids.

Metal chelating nutraceuticals

Transition metals such as iron and copper play important roles in initiation and propagation steps of lipid oxidation. The initiation step of oxygen oxidation requires removal of a hydrogen atom. The presence of metal can accelerate the initiation step of lipid oxidation by the mechanism of $RH + M^{n+} \rightarrow R\cdot + H^+ + M^{(n-1)+}$. Metals can decompose the hydroperoxide to form peroxyl radical and alkoxyl radical, and accelerate the lipid oxidation at the exponential rate (Min 1998; Papas 1999b).

$$Fe^{3+}\ (Cu^{2+}) + \rightarrow ROOH \qquad Fe^{2+}\ (Cu^{+}) \rightarrow + ROO\cdot + H^+$$

$$Fe^{2+}\ (Cu^{+}) + \rightarrow ROOH \qquad Fe^{3+}(Cu^{2+}) \rightarrow + RO\cdot + OH^-$$

Metals are also involved in the formation of singlet oxygen.

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

Hydrogen peroxide can react with transition metal ions to form hydroxyl radical.

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

This reaction is dependent on the availability of transition metal ions such as copper and iron. The availability of metal ions is determined by the concentrations of metal-binding proteins, including ferritin, lactoferrin, and ceruloplasmin (Decker 1998).

Metal chelators, one type of antioxidative nutraceuticals, form complex ions or coordination compounds with metals by occupying all metal coordination sites and preventing metal redox cycling. Metal chelators can convert metal ions into insoluble metal complexes or generate steric hindrance, which can prevent the interactions between metals and lipid intermediates. Some reported metal-chelating proteins are shown in Table 4. Metal chelators are phosphoric acid, citric acid, ascorbic acid, polyphenols such as quercetin, carnosine, some amino acids, peptides, and proteins such as transferrin and ovotransferrin (Decker 1995; Halliwell and others 1995; Ramon and Gonzalo 2002).

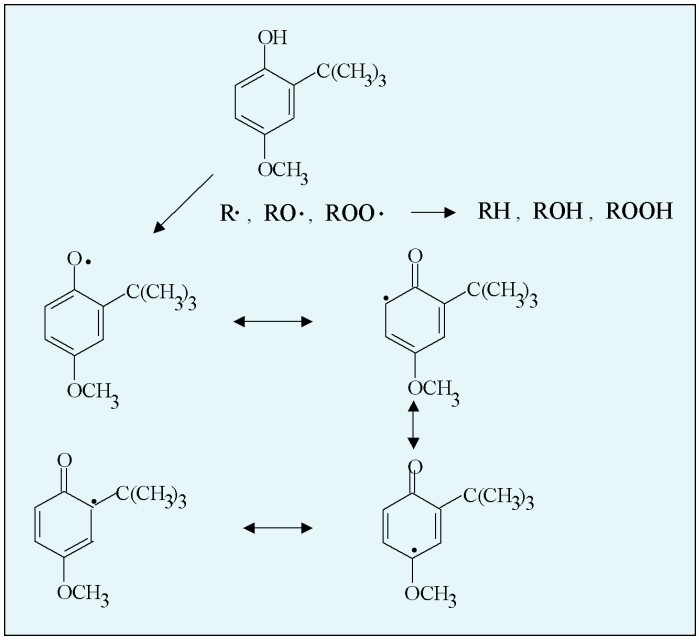


Figure 6—Resonance stabilization of phenolic antioxidant radicals. Antioxidant radicals are stabilized through resonance structures.

Singlet oxygen-quenching nutraceuticals

Singlet oxygen is highly reactive toward any molecules with π

electrons or lone pairs of low ionization energy. There are 2 types of singlet oxygen-quenching mechanisms: physical and chemical quenchings. Physical quenching converts singlet oxygen into triplet oxygen by either energy transfer or charge transfer without generating any other intermediates. Chemical quenching is involved with the generation of intermediates, such as oxidized products. Singlet oxygen reactions with compound (A) to form oxidized products (AO_2) are shown in Figure 7. Chemical quenching between singlet oxygen ($^1O_2$) and quencher (Q) involves the generation of an oxidized product 'QO_2'. Physical quenching converts singlet oxygen ($^1O_2$) to triplet oxygen ($^3O_2$) without production of oxidized product 'QO_2'. Detailed information about the singlet oxygen-quenching mechanisms can be found in an excellent review by Boff and Min (2002). Singlet oxygen quenchers should have electron-rich structures such as double bonds in the molecules to react with singlet oxygen. Carotenoids, which have many double bonds, are well-known singlet oxygen quenchers (Boff and Min 2002). Uric acid is also a powerful quencher of singlet oxygen (Halliwell 1996). Thioredoxin has been reported as a singlet oxygen quencher and a hydroxyl radical scavenger, which acts independently of the redox potential (Kumuda and Chandan 2000).

Antioxidative Nutraceuticals

Tocopherols and tocotrienols

Tocopherols consist of a chroman ring and a long, saturated phytyl chain. Tocols are 2–methyl–2(4′, 8′, 12′–trimethyltridecyl) chroman–6–ols, and tocotrienols have 3 double bonds at position 3′, 7′, and 11′ of the side chain in tocols (Figure 8). The α–, β–, γ–, and σ–tocopherols and tocotrienols differ in the number and position of methyl groups attached to the 5, 7, and 8 of the ring structure (Gregory 1996). Tocopherols and tocotrienols are very nonpolar and exist in lipid phase. Tocopherols are natural constituents of biological membranes. Tocotrienols are found mainly in palm oil, cereal grains, and kale (Watkins and others 1999).

