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INVITED REVIEW
FREE RADICALS IN FOODS

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During the last decade increasing attention has been given to the role of free radicals in biological oxidations. The subject has been of increasing interest to both the food scientist and the physiologist. Free radical scavengers in the form of both indigenous and added antioxidants are necessary for the successful preservation of food; free radicals are increasingly being implicated in the onset of, among others, ischaemic heart disease and for protection against these diseases it is suggested that the dietary intake of the antioxidant vitamins should be increased especially for diets high in polyunsaturated fats.[1,2] Convenience and snack foods which absorb substantial amounts of frying oils are being increasingly consumed. Since poly-unsaturated fatty acids are particularly susceptible to oxidation by free radicals during the storage, cooking and frying of foods, the potential risk of exposure to lipid degradation products[3] is likely to have increased. In foods the non-enzymic and lipoxy-genase catalysed oxidation of polyunsaturated fatty acids, β-carotene and vitamin A can result in the loss of essential nutrients and the development of off-flavours.

KEY WORDS: Foods(s), free radical(s), antioxidant(s), lipid(s), lipid peroxidation, enzyme(s).

INTRODUCTION

Organic molecules in their ground state possess paired electrons having opposite spins, whereas free radicals have one or more unpaired electrons that usually predetermines their greater chemical reactivity. For instance a radical $R^{\cdot}$ will often readily abstract a hydrogen from a substrate LH to form other free radicals. Atmospheric oxygen exists in the relatively stable triplet state as a diradical with two unpaired electrons of parallel spins and cannot readily react with non-radical organic molecules. Therefore such reactions involving molecular oxygen depend on single electron additions to form superoxide, hydroxyl and peroxyl radicals. These radicals are essential species in metabolic reactions like oxidative phosphorylation and photosynthesis and enzyme catalysed reactions requiring the insertion of oxygen atoms as typified by lipoxygenases and other oxygenases. In fresh and processed foods oxygen-derived free radicals, as in the cells of the living system, can be produced by electron transfer reactions. In general the consequences of free radical activity within foods are undesirable, and especially so as free radical initiated oxidation is one of the main of causes of rancidity in fats and oils. Although production of free radicals is now recognised as a normal biochemical event, in food

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systems, control over metabolic activities is reduced as a consequence of harvesting and slaughter. The free radical reactions which occur during the cellular breakdown in foods are similar to some of those which occur in diseased, injured or ageing living tissue. These include, for example, the release of haem iron which can catalyse the oxidation of fats during meat processing or storage. Although the radicals may only have a fleeting existence, they have the ability to trigger chain reactions which amplify the damage caused by the initiating radical. If unchecked, free radicals are capable of causing substantial chemical changes resulting in disruption of organelles and subsequently further chemical and enzymic reactions which may be responsible for the deterioration of food products. Thus free radicals may be responsible for initiation of many biochemical changes within foods including some which are currently ill-defined. Although the precise biochemical mechanisms by which free radical mediated postmortem and postharvest changes occur in foods are generally poorly understood, free radicals are known to be responsible for the oxidation of food components resulting in alteration to the major quality parameters which include colour, flavour, aroma and nutritional value of foodstuffs. Enzyme systems may initiate free radical mediated oxidation in foods, resulting in changes in the sensory quality of the food; for example, peroxidases, lipoxy-genases and microsomal enzymes may be involved not only in lipid peroxidation but more so in the generation of free radicals capable of reacting with a wide range of other substances. Also free radicals may have a useful role during food processing as indicated below.

