Antioxidants for Health and Longevity

21

Ramiah Sivakanesan

Abstract

Aging is an inevitable process due to functional and structural loss in the body accrued over a period of time owing to harmful effects of free radical generated by a variety of events. Aging is associated with changes in cell metabolism which leads to decrease in cell size, number, and atrophy of organs. Cell loss is most evident in the brain and heart, in which regeneration of lost cells does not occur. Many theories explain the process of aging, but the free radical theory provides plausible evidence for its occurrence. Endogenous metabolic events and exogenous factors are responsible for the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen species is collectively used in a broad sense to free radicals like superoxide ($O_{2^{\cdot}}^{-}$), hydroxyl ($OH^{\bullet}$), and lipid peroxyl ($LOO^{\bullet}$) radicals and non-free radicals such as hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), and lipid peroxide (LOOH). Uncontrolled increase in ROS concentration enhances free radical-mediated chain reactions which generally target proteins, lipids, polysaccharides, and DNA. Human body has the capability to counteract the ROS by enzymatic antioxidants superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx); nonenzymatic nutrient antioxidants β-carotene, α-tocopherol, ascorbic acid; and metabolic antioxidants, bilirubin, uric acid, ceruloplasmin, ferritin, transferrin, albumin, and glutathione.

Keywords

Dietary antioxidants · Longevity · Reactive oxygen species

R. Sivakanesan (✉)
University of Peradeniya, Peradeniya, Sri Lanka

S. I. Rizvi, U. Çakatay (eds.), *Molecular Basis and Emerging Strategies for Anti-aging Interventions*, https://doi.org/10.1007/978-981-13-1699-9_21

21.1 Introduction

Aging is a natural physiological process associated with loss in cellular integrity and cell mass guided by programmed cell death or apoptosis. Many believe that this process could be slowed down if not completely halted. Human internal environment is maintained by a number of metabolic events which depend on oxidation of nutrients by the cell and elimination of metabolic end products which are considered to be waste materials.

21.2 Cellular Events in Aging Process and Theories of Aging

21.2.1 General Changes in the Cell

Changes associated with aging are inevitable.

21.2.1.1 Cell Loss and Body Composition Changes

Aging is associated with changes in cell metabolism which leads to decrease in cell size, number, and atrophy of organs. Cell loss is most evident in the brain and heart, in which regeneration of lost cells does not occur. Cell loss in the brain is selective, with the greatest loss occurring in the (a) basal ganglia (b), substantia nigra, and (c) hippocampus.

Body composition changes continuously throughout life. After sixth decade body weight (BW) decreases significantly, around 7 kg per decade in males and 6 kg per decade in females. There is decrease in fatness also. Lean body mass (LBM) decreases by about 6% for each decade of age, the loss accelerating in later life and greater in males. Between 70 and 75 years, about 1 kg LBM is lost.

Body cell mass decreases with age due to decreasing number of cells in organs and increased disuse of skeletal muscle with age. By 70 years of age, skeletal muscle has lost 40% of its maximal weight in early adult life compared with 18% for the liver, 9% for the kidney, and 11% for the lungs. Muscle thus contributes most to the loss of cell mass. Loss of cells in organs such as the liver and kidney will lead to a loss of reserve tissue available to cope with disease conditions.

Loss in BW in the eighth decade of life corresponds more to loss of body cell mass in males and more to loss of body fat in females. Bone density decreases by 12% in males and 25% in females by the ninth decade. Changes in bone density begin at the age of 40 years. Estrogen withdrawal accounts for much of the bone loss among women between 40 and 60 years.

21.2.1.2 Organelle Changes

The endoplasmic reticulum of aged cells is disordered, and its usual association with ribosomes is lost. Hence free ribosomes are present in greater numbers than normal. This results in abnormalities in synthesis of protein to be exported out of the cell. Hence quantitative and qualitative activity of many enzymes decreases (Digital World Medical School 2016).

Abnormalities in size, shape, and cristae in the mitochondria occur with aging. These, together with reduced levels of cytochrome C reductase, impair energy production. An increased rate of organelle breakdown in aged cells is associated with the presence of increased numbers of (a) phagolysosomal vacuoles in the cells and (b) deposition of lipofuscin – a brown pigment believed to be derived from degraded organelle membranes, particularly evident in the heart, brain, and liver (Chandrasoma and Taylor 2001; Digital World Medical School 2016).

Abnormalities develop in some of the cytoplasmic structures in aged cell. The contractility of myofibrils in muscle cells is decreased. The ability of the nerve cells to synthesize acetylcholine declines with aging. The phagocytic efficiency of macrophages is reduced. Cell surface hormone receptors become abnormal, resulting in disturbances in binding of ligands such as insulin (Digital World Medical School 2016). As such the vital activities that sustain the life process is greatly reduced, and the cells lose their viability and physiological capability.

21.2.1.3 DNA Abnormalities

DNA abnormalities are mainly the result of a progressive failure of cellular DNA repair mechanisms. Failure of DNA repair can potentially affect any cellular function and frequently leads to cell death (Digital World Medical School 2016).

21.2.2 Apoptosis

Apoptosis is alternatively referred to as programmed cell death. Apoptosis literally means “a falling away from”. It is a pathway of cellular suicide and is responsible for programmed cell death in several important physiological as well as pathological processes listed below:

(a) Programmed destruction of cells during embryogenesis, as occuring in implantation, organogenesis, and developmental involution
(b) Hormone-dependent physiologic involution, such as involution of the endometrium during the menstrual cycle or the lactating breast after weaning, or pathologic atrophy as in the prostrate after castration
(c) Cell deletions in proliferating populations such as in the intestinal crypt epithelium or cell death in tumors
(d) A variety of mild injurious stimuli (heat, radiation, cytotoxic cancer drugs) that cause irreparable DNA damage that in turn triggers cell suicide pathway (e.g., via the tumor suppressor protein TP53) (Anonymous 2009)

Apoptosis usually involves single cells or clusters that appear on H &E stained sections as round or oval masses with intensely eosinophilic cytoplasm. The nuclear chromatin is condensed, and it aggregates peripherally, under the nuclear membrane, into well-delimited masses of various shapes and sizes. Ultimately karyorrhexis occurs, at a molecular level, reflected in fragmentation of DNA into nucleosome-sized pieces, presumably through activation of endonucleases. The cells rapidly

shrink, form cytoplasmic buds, and fragment into apoptotic bodies composed of membrane-bound vesicles of cytosol and organelles. Fragments are quickly extruded and phagocytosed or degraded.

