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RESEARCH ARTICLE

A REVIEW ON FREE RADICAL, OXIDATIVE STRESS AND ANTIOXIDANT

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Abstract

Oxidation is an important metabolic process in all living organisms that inevitably leads to the formation of free radicals and other reactive oxygen species (ROS). The free radicals thereby generated are extremely reactive and play a major role in multiple cellular processes such as signal transduction.Lipid peroxidation also occurs in food systems, particularly in those containing polyunsaturated fatty acids, and is a causative factor for the development of an undesirable flavour and taste, for decreased shelf life and for the formation of potentially toxic compounds in food products. Antioxidants are employed for preserving food products by retarding the deterioration resulting from oxidation, scavenging free radicals and detoxification of organisms. Currently, synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), *tert*-butylhydroquinone and propyl gallate have been widely employed in food products for preventing the deterioration caused by oxidation and improving shelf life. Their use, however, is restricted in certain countries because of potential health issue.

Introduction:-

Lipids and lipid oxidation:-

Lipids are a heterogeneous group of compounds present in living systems, typically characterised by limited solubility in water and solubility in nonpolar organic solvents such as hydrocarbons, chloroform, benzene, ethers and alcohols (Coultate, 2002). They form the structural components of cell membranes and energy reservoirs, serve as an essential precursor for the production of vitamins and hormones and help lipid solubilisation (Belitz *et al*., 2009). Lipids are formed of fatty acids as the main building blocks and are categorised as saturated fatty acids that do not contain double bonds and unsaturated fatty acids that contain them. Monounsaturated fatty acids are unsaturated fatty acids that contain a single double bond, and polyunsaturated fatty acids are fatty acids that contain more than one double bond. The number of double bonds determines the oxidation potential of the fatty acid into lipid hydroperoxide. Polyunsaturated fatty acids are the most common fatty acids and are more susceptible to oxidation by oxygen or free radicals than saturated fatty acids because of the presence of multiple double bonds in the carbon chain. The double bonds adjacent to methylene ($-CH_2-$) groups containing reactive hydrogen are more susceptible to attack by reactive species, resulting in lipid oxidation (Mylonas and Kouretas, 1998).

For food storage and human health, the oxidation of lipid should be importantly considered because it is one of the key chemical changes that occur during processing, storage, shipment and final preparation of food products

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containing lipids. Lipid oxidation is mainly observed in food products containing polyunsaturated fatty acids. Oxygen free radicals or reactive oxygen species (ROS) often chiefly mediate the oxidation of lipid that occurs in food systems (Noguchi *et al*., 2002).

Free radicals and reactive oxygen species (ROS):-

Free radicals are any chemical species that are capable of existing independently and containing one or more unpaired electrons. Free radicals are derived from the elements oxygen, nitrogen or sulphur, which generate ROS, reactive nitrogen species (RNS) or reactive sulphur species, respectively. The free radicals derived from oxygen are extremely important in several disease states. ROS are a comprehensive class of highly reactive molecules derived from oxygen metabolism. Several ROS types include the superoxide anion radical ($O_2^{-}\cdot$), the hydroxyl free radical ($OH\cdot$) which is an extremely powerful oxidising agent, hydrogen dioxide ($HO_2^{\cdot}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$) and various lipid peroxides (Lü *et al*., 2010). All these types of ROS exhibit strong capacity for reacting with membrane lipids, nucleic acids, proteins and enzymes, resulting in oxidative cellular damage (Chaillou and Nazareno, 2006). In addition, they comprise a major cause of health disorders such as cancer, arthritis, diabetes, inflammation, coronary heart disease and ageing (Di Bernardini *et al.*, 2011a; Memarpoor-Yazdi *et al.*, 2012).Human body produces free radicals and various ROSduring normal essential metabolic processes. Their production is stimulated by external factors such as cigarette smoke, radiation, UV light, air pollutants and industrial chemicals.

Oxidative stress:-

The term 'oxidative stress' is employed for describing oxidative damage resulting from a disruption in the balance between free-radical production and antioxidant defences (Rock *et al*., 1996). Oxidative stress has been linked with the exposure to various environmental factors such as radiation, pollutants, cigarette smoke and toxic chemicals, which has also been implicated in the induction of several human diseases, including ageing (Rao *et al*., 2006).An increase in the oxidative stress is related to damage in numerous molecular species including lipids, proteins and nucleic acids (McCord, 2000).