At the molecular level foods are complex mixtures containing hydrophilic and hydrophobic phases. In the polar phases hydro peroxide homolysis by hydrogen-bonded assistance, a process called "molecule-assisted homolysis" may be enhanced. Lipid phases that are not inert to radical attack may actually participate in reactions where a weakly bonded hydrogen can be readily abstracted. Physical changes during processing of food may have subtle effects on radical reactions. For example, during drying and lowering of water activity, the increase in viscosity and concentration of reactants will increase the time a radical pair is trapped within a "solvent cage", thereby either causing greater radical recombination or propagation with other constituents. Similar effects may occur during freezing and during other concentration processes like reverse osmosis. While free radical activity in foods is generally undesirable, there are a number of important exceptions, such as the desirable development of volatile compounds arising from the oxidation of fatty acids in a range of dairy products. For many years, food scientists have known that undesirable free radical activity may be controlled within processed food systems by the use of naturally occurring or synthetic antioxidants. Much of the early research on free radicals was carried out by food scientists; by the mid 1940's vitamin E was known to retard the initiation of rancidity in fatty foods which could also be further delayed by the additional presence of vitamin C.[4] Within fresh foods, indigenous antioxidants may maintain their activity in suitable storage conditions, yet our current knowledge of free radical mediated biochemical changes within foods is incomplete. The potential to preserve quality by limiting the manifestations of free radical activity during storage and processing has not been fully exploited by the food industry.

Free Radical Generation and Reactivity

The formation of superoxide and other more reactive oxygen species, which contribute to oxygen toxicity within the aqueous and non-aqueous cellular micro

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environments, arises from the stepwise addition of electrons to oxygen. In aqueous solutions, superoxide is a moderately strong reductant but also a weak oxidant[5] and so superoxide molecules are poorly reactive towards organic molecules, at least as oxidants.[6] For reaction with organic molecules the rate constants are usually low and less than 10^3 $M^{-1}s^{-1}$.[7] O_2^- itself can only oxidise strong reducing agents such as α-tocopherol and ascorbic acid with an overall rate constant of 2.7×10^5 $M^{-1}s^{-1}$.[8] In general superoxide is probably not reactive enough to cause cell damage in living systems, although it will react with metals to produce other reactive species such as the hydroxyl radical. However in postharvest and postmortem foods superoxide generating systems may allow superoxide to either be released or accumulate within both aqueous and non-aqueous phases. Fluxes of superoxide generated *in vitro* have been shown to be associated with damage to cell components: superoxide being the precursor of the more reactive hydroxyl radical which is a powerful oxidant.[9] Nevertheless the stability of the superoxide radical is relatively high and thus is long-lived and therefore has the potential to diffuse and strike specific targets,[10] whereas other radicals may be more reactive and strike targets closer to their site of production. The small size of O_2^- enables it to diffuse through the phospholipid bi-layer. In non-polar solvents O_2^- is a strong base and nucleophile; the non-polar interior of cell membranes may allow O_2^- to deacylate phospholipids and damage lipid side chains. The protonated form of O_2^-, the perhydroxyl radical $HO_2^{\cdot}$, a moderately strong oxidant may be of significance in foods with low pH values, such as citrus and other fruit products. The pKa for the dissociation of $HO_2^{\cdot}$ is 4.8 and in living systems it comprises only a small proportion of the physiological O_2^-; only approximately 0.25% is protonated,[11] and is more likely to be detected only in organelles where the pH is low.

In living cells and hence foods the most abundant transition metal ions are iron and copper. In undamaged living cells, compartmentalisation and binding of cations, for example to proteins, may ensure that control is maintained over the availability of intracellular metals,[4,11] but such control diminishes in stored and processed foods. Superoxide is likely to arise from the leakage of electrons from reduced electron transport systems and organelles. Superoxide is believed to contribute to oxygen toxicity through its role as the precursor of the more reactive hydroxyl radical by bringing about the reduction of transition metal ions. In the presence of hydrogen peroxide, reduced transition metals are oxidised and hydroxyl radicals are formed via the Fenton reaction

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\cdot}OH + OH^-$$

Only trace amounts of transition metals are required as the oxidised metal can be reduced by $O_2^{\cdot -}$ or other reducing agents. The hydrogen peroxide may arise from reduction of oxygen by oxidases and can accumulate in the absence of catalases and peroxidases. The potential for the generation of reducing agents, other than O_2^-, e.g. ascorbate and glutathione, is limited and in any case, these may be rapidly lost in the non-living system. Although there is some evidence to suggest that ascorbate can replace O_2^- as the reducing agent for the production of $OH^{\cdot}$, the relative contributions may be concentration dependent. As ascorbate is rapidly oxidised by reaction with both $O_2^{\cdot -}$ and with $^{\cdot}OH$, hydroxyl radical production would quickly become $O_2^{\cdot -}$-dependent.[12] In foods with added nitrites the nitric oxide formed may react with O_2^- to form the hydroxyl radical.[12a]