21.2.3 Theories of Aging

Many theories have been proposed to explain the basis of aging. Hayflick (1985) in his review article on "theories of biological aging" classified them as follows:

(a) Organ theories (immune or neuroendocrine)
(b) Physiological theories (free radical, cross-linking, and waste-product accumulation)
(c) Genome-based theories (somatic mutation, error theory, and program theory)

21.2.3.1 Organ Theories

Immunological Theory
According to the immunological theory, with aging there is decline in normal immune response accompanied by increased autoimmune manifestation.

Neuroendocrine Theory
The neuroendocrine theory of aging is based on the decline in neurons and endocrine cells which are vital to coordinate the activities. Ten percent decrease occurs in total brain weight with age (Brody 1980).

21.2.3.2 Physiological Theories

The Free Radical Theory
Since free radicals are highly unstable reactive molecules, they tend to propagate chain reactions during which many stable molecules are converted to free radicals through a process of oxidation. Uncontrolled increases in oxidant concentrations tend to enhance free radical-mediated chain reactions which generally target proteins (Stadtman and Levine 2000), lipids (Rubbo et al. 1994), polysaccharides (Kaur and Halliwell 1994), and DNA (Richter et al. 1988; LeDoux et al. 1999). The indiscriminate damages lead to loss in cellular architecture which culminates in the death of the cells (Harman 1981).

The Cross-Linkage Theory of Aging
Bjorksten (1974) claims that free radicals are effective cross-linkers. With aging macromolecules like proteins, DNA, and RNA are linked covalently or by a hydrogen bond between them. Cross-linking of collagen increases viscosity in the extracellular compartment, thereby impairing the flow of nutrients and waste products into and out of cells. Cross-linking of DNA affects its usual function leading to mutation or cell death (Hayflick 1985).

Waste-Product Accumulation

Lipofuscin found in lysosomes indicates cellular wear and tear and may impair the cellular function (Hayflick 1985). Lipofuscin is an intralysosomal undegradable polymeric substance, and aged lipofuscin-rich cardiac myocytes become overloaded with damaged mitochondria, leading to increased oxidative stress, apoptotic cell death, and the gradual development of heart failure (Terman et al. 2008).

21.2.4 Genome-Based Theory

Genes may be instrumental in determining longevity. The genetic material is constantly exposed to endogenous and exogenous materials that could bring about mutational changes in DNA. The somatic mutation theory is built on the concept that accumulation of a sufficient level of mutations in somatic cells will produce physiological decrements characteristic of aging (Hayflick 1985).

21.3 Generation of Free Radicals and Reactive Oxygen and Reactive Nitrogen Species

Living organisms are endowed with the inherent potential to generate energy using molecular oxygen. This inevitably results in the generation of reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) owing to the reactive nature of oxygen. Atoms are most stable in the ground state. An atom is considered to be "ground" when every electron in the outermost shell has a complimentary electron that spins in the opposite direction. The oxygen atom contains unpaired electrons in both the outer and inner shells; however the total number of electrons is even. Molecular oxygen (O_2) is a diatomic molecule containing two unpaired electrons in the outer shell. The Lewis structure for oxygen molecule is :Ö::Ö:. Therefore double bond is necessary to satisfy the octet rule for both oxygen atoms, and hence molecular oxygen is not very reactive with the two electrons involved in a chemical bond.

Reactive oxygen species (ROS) refers to a variety of molecules and free radicals (chemical species with one unpaired electron) derived from molecular oxygen. Reactive oxygen species is collectively used in a broad sense to free radicals ($O_2^{\cdot -}$,$OH^{\cdot}$) and non-free radicals (H_2O_2, $^1O_2$) of the biological system. Free radicals are thus atoms, molecules, or ions with unpaired electrons. Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron (Halliwell and Gutteridge 2007; Bahorun et al. 2006). These are formed in our body during various physiological and pathological processes.

Oxygen-derived free radicals include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), peroxyl ($ROO^{\bullet}$), and lipid peroxyl ($LOO^{\bullet}$) and non-radicals like hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and lipid peroxide (LOOH). The non-radical derivatives are generally referred to as oxidants but can easily lead to free radical reactions in living organisms (Genestra 2007). The

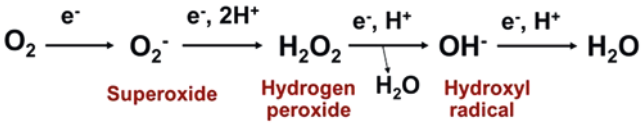

Fig. 21.1 Generation of free radicals from oxygen

nitrogen-derived free radicals are nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), and peroxynitrite ($ONOO^{-}$) (Koppenol et al. 1992). Sequential reduction of molecular oxygen leads to the formation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radical as part of normal aerobic process (Fig. 21.1). Free radicals, ROS, and RNS do play a role in physiological function. They are unstable, highly reactive species which possess the ability to oxidize other molecules in an attempt to attain a stable state. In such a process the oxidized molecules become unstable and reactive and continue the oxidation process causing damage to the oxidized molecules.

Free radicals are formed during normal biochemical reaction involving oxygen. Metals containing proteins, as well as other sources of metals, are potent electron-transferring agents. Endogenous free radicals are generated in the biological system during normal cellular metabolism such as mitochondrial electron transport and endoplasmic reticulum oxidation; enzymatic activity including NADPH oxidase, xanthine oxidase, monoamine oxidase, tyrosine hydroxylase, L-amino oxidase, diamine oxidase, glycolate oxidase, alpha-hydroxy acid oxidase, nitric oxide synthase, and L-gulonolactone oxidase; and events like prostaglandin synthesis, autoxidation of adrenaline, activated phagocytic cells, and cytochrome P_{450} activity (Tandon et al. 2005; Bandyopadhyay et al. 1999; Babior 1978; Slater 1984; Sinclair et al. 1991). Energy required for the cellular activities is generated principally in mitochondria by aerobic oxidation whereby molecular oxygen is completely reduced to water. Nearly 3–5% of the daily oxygen utilized is converted to superoxide, hydrogen peroxide, and hydroxyl radicals.

Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer, and aging. Exogenous ROS/RNS results from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus [an immunosuppressive drug], gentamycin, bleomycin [an antitumor antibiotic]), industrial solvents, cooking (smoked meat, used oil, fat), and radiation (Pham-Huy et al. 2008).

Perez-Campo et al. (1998) have reviewed the relationship between oxidative stress and maximum life span (MLSP) in different vertebrate species. They are of the view that MLSP correlates negatively with the antioxidant status in animals and human beings show the minimum levels of antioxidants.

21.4 Harmful Effects of Free Radicals

A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. When free radicals steal an electron from a surrounding compound or molecule, a new free radical is formed in its place. Newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules. Thus the chain reaction continues and can be "thousands of events long" (Karlsson 1997).

21.4.1 Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. Polyunsaturated fatty acids (PUFAs) are abundant in cell membranes and low-density lipoproteins (LDL). The PUFAs are responsible for the fluidity of cellular membranes which governs its semi-permeability. Free radicals seize electrons often from PUFAs in cell membranes, which results in cell damage via a free radical chain reaction mechanism (Fig. 21.2). In addition, end products of lipid peroxidation may be mutagenic and carcinogenic. The effect of ROS on the carbon-carbon double bond of PUFAs weakens the carbon-hydrogen bond (CH_2), letting a hydrogen atom by dissociation and leave behind an unpaired electron on the carbon atom (•CH). The resultant carbon radical is stabilized by molecular rearrangement to produce a conjugated diene, which then can react with an oxygen molecule to produce lipid

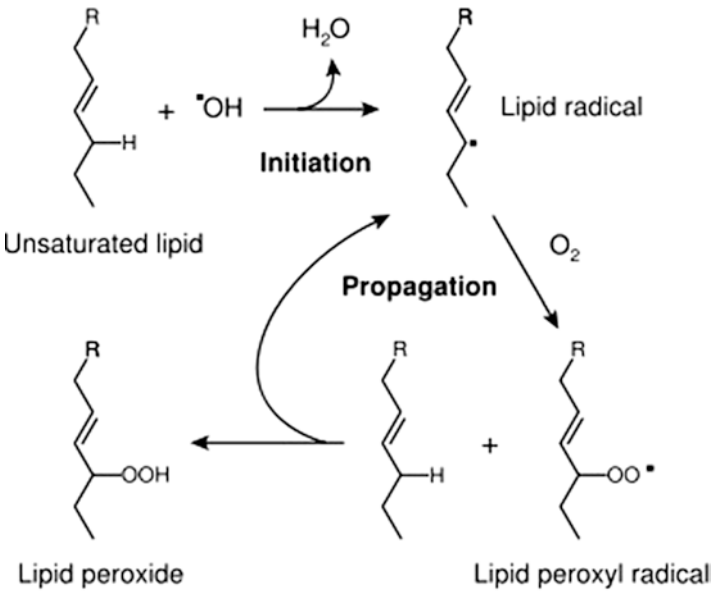

Fig. 21.2 Lipid peroxidation

hydroperoxide (LOOH) and at the same time propagate lipid peroxidation further. A free radical stealing the single electron from the hydrogen associated with the carbon at the double bond leaves the carbon with an unpaired electron and hence becomes a free radical as well (Halliwell and Gutteridge 1985). When lipid peroxidation occurs in biological membranes, their structure and function are in disarray causing highly damaging consequences to the cell. One of the products of lipid peroxidation, malondialdehyde (MDA), has been extensively measured in a variety of conditions. The MDA concentration is significantly higher in normal elderly people (396.39 ± 43.58 nanomoles/dL), elderly hypertensive (551.16 ± 199.52 nanomoles/dL), elderly diabetic (555.87 ± 88.39 nanomoles/dL), and elderly diabetic hypertensive (749.42 ± 260.6 nanomoles/dL) patients compared to normal young subjects (352.26 ± 67.59 nanomoles/dL) (Akila et al. 2007). Diabetes is usually accompanied by increased oxidative stress which result from overproduction of precursors to reactive oxygen radicals and decreased efficiency of inhibitory and scavenger systems. There is evidence that both free radical production and antioxidant defenses are disturbed in diabetes (Lyons 1991; Ceriello 2000). Evidence has been generated also from our own laboratory that there is significant increase in MDA levels among diabetic patients (2.22 ± 1.58 μmol/L) compared to the controls (1.21 ± 0.6 μmol/L) (Udayangani et al. 2015) and proteinuric diabetic patients had even higher MDA levels of 5.2 ± 3.4 μmol/L (Udayangani 2015).

Richard (1985) studied the peroxide-producing potential of tissues in vitro and observed that human brain and kidney tissue homogenates are found to be most resistant to autoxidation, in agreement with humans having the longest MLSP. Based on his observation, he concluded that longevity of different mammalian species is determined in part by intrinsic differences in tissue peroxidation potential due possibly to unusually high concentrations of antioxidants and other defenses against peroxidation reactions.

21.4.2 Oxidation of Nucleic Acid/DNA by ROS

The hydroxyl radical ($OH^{\bullet}$) is the mediator of much of the DNA damage causing strand breaks, which are initiated by abstraction of a deoxyribose hydrogen atom by the hydroxyl radical. ROS forms DNA adduct which is characterized by deletion and mutation and causes genetic effects. The oxidation leads to degradation of bases, single- or double-stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins. Sugars and base moieties are degraded by ROS and cause oxidation of bases and cross-linking to protein (Sies 1985). The hydroxyl radical ($OH^{\bullet}$) oxidizes guanine to 8-hydroxy-2-deoxyguanosine (8-OHdG), which eventually leads to GC→ TA transversions during subsequent DNA replication (Floyd 1990). DNA alteration has been suggested to be responsible in part in the processes of aging (Fraga et al. 1990).

