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## Free Radicals and Antioxidants: Human and Food System

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### ABSTRACT

*This paper presents sources, types, mechanism of action and damaged caused by free radicals in human body as well as in food system. To neutralize the effect of free radicals, the role of antioxidants, their classification and mode of action has been discussed with recent developments.*

**Key words :** ROS, free radicals, antioxidants.

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### INTRODUCTION

It is ironic that oxygen, which is an indispensable element for life can, under certain situations, have severe deleterious effects on the human body. Most of the potentially harmful effects of the oxygen are due to the formation and activity of number of chemical compounds, known as reactive oxygen species (ROS), which have tendency to donate oxygen to other substances. ROS is a collective term used to include oxygen radicals and several non radical oxidizing agents such as HOCl (Hypochlorous acid), hydrogen peroxide, ozone, etc. [Halliwell *et al.*, 1995]. Many such reactive species are free radicals and have a surplus of one or more free floating electrons rather than having matched pairs and are therefore, unstable and highly reactive [Bagchi and Puri, 1998]. A wide variety of oxygen free radicals and other reactive species can be formed in the human body and food system. Transition metal ions accelerate free radical induced damage. Such oxidative damage results in carcinogenesis, aging and atherosclerosis [Yagi, 1987; Cutler, 1984, 1992].

These reactive oxygen species can be combated with the involvement of antioxidants of both exogenous and endogenous origin. Antioxidants are a group of substances which, when present at low concentration in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves. Antioxidants can be better understood by having information about their nature reactivity of reactive oxygen species or free radicals [Ke Cui *et al.*, 2004].

**Reactive oxygen species and free radicals**

Reactive oxygen species is a collective term that includes all reactive forms of oxygen, including both radical and non radical species that participate in the initiation and/or propagation of chain reaction. Free radicals represent a class of highly reactive intermediate chemical entities whose reactivity is derived from the presence of unpaired electron in their structure, which are capable of independent existence for very brief interval of time [Ke Cui *et al.*, 2004]

Free radicals and other reactive species are derived either from normal essential metabolic processes or from external sources, such as exposure to x-rays, ozone, cigarette smoking, air pollutants, industrial chemicals etc.

**Sources of free radical**

- Internal sources
- External source
- Physiological Factors

**Internal sources:**

These can be enzymatic reactions, which serve as a source of free radicals. These include those reactions involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and in the cytochrome P<sub>450</sub> system. Some internal sources of generation of free radicals are mitochondria, xanthine oxidase, phagocytes, reactions involving iron and other transition metals, peroxisomes, Arachidonate pathways, exercise, ischaemia / reperfusion, inflammation.

**External sources:**

These include non-enzymatic reactions of the oxygen with organic compounds. Free radicals also arise in reactions, which are initiated by ionizing radiations. Some external sources of free radicals are cigarette smoke, environmental pollutant, radiations, ultraviolet light, ozone, certain drugs, pesticides, anesthetics and industrial solvents.

**Physiological Factors**

Mental status like stress, emotion etc. and disease conditions are also responsible for the formation of free radicals.

**Types of free radicals**

- Hydroperoxyl radical
- Superoxide radical
- Hydrogen peroxide
- Triplet oxygen
- Active oxygen

**Hydroperoxyl radical:** The hydroperoxyl radical, also known as the perhydroxyl radical, is the protonated form of superoxide with the chemical formula HO<sub>2</sub>. Hydroperoxyl is formed through the transfer of a proton to an oxygen atom. HO<sub>2</sub> can act as an oxidant in a number of biologically important reactions, such as the abstraction of hydrogen atoms from tocopherol and polyunsaturated fatty acids in the lipid bilayer. As such, it may be an important initiator of lipid peroxidation.

**Superoxide :** Superoxide can act either as oxidant or reductant, it can oxidize sulphur, ascorbic acid or NADPH and it can reduce Cytochrome C and metal ions. A dismutation reaction leading to the formation of hydrogen peroxide and oxygen can occur spontaneously or is catalyzed by enzyme superoxide dismutase. In its protonated form (pKa 4.8), superoxide forms and perhydroxyl radical, which is powerful oxidant [Gebicki and Bielski, 1981] but its biological relevance is probably minor because of its low concentration at physiological pH.