There are other mechanisms by which hydroxyl radicals may be produced via the Fenton reaction within foods; for example, there is some evidence to suggest that

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$OH^{\cdot}$ may arise from the addition of cochineal red (E120) to food systems.[13] A major component of this red colouring is carminic acid, which can be reduced to produce semiquinone radicals that then reduce transition metal cations, thus allowing the Fenton reaction to proceed in the presence of H_2O_2.[13]

The Role of Enzymes in Free Radical Mediated Oxidation

Unprocessed foods are biological systems often composed of aerobically respiring cells. The dissociation of organelles and the breakdown of cellular structure during harvesting and storage, or during homogenisation, emulsification or dispersion of food ingredients must initiate many biochemical reactions, whereby especially those involving transfer of single electrons may be uncoupled and result in the release of free radicals. In aerobically respiring living cells most of the oxygen absorbed is catalytically reduced to water by cytochrome c oxidases or blue copper oxidases in the respiration cycle, but up to 5% of the total O_2 may be only univalently reduced to O_2^-.[14] In fresh foods and certainly those stored and processed the tight compartmentalised control of active oxygen species, including O_2^- and H_2O_2, is lost. Thus under these conditions free radical intermediates and active oxygen species may accumulate. In complex food systems it has not yet proved possible to always define in precise chemical terms how such released free radicals react with the very large number of substances present in food materials, although it is now becoming increasingly accepted that free radicals damage texture, flavour and colour. Some enzymes, such as peroxidases and lipoxygenases, are often able to initiate oxidation through the involvement of free radicals. They can withstand thermal processing and therefore in processed foods, where other protective enzymes have been denatured, may still be sufficiently active to initiate changes in quality during long term storage.

During enzyme catalysed oxidation of quite a wide range of substrates the individual reactions between enzyme and substrate and the subsequent release of the product requires in some instances the transfer of single electrons. Until 1969 when superoxide dismutase (SOD) was discovered biochemists had not accepted that enzyme catalysed oxidations could take place by single electron transfer, although Chance[15] had elucidated the single electron reduction of compounds I and II to regenerate native peroxidase. Transition metals like iron, copper or molybdenum are present in the active sites of oxygenases and facilitate the transfer of single unpaired electrons: sometimes a free radical is the prime oxidation product as with xanthine oxidase, lipoxygenase and peroxidase. For other enzyme catalysed reactions transfer of single electrons may be part of a series of steps that ultimately result in the four electron reduction of oxygen to water or the two electron reduction of NAD^+ via FADH.

Xanthine Oxidase

Xanthine oxidase (EC 1.2.3.22, xanthine: oxygen oxidoreductase) is widely distributed in foods. In milk, xanthine oxidase, a molybdenum-containing enzyme, can exist in both free and bound forms. The enzyme contains two molybdenum atoms, two FAD groups and eight Fe-S centres, all of which can readily be involved in transfer of single electrons. The "free" form of the enzyme catalyses the formation of both superoxide and hydrogen peroxide during the oxidation of purines, xanthine itself being oxidised to uric acid. The bound form of the enzyme seems less harmful

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where it acts as a NAD-dependent dehydrogenase. The general reaction catalysed by xanthine oxidase is:

$$H\text{-}OH + RH \longrightarrow ROH + 2H^+ + 2e^-$$

where RH is the substrate and the oxygen introduced is derived from water. Where oxygen is the electron acceptor, sequential single or two electron transfers can form superoxide and hydrogen peroxide, respectively.

$$2e^- + 2O_2 \longrightarrow 2O_2^-$$

$$2e^- + O_2 \longrightarrow O_2^{2-} \xrightarrow{2H^+} H_2O_2$$

The superoxide is believed to be produced at the flavin site of the enzyme and hydrogen peroxide can be produced by two electron reduction of oxygen from either $FADH_2$ or Mo^{IV}.