Tocopherols are typical and important antioxidants in humans. α–tocopherol, which is present at the ratio of 1 to 1000 lipid molecules, is the most abundant among tocopherols. Tocopherols can protect PUFA within the membrane and LDL, and inhibit smooth muscle cell proliferation and protein kinase C activity. Tocopherol has been associated with the reduction of heart disease, delay of Alzheimer's disease, and prevention of cancer (Meydani 2000). γ–Tocopherols can reduce the concentration of nitrogen dioxide (NO_2) better than other tocopherols. Nitrogen dioxide is involved in carcinogenesis, arthritis, and neurologic diseases. Tocotrienols have been shown to have anticancer activity and cholesterol-lowering ability. Some in vitro studies showed that tocotrienols inhibited LDL oxidation better than tocopherols (Watkins and others 1999).

Antioxidant mechanisms of tocopherols include the transfer of a hydrogen atom at 6–hydroxyl group on the chroman ring, and scavenging of singlet oxygen and other reactive species. Tocopherols are regenerated in the presence of ascorbic acids. Phytyl chain in tocopherols can be fit in the membrane bilayer while active chroman ring is closely positioned to the surface. This unique structure enables tocopherols to act as effective antioxidants and to be regenerated through reaction with other antioxidants such as ascorbic acid (Papas 1999c). α–Tocopherol has higher vitamin E activity and singlet oxygen-quenching ability than β–, γ–, and δ– tocopherols, whereas γ–tocopherol has better nitrogen dioxde and peroxynitrite radical-scavenging ability than α–tocopherols (Gregory 1996). Efficiency of scavenging hydroxyl, alkoxyl, and peroxyl radicals by α–tocopherol is approximately 10^{10}, 10^8, and 10^6 (/M/s), respectively (Niki 1996).

Tocotrienols are potential nutraceuticals, and the antioxidant mechanisms of tocotrienols are the same as those of tocopherols. Tocotrienols are more mobile within the biological membrane than tocopherols and have more recycling ability and more inhibition of liver oxidation (Watkins and others 1999).

Ascorbic acid

L–Ascorbic acid is a 6-carbon lactone ring structure with 2,3–

Table 4—Metal-chelating proteins

Proteins	Functions
Ferritin	Iron storage
Transferrin	Iron storage
Lactoferrin	Iron storage
Haptoglobin	Hemoglobin sequestration
Ceruloplasmin	Copper storage
Albumin	Copper storage
Transferrin ferro-oxidase	Iron transport
Hemopexin	Stabilization of heme

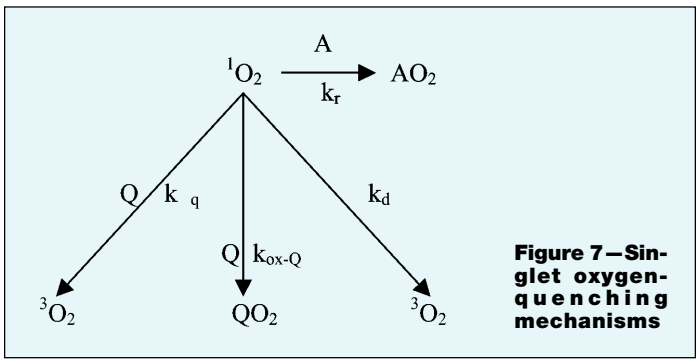
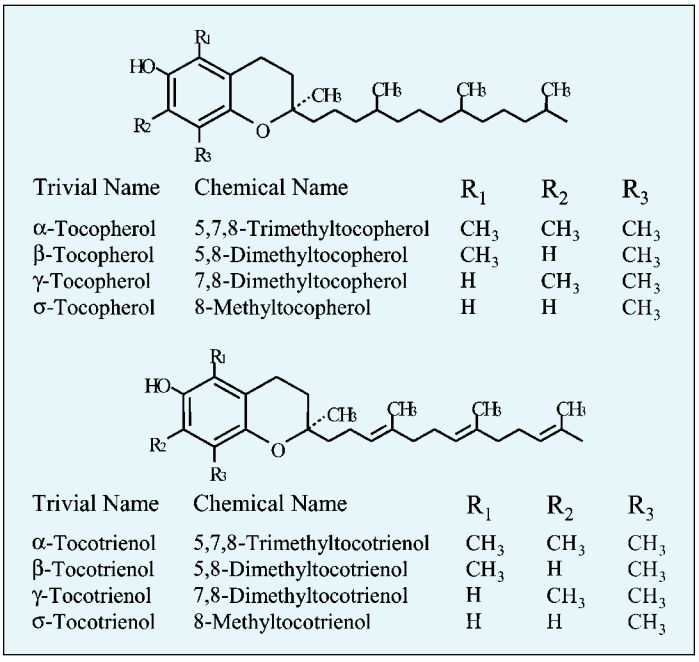