21.4.3 Oxidation of Proteins

The ROS cause oxidation of sulfhydryl groups and modification of amino acids. The proteins may undergo fragmentation, resulting in the loss of their biological activity. ROS can react with several amino acid residues in vitro, generating a wide range of products from modified and less active enzymes to denatured, nonfunctioning proteins (Butterfield et al. 1998; Said and Aiman 2014).

Free radicals are implicated in a number of diseases like diabetes mellitus, cardiovascular diseases, hypertension, atherosclerosis, cancer, and neurodegenerative diseases besides its involvement in aging as summarized by Velavan (2011).

21.5 Antioxidants and Their Role in Preserving Cellular Integrity

A biological antioxidant has been defined as any substance that is present at low concentrations compared to an oxidizable substrate and significantly delays or prevents the oxidation of that substrate (Halliwell and Gutteridge 2007). The beneficial effect of antioxidant depends on their ability to work in aqueous and nonaqueous environment in the body. Antioxidants play a vital role in eliminating or keeping the ROS and/or RNS under check. An imbalance between the excessive formation of ROS and/or RNS and limited antioxidant defenses results in "oxidative stress" leading to various deleterious processes. Based on their location, antioxidants could be grouped as:

(a) Plasma antioxidants: ascorbic acid (vitamin C), bilirubin, uric acid, transferrin, ceruloplasmin, β-carotene
(b) Cell membrane antioxidants: α-tocopherol (vitamin E)
(c) Intracellular antioxidants: superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx)

However according to their nature and action, they are grouped into:

(a) Enzymatic antioxidants: SOD, catalase, GPx, glutathione reductase
(b) Nonenzymatic antioxidants:

Nutrient antioxidants: β-carotene, α-tocopherol, ascorbic acid
Metabolic antioxidants: bilirubin, uric acid, ceruloplasmin, ferritin, transferrin, albumin, glutathione

21.5.1 Antioxidant Protection by Nutrient Antioxidants

Plants are important contributors of various dietary constituents and nutrients. Among them vitamin E, vitamin C, and carotenoids are considered as natural antioxidants. They are very useful to the body and supplement the action of various endogenous antioxidants to combat the deleterious effects of free radicals. All the dietary nutrients are quite specific in its structure and function.

21.5.1.1 Vitamin E

Vitamin E occurs in the diet as a mixture of several closely related compounds, called tocopherols of which α-tocopherol is the most potent form of vitamin E. Tocopherols are considered as nature's best antioxidants. Because of their lipophilic nature, they are present in circulating lipoproteins, cell membranes, and fat deposits. Vitamin E donates extra electrons to needed unpaired electrons in order to stop the damaging potential of the free radical. As a result, vitamin E is converted to tocopheryl radical which is no longer active. Although free radical damage can't be stopped all together, it can be minimized.

Vitamin E is a fat-soluble antioxidant and is the primary defender against effects of free radicals in the body. It protects components of the cell and their membrane from destruction. It is stored in the liver and fat cells. Vitamin E acting as an electron sink is an efficient lipid-soluble antioxidant that functions as "chain breaker" during lipid peroxidation in cell membranes and various lipid particles including LDL (Packer 1998; Kagan et al. 2002).

21.5.1.2 Vitamin C

Vitamin C alternatively termed as ascorbic acid is a water-soluble and versatile free radical scavenger which gives up electrons very easily when they are needed. It helps to regenerate vitamin E from tocopheryl radical. It has the ability to recycle over and over again. Ascorbic acid upon oxidation is converted to dehydroascorbic acid which could be regenerated by glutathione (Fig. 21.3). Vitamin C is a powerful reducing agent which can directly scavenge superoxide, hydroxyl radicals, singlet oxygen, and H_2O_2.

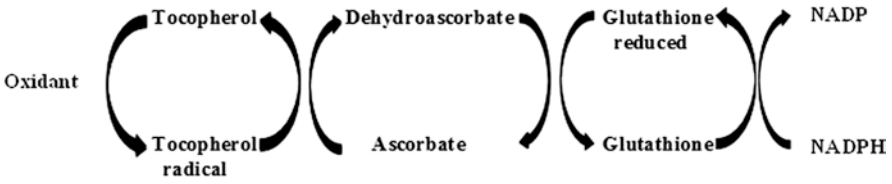

Fig. 21.3 Interaction of antioxidants in biological system

21.5.1.3 Carotenoids

Carotenoids such as α-, β-, and γ-carotene, lycopene, and lutein function as important antioxidants, and they quench $O_2^{-\cdot}$ and $ROO^{\cdot}$ radicals. Among carotenoids the most potent one is β-carotene.

Synergistic interaction by vitamin C, vitamin E, and carotenoids has shown to prevent lipid peroxidation (Niki et al. 1995).

21.5.2 Antioxidant Protection by Enzymatic Antioxidant

21.5.2.1 Superoxide Dismutase

Superoxide dismutase (SOS) is a metalloenzyme present in most of the cells. It converts superoxide radical to H_2O_2.

$$2O_2 \bullet^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

The three SOD forms are:

(a) Cu-Zn SOD present in the cytoplasm
(b) Mn-SOD found in the mitochondria
(c) Cu-SOD in extracellular SOD

21.5.2.2 Catalase

Catalase is responsible for converting the H_2O_2 to harmless water and oxygen. It is found in high concentration in the liver and erythrocytes. The brain, heart, and skeletal muscle contain only low amounts.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

21.5.2.3 Glutathione Peroxidase

Glutathione peroxidase (GPx) is a selenium-dependent enzyme which detoxifies hydrogen peroxide using glutathione. Glutathione (GSH), a tripeptide found in most cells, is oxidized (GSSG) in the reaction catalyzed by glutathione peroxidase and prevents the damage to biomolecules by H_2O_2. Glutathione reductase (GR) keeps the glutathione pool in cell in a reduced state using NADPH derived from hexose monophosphate shunt (Fig. 21.3).

$$H_2O_2 + GSH \xrightarrow{\text{GPX}} H_2O + GSSG$$

$$GSSG + NADPH^+ \xrightarrow{\text{GR}} GSH + NADP$$

21.5.3 Other Dietary Antioxidants

Zinc, copper, and selenium protect against oxidative stress indirectly serving as cofactors for antioxidant enzymes superoxide dismutase and glutathione peroxidase.