Oxidative damage to biomolecules:-

Oxidative damage to DNA:-

Free-radical-induced DNA damage could be explained as either chemical or structural in terms of the characteristic manner of numerous modifications, including deletions, alterations of the purine and pyrimidine bases, breaking of strand, cross-linking of DNA-protein and rearrangements of chromosomes. DNA damage is related to the generation of the $OH^{\cdot}$*via* the Fenton reaction. This radical reacts with the purine and pyrimidine bases and the deoxyribose backbone leading to DNA oxidation. (Dizdaroglu *et al.*, 2002; Valko *et al.*, 2004).

Oxidative damage to proteins:-

Protein oxidation is predominantly mediated by ROS and reactive nitrogen species (RNS), which are also capable of generating other free radicals by reacting with transition metal ions. In an *in vivo*context, oxidative damage to proteins potentially alters the functioning of receptors, enzymes and transport proteins and could generate new antigens that interfere with immune responses. The oxidation of proteins could occur in three ways: oxidative alteration of a specific amino acid, free-radical-mediated cleavage of peptides and protein cross-linking as a consequence of reacting with lipid peroxidation products (Lobo *et al.*, 2010).

Oxidative damage to lipids:-

Oxidation of lipids is a free-radical-mediated process that results in various structural and functional changes. In addition, many lipid by-products are generated during lipid peroxidation.

The presence of factors such as heat, enzymes, light, metals and microorganisms increases the susceptibility of lipids to the oxidative process. Lipids are susceptible to several processes such as autoxidation, photo-oxidation and enzymatic oxidation under different conditions, most of which include one or the other types of free radicals or oxygen species. Of the above mentioned processes, autoxidation is discussed here in detail (Vercellotti *et al*., 1992; Shahidi, 2000). Autoxidation is defined as the spontaneous reaction of lipids with atmospheric oxygen, leading to complex chemical changes that result in the deterioration and off-flavour of food products (Gordon *et al.*, 2001; Sikorski and Kolakowska, 2010).

A chain reaction of free radicals is known to cause ROS-mediated lipid oxidation. The mechanism of free-radical chain reaction or autoxidation is conveniently divided into three distinct stages, namely initiation, propagation and termination, as illustrated in Figure 1 (Young and McEneny, 2001; Dalrymple *et al.*, 2010).

Initiation stage:-
The oxidisation of substrate molecules mediated free radical chain reaction is triggered by the initiators, resulting in hydrogen atom loss from the substrate molecule or unsaturated fatty acids (LH). This results in the formation of a lipid free radical (L•). In an unsaturated fatty acid (LH), the initiation step often occurs at a methylene group.

$$LH + OH\bullet \rightarrow L\bullet + H_2O$$

Propagation stage:-
The generated free radical (L•) is highly unstable and reacts with oxygen to form a peroxyl radical (LOO•). In addition, this product reacts with another unsaturated lipid (LH) molecule by the abstraction of a hydrogen atom from it, resulting in lipid hydroperoxide (LOOH) and new lipid free radical (L•) formation. Thus, a cascade of reactions is commenced until the free radical is neutralised by other free radicals.

$$L\bullet + O2 \rightarrow LOO\bullet$$
$$LOO\bullet + LH \rightarrow LOOH + L\bullet$$

Termination Stage:-
In the final stage, two free radicals combine to form a stable non-radical product, thereby terminating the cascade reaction.

$$L\bullet + L\bullet \rightarrow L\text{-}L$$
$$LOO\bullet + L\bullet \rightarrow LOOL$$

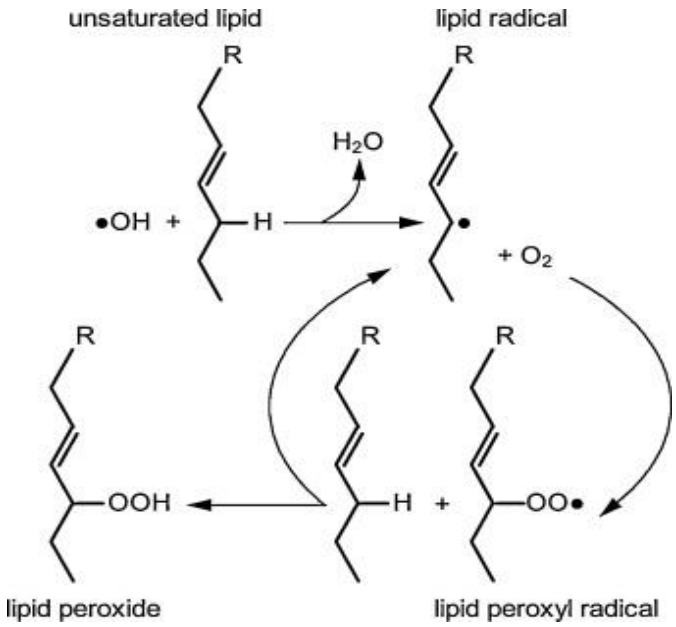


Figure 1:-Illustration of the mechanism of free-radical chain reaction representing the initiation, propagation and termination stages adapted from Dalrymple *et al.*, 2010.