Figure 7—Singlet oxygen-quenching mechanisms



Trivial Name	Chemical Name	R_1	R_2	R_3
α-Tocopherol	5,7,8-Trimethyltocopherol	CH_3	CH_3	CH_3
β-Tocopherol	5,8-Dimethyltocopherol	CH_3	H	CH_3
γ-Tocopherol	7,8-Dimethyltocopherol	H	CH_3	CH_3
σ-Tocopherol	8-Methyltocopherol	H	H	CH_3

Trivial Name	Chemical Name	R_1	R_2	R_3
α-Tocotrienol	5,7,8-Trimethyltocotrienol	CH_3	CH_3	CH_3
β-Tocotrienol	5,8-Dimethyltocotrienol	CH_3	H	CH_3
γ-Tocotrienol	7,8-Dimethyltocotrienol	H	CH_3	CH_3
σ-Tocotrienol	8-Methyltocotrienol	H	H	CH_3

Figure 8—Structures of tocopherols and tocotrienols

enediol moiety. The antioxidant activity of ascorbic acid comes from 2,3–enediol. L–Ascorbic acid first changes to semi-dehydroascorbic acid through donating 1 hydrogen atom and electron, and then L–dihydroascorbic acid by donating a 2nd hydrogen atom and electron (Figure 9). Both L–ascorbic acid and L–dihydroascorbic acid retain the vitamin C activity. Ascorbic acid is highly susceptible to oxidation in the presence of metal ions such as Cu^{2+} and Fe^{3+}. Oxidation of ascorbic acid is also influenced by heat, light exposure, pH, oxygen concentration, and water activity (Gregory 1996).

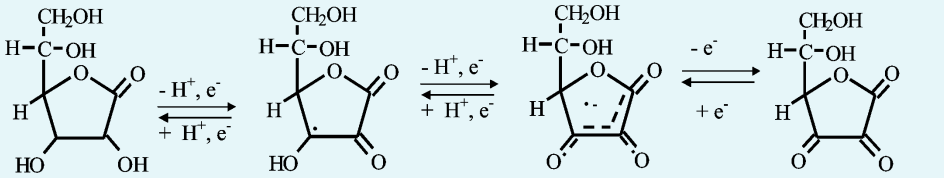


Figure 9—Sequential 1-electron oxidations of L-ascorbic acid

Ascorbic acid may be related to the prevention of some cancers, heart disease, and the common cold. Ascorbic acid and tocopherol supplementation can substantially reduce oxidative damage. The effects are greater in nonsmokers than in smokers. Smoking induces oxidative stresses from numerous free-radical compounds in the gas phases and the ascorbic acid radical could be prooxidant in smokers (Kaur and Kapoor 2001).

The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen. Scavenging aqueous radicals and regeneration of α–tocopherol from the tocopheroxyl radical species are also well known antioxidant mechanisms of ascorbic acid. Ascorbic acid is an excellent electron donor because of the low standard 1-electron reduction potential (282 mV), the generation of relatively stable semi-dehydroascorbic acid, and the easy conversion of dehydroascorbic acid to ascorbic acid. (Rumsey and others 1999). The reaction rate constants of ascorbic acid with other radicals are shown in Table 5. The kinetics of electron or hydrogen atom transfer reactions are rapid, resulting in ascorbic acid being an excellent antioxidant. However, ascorbic acid can act as a prooxidant under certain conditions, including reducing ferric iron to more active ferrous iron.

Regeneration of tocopherol radicals to tocopherols by ascorbic acid has been known since the 1940s. Ascorbic acid can donate a hydrogen atom to a tocopheroxyl radical at the rate of 2×10^5/M/s because of the difference of 1-electron reduction potential between ascorbic acid (282 mV) and (480 mV). The phenol group of tocopherol is located near the interface of a biological membrane-water phase, and ascorbic acid can access easily to the antioxidant active site of tocopherols and regenerate tocopherols from tocopherol radicals (Figure 10) (Buettner and Jurkiewicz 1996).

Carotenoids

Carotenoids are a group of tetraterpenoids. The basic carotenoid structural backbone consists of isoprenoid units formed either by head-to-tail or by tail-to-tail biosynthesis. There are primarily 2 classes of carotenoids: carotenes and xanthophylls. Carotenes are hydrocarbon carotenoids and xanthophylls contain oxygen in the form of hydroxyl, methoxyl, carboxyl, keto, or epoxy groups. Lycopene and β–carotenes are typical carotenes whereas lutein in green leaves and zeaxanthin in corn are typical xanthophylls. The structures of carotenoids are acyclic, monocyclic, or bicyclic. For example, lycopene is acyclic, γ–carotene is monocyclic, and α– and β–carotenes are bicyclic carotenoids (deMan 1999). Double bonds in carotenoids are conjugated forms and usually the all *trans* forms of carotenoids are found in plant tissues (Figure 11).