21.5.4 Antioxidant Protection by Non-nutrient Antioxidants

Phytochemicals are non-nutrient compounds found in plant-derived foods that have biological activity in the body. They contribute taste, aromas, colors, and other characteristics to food. Polyphenols are the important phytochemicals that can work either indirectly or directly by stopping free radicals from propagating.

The most common group of plant phenolics are flavonoids and nearly 4000 have been identified in plants. They are sometimes referred to as "super antioxidants." In addition to the antioxidant potential, they show antiviral, antiallergic, anti-inflammatory, antithrombogenic, and anticarcinogenic effects. Flavonoids share a common structure (two benzene rings and a central pyran ring) which determines their antioxidant functioning. They are grouped into flavonols, flavanols, flavonones, anthocyanins, isoflavones, and flavones.

Our recent study on soy-incorporated traditional breakfast food items in diabetic individuals revealed that the serum antioxidant capacity measured as the ferric reducing ability of plasma (FRAP) significantly increased from 817.80 ± 176.5 to 1059.75 ± 200.6 μmol/l over an experimental period of 120 days. In the control group of diabetics, the FRAP value was 820.61 ± 147.1 μmol/l at the commencement and decreased to 723.64 ± 101.3 μmol/l during the same experimental period. Soybean is a relatively good source of vitamin E (7.15 μg of tocopherol/g of seeds) (Vasantharuba et al. 2007) and ascorbic acid (37.84 mg/100 g) (Okuwa and Orji 2007). Further the flavonoid content is 3.84 mg/100 g and is relatively higher compared to tannins (0.46 mg/100 g), phenols (0.04 mg/100 g), and saponins (0.17 mg/100 g) (Okuwa and Orji 2007). These antioxidants would have enhanced the total antioxidant capacity.

21.5.5 Human and Animal Studies on the Role of Antioxidants in Longevity

With the current understanding from human and animal studies, evidence supports that vitamins A and E may only provide life span benefits when started early in life (Chong-Han 2010).

21.5.5.1 Thai Traditional Formula

A Thai traditional formula has been claimed to prevent and/or cure disease. Based on this assertion, Luanchoy et al. (2014) carried out phytochemical screening tests in the six herbs included in the formula for longevity and documented the presence of phenolic compounds, tannins, and flavonoids in *Cyperus rotundus* and *Albizia*

procera; phenolic compounds and flavonoids in *Piper nigrum*, *Diospyros rhodocalyx*, and *Streblus asper*; and phenolic compounds, but neither flavonoids nor tannins, in *Tinospora crispa* extract. Further their investigations revealed only *Albizia procera* possessed highly potent antioxidant activity, although its potency was lower than that of vitamin C and Trolox.

21.5.5.2 Blue Mountains Eye Study

Ava Grace Tan et al. (2008) through a prospective population-based cohort study provided evidence of long-term beneficial association between antioxidants, mainly vitamin C (either alone or in combination with other antioxidants), and nuclear cataract development, a well-known biological marker of aging. Their findings that subjects in the highest quintile of total intake of vitamin C contributed by diet and supplements had a reduced risk of incident nuclear cataract is a convincing evidence for the protective role of vitamin C. Furthermore, intake of vitamins C and E, β-carotene, and zinc in combination above median value was also associated with a reduced risk of incident nuclear cataract. The reason for the damage to crystalline proteins, lens fiber membranes, and lipids resulting in lens opacities is due to the oxidative consequence of superoxides and hydroperoxides (Boscia et al. 2000).

21.5.5.3 Oyster Mushroom Supplements

Sánchez et al. (2015) studied the effect of selected oyster mushroom supplements on the longevity of the Mexican fruit fly, *Anastrepha ludens*. They reported that *Pleurotus djamor* ECS-0142 strains with the highest antioxidant capacity when supplemented at 1% level showed slightly but significantly greater survival than those on the control diet. However 5–20% concentrations of mushrooms in the diet resulted in a decrease in life expectancy.

21.5.5.4 Mediterranean Diet

The traditional Mediterranean diet is long known for its health-preserving ability. The traditional Mediterranean diet is built on health-promoting characteristic such as high consumption of legumes, cereals, fruits, and vegetables, moderate consumption of milk and dairy products and ethanol and intake of fat with high monounsaturated to saturated ratio, as well as low consumption of meat and meat products (Trichopoulou et al. 1995). Based on the fact that the Mediterranean diet contributes a significant amount of flavonoids through fruits, vegetables, and beverages and polyphenolic compounds from olive oil, Trichopoulou and Vasilopoulou (2000) concluded that the diet has considerable antioxidant properties. Even though they claimed that there is no direct evidence that the antioxidants are central to the benefits of the Mediterranean diet, based on epidemiological data they suggested that there is indirect evidence to indicate antioxidants may play a major role.

21.5.5.5 Antioxidant Profiles in Italian Centenarians

Plasma levels of vitamin C, uric acid, vitamin A, and vitamin E and activities of SOD and GPx were estimated in healthy subjects of different age groups. In subjects

≤60 years, 61–80 years, 81–99 years, and ≥100 years, the vitamin C (μM) concentrations were, respectively, 49.5 ± 14.5, 49.8 ± 15.8, 35.7 ± 9.8, and 29.6 ± 4.5. Subjects who were 81 years or more had significantly lower vitamin C concentration than the subjects in other age groups (Polidori et al. 2007). Similarly the uric acid concentrations were significantly lower in 81–99 years (243.2 ± 64.2 μM) and ≥100 years (218.6 ± 57.4 μM) old subjects than subjects in the age categories ≤60 years (324.1 ± 88.0 μM) and 61–80 years (293.7 ± 79.9 μM). However, plasma levels of the fat-soluble vitamins A (3.5 ± 1.8 μM in centenarians higher than other age groups) and E (49.9 ± 8.3 μM in centenarians higher than other age groups) were significantly higher in centenarians compared to younger groups. This study concluded that in Italian population, elevated levels of plasma vitamins A and E seem to be important for longevity.