The mechanism described above is initiated in the presence of various physical or chemical factors, including temperature, ROS and photosensitisers such as chlorophyll, transition metal ions, heating or radiation.

Hydroperoxide, the primary product of lipid autoxidation, is known to be unstable and susceptible to decomposition, which in turn generates aldehydes such as malondialdehyde, hydrocarbons, ketones, alcohols and epoxy compounds; these are known as secondary oxidation products. The estimation of these compounds, together with free radicals, constitutes the basis for measuring the oxidative deterioration of food lipids (Halvorsen and Blomhoff, 2011).

Antioxidants:-

Antioxidants are substances with the potential to delay or inhibit the oxidation of lipids or other oxidisable biomolecules when present at low concentrations(Halliwell, 2007; Puchalska *et al.,* 2014). Antioxidants possess a strong capacity for preventing the harmful action of free radicals by scavenging them and effectively detoxify organisms in multiple ways; these include inhibiting the formation of free lipid radicals, interrupting the initiation or propagation of autoxidation chain reactions (Young and Woodside, 2001), functioning as a reducing agent, converting hydroperoxide into stable or less-reactive compounds, converting prooxidant metals into stable products by functioning as a metal chelator and inhibiting prooxidative enzymes such as lipoxygenases(Pokorný, 2007; Kancheva, 2009; Puchalska *et al.,* 2014).

Antioxidants have free radical scavenging capacity. They improve the shelf life of food and pharmaceutical products by delaying the process of lipid peroxidation, a chief cause of food and pharmaceutical product deterioration during processing and storage (Halliwell, 1996)

Mechanism of action of antioxidants:-

There are two principal mechanisms underlying antioxidant activity (Rice–Evans and Diplock, 1993). The first mechanism relates to chain-breaking antioxidant activity in which the primary antioxidant (AH) donates an electron to the free radical (R•) present in the system.

$$R\bullet + AH \rightarrow RH + A\bullet$$

The second mechanism includes complex formation between the lipid radical and the antioxidant radical (free-radical acceptor) and ROS initiators removal (secondary antioxidants) by quenching the chain-initiating catalyst.

$$R\bullet + A\bullet \rightarrow RA$$

Different mechanisms are exerted by antioxidants on biological systems, including electron donation, metal-ion chelation or through co-antioxidants (Krinsky, 1992).

Antioxidant defence systems:-

The human body has a host of mechanisms for counteracting damage by free radicals and other ROS, which act on different oxidants and in different cellular compartments. The antioxidant defence system is categorised into two main groups: enzymatic antioxidants and non-enzymatic antioxidants.

Enzymatic antioxidants:-

The first major line of defence against free radicals is the intracellular enzyme system composed of three major enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes help in the reduction of the most harmful oxidants concentration in tissues. Several essential minerals are required for the formation and activity of these enzymes, including selenium, copper, manganese and zinc.

Superoxide dismutases (SOD):-

Superoxide dismutases (SODs) are enzymes that occur in all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005). SODs catalyse the dismutation of superoxide anion into oxygen and hydrogen peroxide, as shown below (Rahman, 2007):

$$2\ O_2\cdot + 2H \rightarrow H_2O_2 + O_2$$

SODs are classified into three major types according to the metal-dependent redox-active centre required for the dismutation reaction; they include Cu/Zn type (which binds both copper and zinc), Fe and Mn type (which binds either iron or manganese) and Ni type (which binds nickel). Humans, like all other mammals, contain the three types of SOD; Mn-SOD is found in the mitochondrial matrix and extracellular fluids, while Cu/Zn-SOD is localised in the cytoplasm (Cao *et al*., 2008). The higher plants contain two types of SOD, which are centralised in different cellular compartments; Mn-SOD is found in the matrix, while Fe-SOD is chiefly confined to the chloroplast (Corpas *et al.*,

2006). SODs exhibit various functions, including maintaining the concentration of superoxide radicals at low levels and defending against oxidative stress.