The antioxidant potentials of carotenoids have been reported for the prevention of free radical initiated diseases, including atherosclerosis, cataracts, age-related muscular degeneration, and multiple sclerosis. Consumption of fresh tomatoes, tomato sauce, and pizza is significantly related to a low incidence of prostate cancer (Giovannucci and others 1995). Lycopene, which is the main carotenoid of tomatoes and tomato products, has several health benefits including decreasing the development of cervical, colon, prostate, rectal, stomach, and other types of cancers (Giovannucci and Clinton 1998; Giovannucci 1999). Tomato juice with 40 mg of lycopene can reduce the endogenous levels of lymphocyte DNA breakage in a group of male smokers (Pool–Zobel and others 1997). Carotenoids including lycopene and β–carotene inhibit the formation of oxidized products of LDL cholesterol, which are associated with coronary heart disease (Weisburger 1999). β–Carotene is involved in the protection of the skin against deleterious effects of sunlight. UV ray initiates free radical in the epidermis by inducing lipid oxidation, which results in premature aging of the skin.

Carotenoids are the most efficient singlet oxygen quencher in biological systems. One mole of β–carotene can quench 250 to 1000 molecules of singlet oxygen at a rate of 1.3×10^{10}/M/s

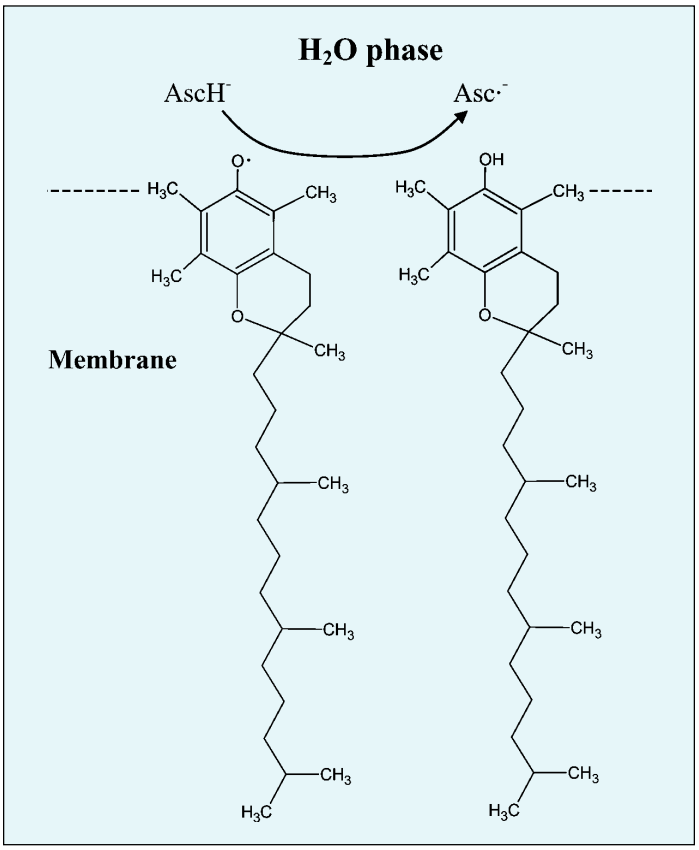


Figure 10—Regeneration of tocopherols by ascorbic acid

(Foote 1976). The rate of singlet oxygen quenching by carotenoids is dependent on the number of conjugated double bonds and on the type and number of functional groups on the ring structure of the molecules (Beutner and others 2000). To act as an effective singlet oxygen quencher, at least 7 conjugated bonds are required, and as the number of conjugated bond increases, quenching efficiency increases (Boff and Min 2002). Singlet oxygen quenching mechanisms by carotenoids are physical quenching without generating oxidizing products ($^1O_2$ + ^{1}Carotenoid → $^3O_2$ + ^{3}Carotenoid).

Contrary to the singlet oxygen quenching ability of carotenoids, hydrogen donating antioxidant activities of carotenoids are controversial. The free radical scavenging mechanism of b–carotene has been proposed to be different from the hydrogen donating phenolic compounds (Liebler 1993; Haila and others 1997). β–Carotene may donate electrons instead of hydrogen atom to free radicals, and become β–carotene radical cation (R + β–carotene → R^-+ β–carotene$^+$) (Liebler 1993; Mortensen and others 2001; Lee and others 2003). Skibsted and co-workers reported the presence of near infrared absorption species from β–carotene using laser flash photolysis. β–Carotene radical cation can absorb near infrared energy. β–Carotene can become radical cation by donating electron not by hydrogen. However, near-infrared absorption species were not observed from xanthophylls containing hydroxyl, keto, and aldehyde groups, which may donate hydrogen atoms instead of electrons to free radicals (Edge and others 1997).

β–Carotene in high oxygen concentration can act as a prooxidant rather than an antioxidant. Antioxidant activity of β–carotene increases at low oxygen concentrations. Not only oxygen concentration but also carotenoid concentration plays an important role in determining antioxidant or prooxidant properties. Relatively high standard 1-electron reduction potential of β–carotene radical cation (1060 mV) (Edge and others 2000) could explain the prooxidant property of β–carotene. β–Carotene may not donate a hydrogen atom to peroxyl radicals effectively, which has a similar standard 1-electron reduction potential of peroxyl radicals (1000 mV), and therefore cannot act as an antioxidant. Burton and Ingold (1984) proposed that β–carotene may react with free radicals by an additional mechanism, and β–carotene molecules become resonance-stabilized, carbon-centered, and conjugated radicals.