21.5.5.6 Green Tea

Consumption of green tea has caught the attention of many. The tea plant *Camellia sinensis* upon processing yields a variety of white, green, and black tea. *C. sinensis* is grown in many Asian countries. The health benefits of green tea is due to flavonoids, mainly catechins, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). Epigallocatechin gallate is an efficient antioxidant, and its concentration in green tea is higher than black tea (Cheng 2000). The concentration of total polyphenols in dried green tea leaves vary from 8% to 12% (Min and Peigen 1991).

Apart from its antioxidant effect, green tea lowers total cholesterol level as well as improves the ratio of LDL cholesterol to HDL cholesterol (Cheng 2006).

Maurya and Rizvi (2008) reported that the tea catechins have strong antiaging activity; hence consumption of green tea, which is rich in catechins, may delay the onset of aging.

21.5.5.7 Resveratrol

Resveratrol, a polyphenol found in grape, is claimed as an antiaging agent. Resveratrol is a calorie-restriction mimetic agent (Barger et al. 2008). Sirtuins are NAD^+ dependent histone/protein deacetylases which are target for resveratrol. Seven sirtuins (SIRT) have been reported in mammals, of which SIRT-1 via its deacetylase activity mediates the beneficial effects on health and longevity of resveratrol (Markus and Morris 2008). Resveratrol, abundantly present in wine, scavenges O_2^- and $OH^\bullet$ in vitro, as well as lipid hydroperoxyl free radicals.

21.6 Natural Sources of Nutrients and Phytochemicals with Antioxidant Capacity

21.6.1 Vitamin C

Vitamin C is widely distributed in fresh fruits and vegetables. Some rich sources of it are presented in Table 21.1.

Table 21.1 Dietary sources of vitamin C

Sources	Amount of vitamin C (mg/100 g food)
Rosehip	2000
Kiwifruit	90
Broccoli	90
Orange juice (3/4 cup)	75
Strawberry	50
Orange	50
Lemon	40
1 potato	25
Banana	9
Apple	6

21.6.2 Vitamin E

Vitamin E in nature is found in dietary articles that are rich in polyunsaturated fat. Hence vegetable oils like sunflower, soybean, and safflower oils are among the best sources of vitamin E. Tocols is the name designated to tocopherols and tocotrienols. Tocopherols exist as four homolog forms, alpha, beta, delta, and gamma, and alpha-tocopherol is the major tocopherol in many edible oils. By far the richest source of vitamin E is wheat germ oil. Oils extracted from cereals like rice bran and corn also provide some vitamin E. Almonds among the nuts are considered as a good source of vitamin E, and peanuts, hazelnuts, and sunflower seeds contain considerable amount of vitamin E. The daily vitamin E requirement is 15 mg, and one tablespoon of wheat germ oil provides 20.3 mg, which is more than the recommended daily allowance. The US Department of Agriculture's nutrient database indicates that wheat germ contains 4.53 mg of vitamin E in 28 g. Germination of soybean seed for 48 h increases the vitamin E content from 7.15 ± 0.34 μg/g of seed to 12.63 ± 0.54 μg/g of seed (Vasantharuba et al. 2007).

21.6.3 Carotenoids

The plant kingdom contributes to our carotenoid requirements. The carotenoid family has many types, but the most common ones are α-carotene, β-carotene, beta-cryptoxanthin, lutein, zeaxanthin, and lycopene. The color of the fruits and vegetables is due to their carotenoid content, and hence they are considered as good sources. Dark leafy green vegetables like spinach, broccoli, and leaf cabbage (kale) are good sources of carotenoids, and those grown in Sri Lanka such as *Alternanthera sessilis*, *Sesbania grandiflora*, and *Centella asiatica* are also good sources. Values for α-carotene, β-carotene, lutein + zeaxanthin, lycopene, and β-cryptoxanthin from approximately 200 references were evaluated and reported by Holden et al. (1999).

21.6.4 Phytochemicals

The naturally occurring chemical compounds found in plants which provide health benefits for humans further than those attributed to macronutrients and micronutrients are grouped as phytochemicals (Hasler and Blumberg 1999). The foods containing considerable amount of such phytochemicals are also referred to as functional foods and are being extensively studied. Among the phytochemicals, polyphenols are recognized as having health-promoting roles. Based on the structure, polyphenols are divided into (a) simple phenolic acids, e.g., ferulic, caffeic, p-coumaric, vanillic, gallic, ellagic, p-hydroxybenzoic, and chlorogenic acids; (b) stilbenes, e.g., resveratrol; (c) curcuminoids, e.g., curcumin; (d) chalcones, e.g., phlorizin and naringenin chalcone; (e) lignans, e.g., matairesinol and secoisolariciresinol; and (f) flavonoids (Bravo 1998; Harborne and Baxter 1999; Williams et al. 2004).

The flavonoids are composed of seven subclasses, namely:

(a) Flavonols, e.g., quercetin [in apples and onions]
(b) Flavanols as monomeric, e.g., catechin [in red wine, grapes, and black tea] and epicatechin [in cocoa and chocolate], oligomeric, and polymeric compounds, e.g., proanthocyanidins, also called condensed tannins [in apple, grape seed, and cocoa]
(c) Anthocyanins, e.g., cyanidin [black berries]
(d) Flavones, e.g., luteolin [in tea, fruits, and vegetables] and apigenin
(e) Flavanones, e.g., naringenin [citrus fruits, tomato] and hesperidin [in orange juice]
(f) Flavanonols, e.g., taxifolin [in red onion]
(g) Isoflavones, e.g., genistein [in soya] and daidzein [in soymilk]

Dietary phytochemicals have been known to possess anti-obesity potential, and their mechanisms of action have been reviewed (González-Castejón and Rodriguez-Casado 2011).