**Hydrogen peroxide:** The univalent reduction of superoxide produces hydrogen peroxide, which is not a free radical because all its electrons are paired. It readily permeates through the membranes and is therefore not compartmentalized in the cell. The main damages caused by this are breaking up of DNA, resulting in single strand breaks and formation of DNA protein crosslink. Numerous enzymes (peroxidases) use hydrogen peroxide as a substrate in oxidation reactions involving the synthesis of complex organic molecule. This is an oxidizing agent but not specially reactive and its main significance lies in it being a source of hydroxyl radical in the presence of reactive transition metal ions.

**Singlet oxygen:** It is not a free radical but it can be formed in some radical reactions and can trigger off others. This arises from hydrogen peroxide molecules. Singlet oxygen on decomposition generates superoxide and hydroxyl radicals.

**Triplet oxygen:** Triplet oxygen can react with elements and ions to form oxides, but usually not with organic compounds, which are in singlet state. However, it reacts easily with free radical molecules produced by the action of other active radicals, radiations, ultra violet light, and heat or by complex formation with oxygen and transition metal to produce active peroxide radicals and trigger auto-oxidation of unsaturated fatty acids and others.

#### **Damages caused by free radicals**

If free radicals are not inactivated, their chemical reactivity can damage all cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. Their destructive effect on protein may play a role in the causation of diseases, like cataracts. Free radical damage to DNA is also implicated in the causation of cancer and its effect on LDL cholesterol is very likely responsible for heart disease. Free radicals are also responsible for ageing [Bagchi and Puri, 1998].

**Oxidative damages to proteins:** Oxidative attack on proteins results in site-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross linked reaction products, altered electrical charges and increased susceptibility to proteolysis [Farr and Kogoma, 1991].

**Oxidative damage to DNA:** Activated oxygen and agents that generate oxygen free radicals, such as ionizing radiations, induce numerous lesions in DNA that causes deletion, mutations and other lethal genetic effects. Characterization of this damage to DNA has indicated that both sugar and base moieties are susceptible to oxidation, causing base degradation, single strand breakage and cross links to proteins [Imlay and Linn, 1986].

**Free radical and ageing:** Free radical reactions are expected to produce progressive adverse changes that accumulate with age throughout the body. Aging pigments (lipofuscin granules) are accumulated in the subsarcolemmal region of the muscle fibers and becomes more abundant with increasing age.

**Free radical and diseases:** Free radicals result in number of human degenerative diseases affecting a wide variety of physiological functions such as atherosclerosis, diabetes, ischemia/reperfusion (I/R) injury, inflammatory diseases (rheumatoid arthritis, inflammatory bowel diseases and pancreatitis), cancer, neurological diseases, hyper tension etc. [Ke Cui *et al.*, 2004]. Free radicals are however, not always harmful. They also serve useful purpose in the human body. The oxygen radicals in the living system are probably necessary compounds in the maturation process of cellular structure. White blood cells release free radicals to destroy invading pathogenic microbes as a part of body defense mechanism against diseases. Hence, complete elimination of these radicals would not only be impossible but also harmful [Bagchi and Puri, 1998].

### Mechanism for the formation of free radicals

Free radicals can be formed by three ways -

- By homolytic cleavage of covalent bond of normal molecule, with each fragment retaining one of paired electrons.



- By the loss of single electron from normal molecule.



- By addition of single electron to normal molecule.



A radical might donate its unpaired electron to other molecule. It might take electron from other molecule in order to pair or it might simply join to the molecule. When radical gives one electron or takes one electron or simply adds on to the anion to become a radical. Thus the future of the reactions that usually proceed as chain reaction is such that one radical begets another [Halliwell and Gutteridge, 1984].

### Antioxidants

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. [Mandal et al, 2009]

According to literature, these are "substance that when present in low concentration compared to those of the oxidisable substrates significantly delay or inhibit the oxidation of that substance [Murthy, 2001].

The antioxidant can also be defined as "A compound capable of inhibiting oxygen mediated oxidation of diverse substances from simple molecule to polymer and complex bio-systems [Chintang Ho, 1994].