Depending on the redox potentials of free radicals and chemical structures of carotenoids, especially the presence of oxygen-containing functional groups, either hydrogen atoms or electrons may transfer from carotenoids to free radicals (Edge and others 1997). β–Carotene can scavenge superoxide anions with the following equation (Edge and others 1997).

$$\cdot O_2^- + CAR + 2H^+ \rightleftharpoons CAR^+ + H_2O_2$$

Table 5—Rate constants for reaction of equilibrium mixture of ascorbic acid/semidehydroascorbic acid/dehydroascorbic acid at pH 7.4

Radical	k (/M/s)	
HO·	1.1×10^{10}	
RO· (*tert*-butyl alkoxyl radical)	1.6×10^{9}	
ROO· (alkyl peroxyl radical)	$1\text{-}2 \times 10^{6}$	
GS· (glutathiyl radical)	6×10^{6}	(pH 5.6)
Tocopheroxyl radical	2×10^{5}	
Ascorbate$^-$· (dismutation)	2×10^{5}	
O_2^-· /H_2O·	1×10^{5}	

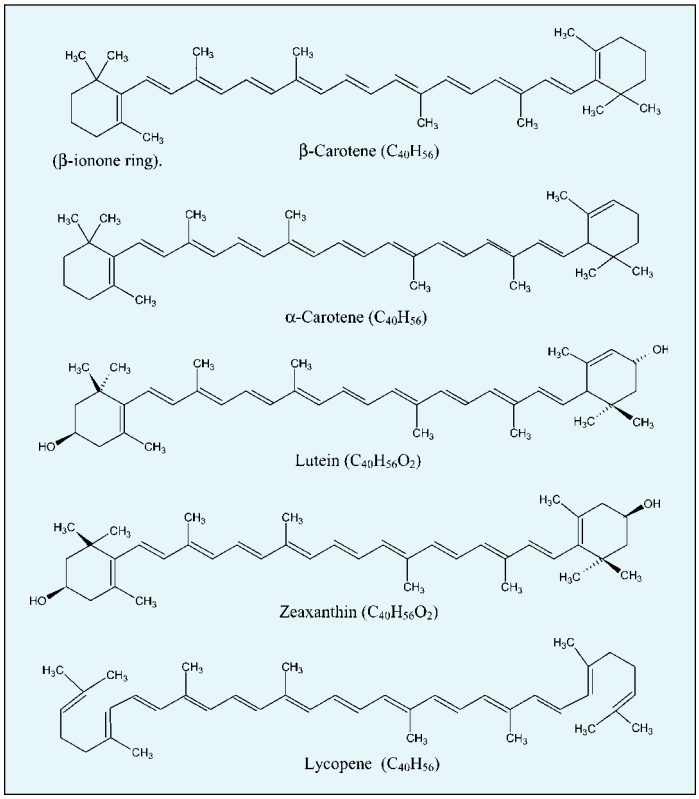


Figure 11—Structures of carotenoids. Lycopene and α- and β-carotenes are carotenes, and lutein and zeaxanthin are xanthophylls.

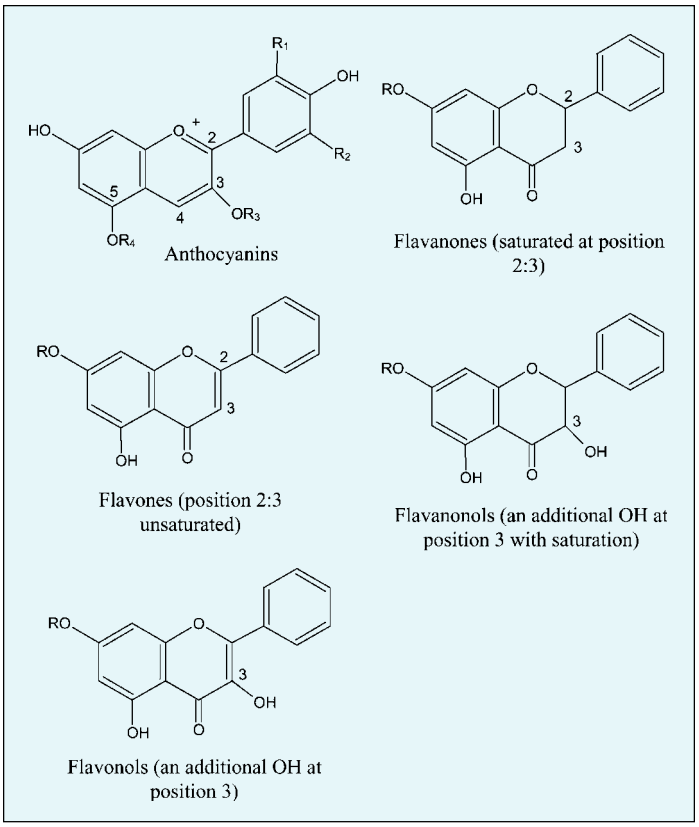


Figure 12—Structures of flavonoids

Polyphenols

Phenolic compounds or polyphenols are ubiquitous in plants with more than 8000 structures reported (Bravo 1998). The classes of phenolic compounds are shown in Table 6. Flavonoids, the most important single polyphenol group, are glycosides with a benzopyrone nucleus. The flavonoids including flavones, flavonols, flavanones, flavanonols, and anthocyanins are based on the common structures of carbon skeletons (Figure 12). The flavones have a double bond between C2 and C3, whereas the flavanones have a saturated C2–C3. Flavononols have an additional hydroxyl group at the C3 position, and flavanonols are saturated between C2 and C3 with a hydroxyl group at the C3 position. The most ubiquitous flavonoid is quercetin, 3, 5, 7, 3′, 4′–pentahydroxy flavone. Each flavonoid group is different, depending on the number of hydroxyl, methoxyl, and other substituents on the 2 benzene rings.