The phytochemicals present in foods have been reported to possess some possible health benefits. Isoflavones can reduce blood pressure and increase blood vessel dilation; anthocyanins also cause blood vessel dilation and improve insulin sensitivity; proanthocyanidins inhibit LDL oxidation and inflammation; catechins and epicatechins through vasodilation improve blood flow to the brain, in addition to improving insulin sensitivity (Heneman and Zidenberg-Cherr 2008).

21.7 Conclusion

Aging, an inevitable process, is the result of free radical-mediated damage to cellular fabric. Antioxidants provide relief from oxidant stress. Natural antioxidants present in the diet that we consume contribute to the body's antioxidant defense system along with the endogenous antioxidants. As age advances, the antioxidant protection diminishes when dietary intake dwindles. There are convincing

evidences from a number of studies to indicate that aging could be slowed down by antioxidants giving the hope that life can be prolonged if adequate antioxidant capacity of the body is maintained. Thus dietary antioxidants could prove its value for an aging population. Fruits, vegetables, and other plant-based foods are rich in bioactive phytochemicals with antioxidant potential, especially the phenolics, that may provide desirable health benefits beyond basic nutrition to reduce the risk of the development of chronic diseases due to oxidant stress. There are many more effects of antioxidants which are not covered in this chapter.

References

Akila VP, Harishchandra H, D'souza V, D'souza B (2007) Age related changes in lipid peroxidation and antioxidants in elderly people. Indian J Clin Biochem 22(1):131–134. https://doi.org/10.1007/BF02912896

Anonymous (2009) Apoptosis. http://jpck.zju.edu.cn/jcyxjp/redir.php?catalog_id=515&object_id=1122. Accessed on 12 Dec 2017

Babior BM (1978) Oxygen-dependent microbial killing by phagocytes. New Engl J Med 298:659–668. https://doi.org/10.1056/NEJM197803232981205

Bahorun T, Soobrattee MA, Luximon-Ramma V, Aruoma OI (2006) Free radicals and antioxidants in cardiovascular health and disease. Internet J Med Update 1:1–17. https://doi.org/10.4314/ijmu.v1i2.39839

Bandyopadhyay U, Das D, Banerjee RK (1999) Reactive oxygen species: oxidative damage and pathogenesis. Curr Sci 77:658–665

Barger JL, Kayo T, Vann JM, Arias EB, Wang J, Hacker TA (2008) A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS One 3:e2264

Bjorksten J (1974) In: Rockstein M (ed) Theoretical aspects of aging. Academic, New York, p 43

Boscia F, Grattagliano I, Vendemiale G, Micelli-Ferrari T, Altomare E (2000) Protein oxidation and lens opacity in humans. Invest Ophthalmol Vis Sci 1:2461–2465

Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr Rev 56:317–333

Brody H (1980) In: Schimke RT (ed) Biological mechanisms in aging. U.S. Department of Health and Human Services, Washington, DC, p 563

Butterfield DA, Koppal T, Howard B, Subramaniam R, Hall N, Hensley K, Yatin S, Allen K, Aksenov M, Aksenova M, Carneyc J (1998) Structural and functional changes in proteins induced by free radical mediated oxidative stress and protective action of the antioxidants N-tert-butyl-alpha phenylnitrone and vitamin E. Ann N Y Acad Sci 854:448–462

Ceriello A (2000) Oxidative stress and glycemic regulation. Metabolism 49:27–29

Chandrasoma P, Taylor CR (2001) Changes associated with aging. In: Concise pathology, 3rd edn. McGraw-Hill, pp 246–249

Cheng TO (2000) Tea is good for the heart. Arch Intern Med 60:2397

Cheng OT (2006) All teas are not created equal- the Chinese green tea and cardiovascular health. Int J Cardiol 108:301–308

Chong-Han K (2010) Dietary lipophilic antioxidants: implications and significance in the aging process. Crit Rev Food Sci Nutr 50(10):931–937. https://doi.org/10.1080/10408390903044073

Digital World Medical School (2016) Cell, tissue and organ changes associated with aging. http://www.digital-world-medical-school.net. Accessed on 20 Dec 2017

Floyd RA (1990) The role of 8-hydroxyguanosine in carcinogenesis. Carcinogenesis 11:1447–1450

Fraga CG, Shigenaga MK, Park JW, Deagan P, Ames BN (1990) Oxidative damage to DNA during aging: 8-hydroxy-2-deoxyguanosine in rat organ DNA and urine. Proc Natl Acad Sci U S A 87:4533–4537