According to US Food and Drug Administration (FDA), antioxidants are defined as substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation [Halliwell, 1990; Halliwell and Gutteridge, 1984].

While antioxidants are of interest to the food industry because they prevent rancidity in food [Loliger, 1991], antioxidants are also of interest to biologist and clinicians because they may help to protect human body against damage by reactive oxygen species (ROS).

Antioxidants defense both enzymatic and non enzymatic reactions protect the body against oxidative damage. Non enzymatic antioxidants are frequently added to the food to prevent lipid oxidation. Several lipid antioxidants can exert pro-oxidant effect towards other molecule under certain circumstances thus the antioxidants for food and therapeutic use must be characterized carefully.

### **Classification of antioxidants**

- A) Natural antioxidants
- B) Synthetic antioxidants

**A) Natural antioxidants:** Naturally occurring antioxidants of high or low molecular weight can differ in their composition, in their physical and chemical properties, in their mechanism and in their site of action. They can be divided into following categories:

**i) Enzymes:** Enzyme such as superoxide dismutase (SOD), catalase and glutathione peroxidase attenuate the generation of reactive oxygen species by removing potential oxidants or by transferring ROS/RNS (reactive nitrogen species) into relatively stable compounds. SOD which was discovered in late 60s, catalyses the transformation of the superoxide radical into hydrogen peroxide, which can than be transformed by enzyme catalase into water and molecular oxygen. While superoxide anion in itself is not particularly reactive, it can reduce transition metal ions, such as iron and gets converted to most reactive radicals - the hydroxyl radical. Thus, elimination of superoxide radical can attenuate the formation of hydroxyl radical. Glutathione peroxidase (GPx) reduces lipid peroxides (ROOH), formed by the oxidation of polyunsaturated fatty acids (PUFA), to a stable, non toxic molecule - hydroxyl fatty acid (ROH). Together with phospholipase, GPx can also convert phospholipids hydro peroxide (PL-OOH) into phospholipids hydroxide (PL-OH) [Ursini et al., 1982].

**iii) Low molecular weight antioxidants:** These are subdivided into lipid-soluble antioxidants (tocopherol, carotenoids, quinones, bilirubin and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and polyphenols). These delay or inhibit cellular damage mainly through free radical scavenging property.

**Lipids soluble antioxidants:** These antioxidants tend to accumulate in lipid plasma lipoprotein (eg. LDL); upon supplementation. This group of antioxidants are supposed to act as highly efficient scavengers, such as against lipid peroxy radical, which are formed within the lipoprotein as a consequence of free radical chain reaction of lipid peroxidation.

**Water soluble antioxidants:** These antioxidants cannot enter the lipid moiety of low density lipoprotein (LDL); these will be less efficient as these are principally unable to encounter most of these lyophilic radicals; however, such a compound may act in a synergistic manner with lipophilic antioxidants by regenerating them.

**B) Synthetic antioxidants** These are most effective antioxidants and are synthetic chemicals, approved by Food and Drug Administration for addition to foods, e.g. BHA (Butylated hydroxy anisole), BHT (butylated hydroxy toluene), TVHQ (tertiary butylated hydroxy quinone) etc .

### Mode of action of antioxidants

In general, the antioxidants act by the following routes:

- Chain breaking reaction e.g.  $\alpha$ -tocopherol, which act in lipid phase to trap free radical.
- By reducing concentration of reactive oxygen species e.g. Glutathione.
- By scavenging initiating radicals e.g. superoxide dismutase which act in the lipid phase to trap superoxide free radicals .
- By chelating transition metal catalyst: a group of compound which act by sequestration of transition metals that are well established prooxidants. In this way transferring, lactoferrin and ferritin function to keep iron induced oxidant stress in check and ceruloplasmin and albumin as copper sequestrants.

### CONCLUSION

On the basis of above study following conclusions can be drawn:

1. Free radicals are very harmful to human health and can cause several degenerative diseases like diabetes, cancer, atherosclerosis, hypertension etc.
2. Various kinds of antioxidants particularly from natural sources such as enzymes, tocopherol, carotenoids, ascorbic acid, polyphenols etc. inhibit the cellular damage mainly through free radical scavenging property.

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