Isoflavones, which do not have the common flavonoid structures, are chemically related with flavonoids. Soybeans contain significantly high isoflavone levels and are the major dietary source of isoflavones in humans. Isoflavones found in soybeans are aglycone forms, including genistein, daidzein, and glycitein, and their glycoside, malonyl glucoside, and acetyl glucoside derivatives (Figure 13). Genistein and its derivatives are found in the highest content in soybeans, followed by daidzein and its derivatives and glycitein and its derivatives (Hendrich and others 1999).

It has been reported that phenolic compounds have antioxidant, antimutagenic, and free-radical scavenging activities. Epidemiologic studies showed that increased consumption of phenolic compounds reduces the risk of cardiovascular disease and certain type of cancer. Moderate consumption of red wine, which contains high content of polyphenols, is associated with a low risk of coronary heart disease (Bravo 1998; German and Walzem 2000).

Consumption of soy and soy products are related with biological effects, including anticarcinogenic, antiatherosclerotic, and antihemolytic effects. The bioactive components are isoflavones (Hendrich and others 1999). Soybean is the unique source of isoflavones with 1 to 3 mg/g and with 0.025 to 3 mg/g soy products (Wang and Murphy 1994). Antioxidant activities of isoflavones, especially genistein, were reported in vivo and in vitro (Naim and others 1976), in simple lipid system such as liposomes, and in more complex system such as lipoproteins (Patel and others 2001). Addition of purified forms of isoflavones inhibited copper-dependent LDL oxidation (Hwang and others 2000). Oral intake of the isoflavone genistein is associated with an increased resistance of LDL oxidation and inhibition of plasma lipid oxidation products (Wiseman and others 2000).

Antioxidant mechanisms of polyphenolic compounds are based on hydrogen donation abilities and chelating metal ions (Bravo 1998). After donating a hydrogen atom, phenolic compounds become resonance-stabilized radicals, which do not easily participate in other radical reactions. However, phenolic compounds act as prooxidants under certain conditions, such as high concentrations of phenolic compounds or metal ions, and high pH. Chemical structures also affect the antioxidant activities.

Flavonoids have the most potent antioxidant activities because of the chemical structures with *o*–diphenolic group, a 2–3 double bond conjugated with the 4–oxo function, and hydroxyl groups in positions 3 and 5. Antioxidant activities of flavonoids are influence by hydroxylation and the presence of sugar moiety (Bravo 1998). Flavonoids are effective hydroxyl radical and peroxyl radical scavengers. Flavonoids can make complexes with metals and inhibit metal initiating lipid oxidation (Hendrich and others 1999).

The antioxidant mechanisms of isoflavones are not clearly understood and have been suggested to be different from conventional antioxidants. The structural similarities of genistein and daidzein to naturally occurring estrogens suggest that these compounds may protect against hormone-dependent cancers (that is, prostate and mammary) by modulating the activity of estrogen (Hendrich and others 1999). Antioxidant activities of isoflavone on lipoxygenase-catalyzed lipid oxidation were dependent on the concentrations and structures of isoflavones (Naim and others 1976). Glucose linkage to aglycone reduced the antioxidant activities of isoflavone. Isoflavones are not consumed during lipid oxidation and show synergic antioxidant effects with ascorbic acid (Patel and others 2001). Patel and others (2001) suggested antioxidant mechanisms of isoflavone as analogous of tocopherol-mediated peroxidation. Hwang and others (2000) suggested that isoflavones may prevent lipid oxidation through stabilizing LDL structures instead involving in lipid oxidation chain reaction.

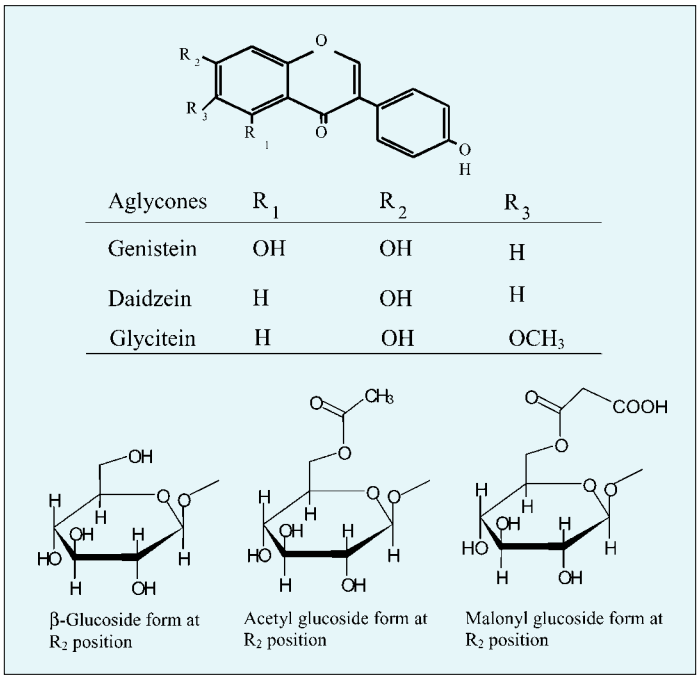


Figure 13—Isoflavone structures. Genistein, daidzein, and glycitein are aglycones, and β-glucoside, acetyl glucoside, and malonyl glucosides of each aglycone are found in soybean.