Genestra M (2007) Oxyl radicals, redox-sensitive signaling cascades, and antioxidants. Rev Cell Sig 19:1807–1819. https://doi.org/10.1016/j.cellsig.2007.04.009
González-Castejón M, Rodriguez-Casado A (2011) Dietary phytochemicals and their potential effects on obesity: a review. Pharmacol Res 64:438–455. https://doi.org/10.1016/j.phrs.2011.07.004
Halliwell B, Gutteridge JM (1985) The importance of free radicals and catalytic metal ions in human diseases. Mol Asp Med 8(2):89–193
Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine, 4th edn. Clarendon Press, Oxford
Harborne JB, Baxter H (1999) The handbook of natural flavonoids, vol 2. Wiley, Chichester, p 1838. ISBN 0 471 95893
Harman D (1981) The aging process. Proc Natl Acad Sci U S A 78(11):7124–7128
Hasler CM, Blumberg JB (1999) Symposium on phytochemicals: biochemistry and physiology. J Nutr 129:756S–757S
Hayflick L (1985) Theories of biological aging. Exp Gerontol 20:145–159
Heneman K, Zidenberg-Cherr S (2008) Some facts about phytochemicals. UC Cooperative Extension Center for Health and Nutrition Research Nutrition and Health Info Sheet. http://nutrition.ucdavis.edu/content/infosheets/fact-pro-phytochemical.pdf
Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat S, Davis CS, Douglass LW, Gebhardt S, Haytowitz D, Schake S (1999) Carotenoid content of U.S. foods: an update of the database. J Food Compos Anal 12:169–196
Kagan VE, Kisin ER, Kawai K (2002) Towards mechanism-based antioxidant preventions. Ann N Y Acad Sci 959:188–198
Karlsson J (1997) Introduction to nutraology and radical formation. In: Antioxidants and exercise. Human Kinetics Press, Champaign, pp 1–143
Kaur H, Halliwell B (1994) Evidence for nitric oxide-mediated oxidative damage in chronic inflammation. Nitro tyrosine in serum and synovial fluid from rheumatoid patients. FEBS Lett 350(1):9–12
Koppenol WH, Moreno JJ, Pryor WA, Ischiropoulos H, Beckman JS (1992) Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. Chem Res Toxicol 5:834–842. https://doi.org/10.1021/tx00030a017
LeDoux SP, Driggers WJ, Hollensworth BS, Wilson GL (1999) Repair of alkylation and oxidative damage in mitochondrial DNA. Mutat Res 434(3):149–159
Luanchoy S, Tiangkul S, Wongkrajang Y, Temsiririrkkul R, Peungvicha P, Nakornchai S (2014) Antioxidant activity of a Thai traditional formula for longevity. Mahidol Univ J Pharm Sci 41(3):1–5
Lyons TJ (1991) Oxidized low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes? Diabet Med 8(5):411–419. https://doi.org/10.1111/j.1464-5491.1991.tb01624.x
Markus MA, Morris BJ (2008) Resveratrol in prevention and treatment of common clinical conditions of aging. Clin Interv Aging 3:331–339
Maurya PK, Rizvi SI (2008) Protective role of tea catechins on erythrocytes subjected to oxidative stress during human aging. Nat Prod Res 23(12):1072–1079. https://doi.org/10.1080/14786410802267643.
Min Z, Peigen X (1991) Quantitative analysis of the active constituents in green tea. Phytother Res 5:239–240
Niki E, Noguchi N, Tsuchihashi H, Gotoh N (1995) Interaction among vitamin C, vitamin E, and beta-carotene. Am J Clin Nutr 62(Suppl 6):1322S–1326S
Okuwa DE, Orji O (2007) Phytochemical composition and nutritional quality of Glycine max and Vigna unguiculata (L) walp. Am J Food Technol 2:512–520. https://doi.org/10.3923/ajft.2007.512.520
Packer Long A (1998) Biological oxidants and antioxidants: molecular mechanisms and health effects. AOCS Press, Champaign

Perez-Campo R, López-Torres M, Cadenas S, Rojas C, Barja G (1998) The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. Comp Physiol B 168(3):149–158

Pham-Huy LA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci 4(2):89–96

Polidori MC, Mariani E, Baggio G, Deiana L, Carru C, Pes GM, Cecchetti R, Franceschi C, Senin U, Mecocci P (2007) Different antioxidant profiles in Italian centenarians: the Sardinian peculiarity. Eur J Clin Nutr 61:922–924. https://doi.org/10.1038/sj.ejcn.1602596

Richard GC (1985) Peroxide-producing potential of tissues: inverse correlation with longevity of mammalian species. Proc Natl Acad Sci USA 82:4798–4802

Richter C, Park JW, Ames BN (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci U S A 85(17):6465–6467

Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA (1994) Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. J Biol Chem 269(42):26066–26075

Said MA-D, Aiman IA-Q (2014) Review article: oxidative stress versus antioxidants. Am J Biosci Bioeng 2(5):60–71. https://doi.org/10.11648/j.bio.20140205.11

Sánchez JE, Jiménez-Pérez G, Liedo P (2015) Can consumption of antioxidant rich mushrooms extend longevity? Antioxidant activity of Pleurotus spp. and its effects on Mexican fruit flies' (*Anastrepha ludens*) longevity. Age 37:107. https://doi.org/10.1007/s11357-015-9847-0

Sies H (1985) Oxidative stress: introductory remarks. In: Sies H (ed) Oxidative stress. Academic, London, pp 1–7

Sinclair AJ, Barnett AH, Lunec J (1991) Free radicals and antioxidant systems in health and disease. J Am Med Assoc 7:409–417

Slater TF (1984) Free radical mechanisms in tissue injury. Biochem J 222:1–15

Stadtman ER, Levine RL (2000) Protein oxidation. Ann N Y Acad Sci 899:191–208

Tan AG, Mitchell P, Flood VM, Burlutsky G, Rochtchina E, Cumming RG, Wang JJ (2008) Antioxidant nutrient intake and the long-term incidence of age-related cataract: the Blue Mountains Eye Study. Am J Clin Nutr 87:1899–1905

Tandon V, Gupta BM, Tandon R (2005) Free radicals/reactive oxygen species. JK-Practitioner 12:143–148

Terman A, Kurz T, Gustafsson B, Brunk UT (2008) The involvement of lysosomes in myocardial aging and disease. Curr Cardiol Rev 4:107–115. https://doi.org/10.2174/157340308784245801

Trichopoulou A, Vasilopoulou E (2000) Mediterranean diet and longevity. Br J Nutr 84(Suppl 2):S205–S209

Trichopoulou A, Kouris-Blazos A, Vassilakou T, Gnardellis CH, Polychronopoulos E, Venizelos M, Lagiou P, Wahlqvist M, Trichopoulos D (1995) The diet and survival of elderly Greeks; a link to the past. Am J Clin Nutr 61(Suppl):1346S–1350S

Udayangani RMD (2015) Anaemia and oxidative stress in long term diabetes mellitus. M.Sc research project report

Udayangani RMD, Sivakanesan R, Kuruppuarachchi PL (2015) Effect of glycaemic control on serum concentration of thiobarbituric acid reactive substance in long term diabetes mellitus patients. Proceedings of the Peradeniya University International Research Sessions (iPURSE), p 188

Vasantharuba S, Wijesinghe DGNG, Sivakanesan R (2007) Changes in vitamin E and essential fatty acid contents and their interrelationship in soybean (*Glycine max* L. merr.) seeds during germination and storage. Trop Agric Res 19:119–127

Velavan S (2011) Free radicals in health and diseases – a mini review. Pharmacol Online 1:1062–1077

Williams RJ, Spencer JP, Rice-Evans C (2004) Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med 36:838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001