Catalase (CAT):-

Catalase (CAT) is a common enzyme found in virtually all living organisms that are exposed to oxygen and is localised in the peroxisomes and cytosol. The major function of this enzyme is catalysing the decomposition of hydrogen peroxide into water and oxygen through a scavenging reaction (Chelikani *et al.*, 2004).The enzyme typically occurs in all organs in animals but is particularly concentrated in the liver (Eisner and Aneshansley, 1999).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione peroxidases (GPx):-

Glutathione peroxidases (GPx) contains four selenium cofactors and catalyses the oxidation of GSH (reduced glutathione) to GSSG (oxidised glutathione) and one molecule of water at the expense of H_2O_2(Rahman, 2007).

$$H_2O_2 + 2GSH \rightarrow GSSG + H_2O$$

Oxidised glutathione is harmful to cells as it reacts with proteins and oxidised thiol groups to form disulphide linkages and consequently promotes disulphide bond formation in proteins. This is countered in cells by the NADPH-mediatedreduction of GSSG to GSH in the presence of the enzyme glutathione reductase; this enzyme is localised in the cytosol and mitochondria and its distribution mirrors that of GPx(Willcox *et al*., 2004).

$$GSSG + NADPH + H \rightarrow 2GSH + NADP$$

Non-enzymatic antioxidants:-

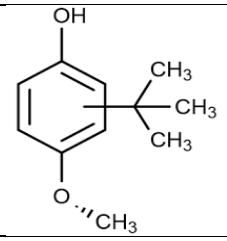
The second line of defence against the harmful effects of free radicals is the presence of extracellular antioxidants, of which there are quite a few, including the vitamins A, C and E, enzyme cofactors (Q10), nitrogen compounds (uric acid) and peptides (glutathione). The water-soluble antioxidants (vitamin C and phenolic compounds), lipid-soluble antioxidants (vitamin E and carotenoids) and proteins (transferrin, LF and albumin) are capable of blocking the action of free radicals in the body by chelating transition metal ions (Bagchi and Puri, 1998; Becker *et al*., 2004).

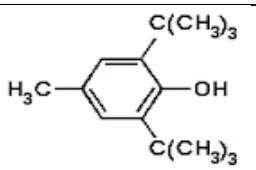
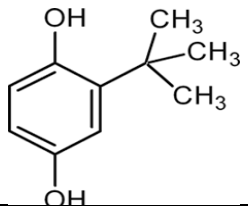
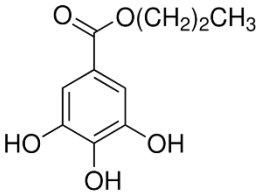
Sources of antioxidants:-

Synthetic antioxidants:-

Synthetic antioxidants have been developed for providing a system for measuring antioxidant activity, comparison with natural antioxidants and inclusion into food products. Certain synthetic antioxidants have been reported to chiefly consist of phenolic compounds and are usable in food products for suppressing free radical generation, preventing lipid oxidation and improving shelf life. Limited types of synthetic antioxidants are approved for use as food additives because of their harmful impact and side effects in human beings; these include butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) (Pihlanto, 2006; Shahidi and Zhong, 2010; Zhong *et al.*, 2011). A few synthetic antioxidants and their applications are listed in Table 1.

Table 1:-Illustration of chemical structures and applications of synthetic antioxidants adapted from Carocho and Ferreira, 2013.

Compound name	Structure	Application	Reference
BHA		Food antioxidants	Branen (1975)

BHT		Food antioxidants	Botterweck*et al*. (2000)
TBHQ		Animal processed Food antioxidants	Gharavi and El-Kadi (2005)
PG		Food antioxidants	Soares*et al.* (2003)

BHA, butylated hydroxyl anisole; BHT, butylated hydroxyl toluene; PG, propyl gallate; TBHQ, *tert*-butylhydroquinone

The activity of phenolic antioxidants is dependent on resonance, which stabilises phenolic radicals and is determined by the substitutions at *ortho* and *para* positions of the aromatic ring and the size of the substituting groups, as shown by Shahidi*et al*. (1992). According to Hudson and Lewis (1983), the presence of carbonyl and–COOH groups in a wide range of phenolic compounds could result in the inhibition of oxidative rancidity through metal ion chelation.

Natural antioxidants:-

An increase in the demand for safe and naturally occurring antioxidants as alternatives to synthetic ones has been observed in recent years because of rising limitations on the use of synthetic antioxidants and their negative impact on human health (Gülçin, 2012).

Plant materials are rich sources of natural antioxidants because they include an aromatic ring as part of their molecular structure; these could be linked to a range of cyclic ring structures and contain one or more hydroxyl groups that provide unstable hydrogen and support the formationof free radicals (Shahidi, 1997). Natural antioxidants could function as reducing agents, as inhibitors of free-radical autoxidation chains and of singlet oxygen formation and as inactivators of prooxidant metals.

Plant and food additives are good sources of natural antioxidants because they include vitamins (vitamins E and C and β-carotene), plant phenols (flavonoids and other phenolic compounds) and peptides derived from proteins (Mukhopadhyay, 2007). Natural antioxidants from these sources provide oxidative stability to food products.

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