Lipoic acids

Some sulfur-containing compounds, including glutathione, lipoic acid, and dihydrolipoic acid, have shown antioxidant activities. The chemical structure of lipoic acid is 1,2–dithilane–3–pentanoic acid (Figure 14). Lipoic acid is present in meat, liver, and heart (Bast and Haenen 2001).

Lipoic acids can prevent oxidative damages of proteins. Antioxidant activity of lipoic acid can help to reduce diabetic late complication, which can be developed through oxidative stress. Lipoic acid plays an important role in reducing blood glucose con-

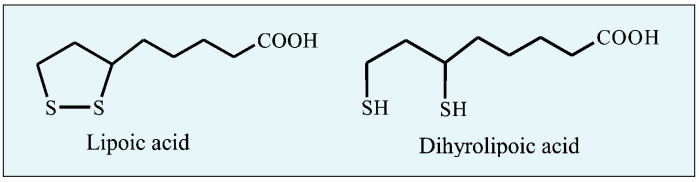


Figure 14—Structures of lipoic acids

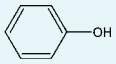
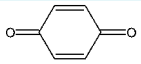
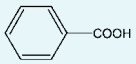
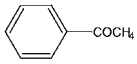
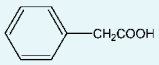
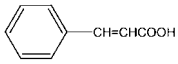
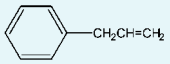
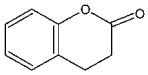
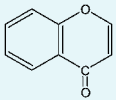
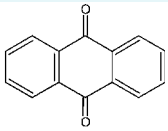
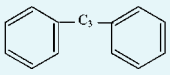
centration. Lipoic acid regenerates GSH in liver, kidney, and lung tissue and also regenerates vitamins C and E. A dietary study of lipoic acid showed a decrease in age-related decline in oxygen consumption and radical formation, improvement of mitochondrial membrane potential, and increases of ascorbic acid and GSH levels (Hagen and others 1999). Lipoic acids may improve age-related decline in memory and cognitive function and brain-related ailments, including Alzheimer's disease and Parkinson's disease (Kramer and Packer 2001).

Reduced (dihydrolipoic acid) and oxidized forms of lipoic acid both act as antioxidants and scavenge the reactive oxygen species. Lipoic acids are excellent antioxidants, showing abilities for radical scavenging, metal chelating, interaction with other antioxidants, metabolic regeneration, and gene regulation (Bast and Haenen 2001).

The standard 1-reduction potential of lipoic acid/dihydroxy lipoic acid is –320 mV, which is significantly lower than that of GSSG/GSH and dehydroascorbic acid/ascorbic acid, 250 and 282 mV, respectively.

Dihydrolipoic acid is a reductant and regenerates GSH from GSSG and ascorbic acid from dehydroascorbic acid at the rate constant of 32 and 875 /M/min, respectively.

Table 6—Classes of phenolic compounds

Class	Basic skeleton	Basic structure
Simple phenols	C_6	
Benzoquinoes	C_6	
Phenoic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropens	C_6-C_3	
Coumarins	C_6-C_3	
Chromones	C_6-C_3	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	

Bioavailability of Antioxidative Nutraceuticals

Definition of bioavailability is the amount or the percentage of an ingested nutrient that is absorbed and thus available to the body for metabolic use. Bioavailability of antioxidative nutraceuticals is influenced by many factors, including types of nutraceuticals, geometric isomers, processing methods, and matrices surrounding the compounds (Papas 1999b).

Tocopherols and tocotrienols in human blood and tissues are in their free and unesterified form. Esterification of tocopherols, which blocks the 6–hydroxyl group of tocopherols, makes tocopherols more stable to oxidizing agents such as air, light, and metals, and used for fortifying foods or vitamin supplements. Esters of tocopherols are hydrolyzed by lipases and tocopherols are absorbed in their free, unesterified form. α–Tocopherol is preferentially secreted by the liver into the blood lipoprotein, even though α– and β–tocopherols are equally well absorbed. A tocopherol-binding protein plays an important role in this preferential incorporation with α–tocopherol (Papas 1999b). Even though tocotrienols have a higher radical scavenging activity than tocopherols, they are less bioavailable after oral ingestion (Packer and others 2001). It has been known that α–tocotrienol is preferentially absorbed compared with δ– and γ–tocotrienols (Ikeda and others 1996).

Ascorbic acid in foods is mainly (80% to 90%) in the reduced form and is absorbed in human intestine by a sodium-dependent active transport system. Ascorbic acid is suggested to be absorbed better than dehydroascorbic acid in humans (Gregory 1996).

Food processing can affect bioavailability. Absorption of lycopene from fresh tomatoes and β–carotene from fresh carrots is significantly lower than from tomato juice or cooked carrots. Heat processing breaks the carotenoid protein complexes and converts *cis* to *trans* β–carotene, which can affect bioavailability.

Excessive heating can promote oxidation or formation of complexes of antioxidants with carotenoids and proteins. Even when *cis* and *trans* forms of β–carotene are ingested, the concentration of *trans* β–carotene in blood and tissue is higher than *cis* β–carotene (Deming and others 2002).

Naturally occurring geometrical isomers of lycopene are primarily in the all-*trans* configuration. In dietary studies, ingested lycopene is predominately (about 95%) in the all-*trans* form. However, Clinton and others (1996) have shown that *cis*-isomers of lycopene represent approximately 50% of total lycopene in blood and up to 80% in prostate tissues. It has been suggested that *cis*-isomers of lycopene are more bioavailable than all *trans*-isomers, most likely because of the greater solubility of *cis*-isomers in the bile acid micelles, a shorter length to fit into micelles, and a lower tendency to aggregate (Boileau and others 1999; Boileau and others 2002). The mechanisms explaining the isomerization of all-*trans* to *cis*-lycopene isomers in vivo after food consumption and the physiological importance of *cis*-lycopene are not fully understood (Nguyen and Schwartz 1999; Boileau and others 2002).

Major forms of isoflavones in foods are β–glycosides. However, glucosides are not detected in human blood and urine whereas aglycones of isoflavones are found. Hydrolysis of flavonoid glycosides can be done by microorganism glucosidases in the colon, not by the host mammalian. Aglycones of isoflavone can be absorbed in the gut better than their glucoside derivatives. Sugar moiety of glycosides is an important factor of absorption and bioavailability of isoflavones (Hendrich and others 1999).

Foods Containing Antioxidative Nutraceuticals

Fruits, vegetables, spices, herbs, and beverages such as tea and wine are typical foods containing various antioxidative nutraceuticals. In fruits, grapes contain polyphenolic compounds such as caftaric acid, tartaric acid ester of caffeic acid, flavon–3–ol catechin, and anthocyanins.

Berries, including blueberries, strawberries, blackberries, and crowberries, contain large amounts of phenolic compounds such

as hydroxylated benzoic acids and cinnamic acid, and flavonoids, including anthocyanins, proantocyanins, flavonols, and catechins. Citrus fruits have polyphenols such as hydroxycinnamic acid including *p*–coumaric, caffeic, and ferulic acids, limonoids, and naringin. Even citrus seeds and peels have antioxidant activity (Bravo 1998).

Tomato, beans, broccoli, beet, mushroom, corn, white cabbage, kale, cauliflower, spinach, garlic, onion, cacao beans, and soybean are typical vegetables containing nutraceuticals. Sage, rosemary, oregano, and thyme are some examples of spices and herbs with nutraceuticals. Discussing specific nutraceuticals in each fruit, vegetable, spice, and herb is beyond the object of this review; excellent reviews on these topics can be found in Potter and Steinmetz (1996), Bravo (1998), and Kaur and Kapoor (2001).

Tea contains large amounts of flavonoids, including catechin, epicatechin, quercetin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate. Catechin and quercetin inhibit LDL oxidation and protect lymphoid cells against cytotoxic effects of oxidized LDL. Catechin delays the oxidation of human plasma with enxogenous antioxidants such as α–tocopherol and β–carotene.

Tea drinking can inhibit the oncogene expression in the lungs. Tea flavonoid–epigalocatechin gallate inhibit the oncogene expression in skin. Tea polyphenol extracts stimulated the expression of detoxifying enzymes in cultured human heptatoma cell line (Balentine and others 1997; Ahmad and Mukhtar 1999).

In some parts of France, coronary heart disease mortality is low despite a high intake of saturated fats and relatively high plasma cholesterol levels; this is called the "French paradox." High consumption of wine was found to be related with the French paradox. Wine contains large amount of phenolic compounds, which inhibit LDL oxidation. Phenolic compounds in wine are *p*–coumaric, cinnamic, caffeic, gentisitc, ferulic, and vanillic acids (Sun and others 2002).

Evidence supporting the protective effects of high fruit and vegetable consumption on the risks of many cancers and age-related diseases is extensive and consistent. For example, high consumption of fruits and vegetables is related to the prevention of osteoporosis by maintaining the body's bone density (Tuker and others 1999), reducing the risk of cardiovascular disease (Liu and others 2000), prostate cancer (Cohen and others 2000), and lung cancer (Michaud and others 2000).

Recommended daily allowances suggested by United States of Department of Agriculture (USDA) include fruit and vegetable groups for maintaining healthy life of human beings (USDA/CNPP 2000). Depending on the age, sex, body size, and level of physical activity, serving sizes per each day of fruit and vegetable groups vary from 2 to 4 and from 3 to 5, respectively (USDA/CNPP 2000).

Conclusions

Aging is a complex multifactorial process in which free radical oxidative damage plays a very important role, but free radical oxidative damage may not be the exclusive mechanism in aging. Antioxidant defense mechanisms in humans, such as antioxidative enzymes, tocopherol, and ascorbic acid, are linked to each other and balance with reactive oxygen species. The increased concentration of dietary foods containing antioxidative nutraceuticals with 3 to 5 servings from the vegetable group and 2 to 4 servings from the fruit group (USDA/CNPP 2000) can help humans reduce the deleterious reactive oxygen species and free radicals, and balance the oxidative stress to slow the aging process.

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