Peroxidases

Peroxidases (EC 1.11.1.7) catalyse the overall reaction:

$$ROOH + AH_2 \longrightarrow H_2O + ROH + A$$

Hydrogen peroxide is generally the oxidising substrate which results in the formation of compound I from peroxidase, which then in turn oxidises AH_2 by abstraction of a single electron to form the free radical and compound II. Both peroxidase compounds I and II, where the haem Fe exists in nominal oxidation states of 5 and 4 respectively, are able to easily oxidise a wide range of phenolic substrates to form free radicals.

$$\underset{\text{compound I}}{Per\text{-}Fe^{V} - O} + e^- + H^+ \longrightarrow \underset{\text{compound II}}{Per\text{-}Fe^{IV} - OH}$$

$$\underset{\text{compound II}}{Per\text{-}Fe^{IV} - OH} + e^- + H^+ \longrightarrow \underset{\text{native peroxidase}}{Per\text{-}Fe^{III} - H_2O}$$

Hydrogen abstraction readily occurs from phenolic substrates because of the resonance stabilisation of the resultant phenoxyl radicals. These separate cationic radicals are then able to react non-enzymically with a wide range of other susceptible substrates to generate other radicals and promote a large number of separate reactions. In this way peroxidases have a propensity for initiating the oxidation of a very wide range of compounds, generally unlike other oxidase enzymes. It is this propensity to stimulate the oxidation of a large number of substances, sometimes ill-defined, which makes it difficult to identify precisely the action of peroxidases not only in foods but also in living plants. Because of the ease of hydrogen abstraction many of the natural susceptible substances must include phenolics, such as coumaric and caffeic acids and anthocyanins. In the same way flavins, pyridinium compounds and other aromatic compounds are likely to be oxidised by the peroxidase-generated free radicals. Vitamin C is oxidised directly by peroxidase to produce the resonance stabilised ascorbate radicals which can disproportionate to dehydroascorbate and ascorbic acid. Other radicals and in particular those of test substrates, such as guaicol and o-dianisidine, can combine to form dimers or higher polymers. Nevertheless in foods the amount of hydrogen peroxide present is generally small and therefore the rates of oxidation are likely to be low, but oxidation can become apparent during storage at ambient temperatures.

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Peroxidases also catalyse oxidatic reactions in the absence of hydrogen peroxide. The oxidatic reaction, accompanied by the uptake of oxygen, is exemplified by the oxidation of the synthetic substrate dihydroxyfumaric acid (DHF) by compounds I and II.[16] Superoxide is believed to be involved in the oxidatic reaction which is inhibited by superoxide dismutase. In plants a similar scheme is believed to operate for the oxidation of NADH, pyridoxyl compounds and indole-3-acetic acid (IAA). During the oxidation of IAA the initial reaction requires the oxidation by compound I to form first IAA$^{\cdot}$ and secondly indole-3-methyl radicals which are then oxygenated by compound III, Per-FeII-O_2.[17] Although IAA is a plant hormone, clearly it and possibly other indole compounds could be a source of peroxidase-generated free radicals during food storage, which indirectly might cause loss of quality by initiating the formation of other free radicals. In heat processed foods, where catalase and superoxide dismutases are likely to have been inactivated, the deleterious effects of the more thermostable peroxidases are likely to be more apparent.

Lipoxygenases

Lipoxygenases (EC 1.1 3.11.12, linoleate: oxygen oxidoreductase) catalyse the oxidation of polyunsaturated fatty acids containing a *cis, cis*-1, 4-pentadiene system to produce first free radicals and subsequently after oxygenation conjugated hydroperoxy unsaturated acids. Generally oxidation of unsaturated lipids through the action of lipoxygenase may lead to either beneficial or detrimental changes in the sensory characteristics of foods. In harvested fruit and vegetables, the hydroperoxides produced are degraded by hydroperoxide lyases to form volatile carbonyl compounds which contribute to characteristic aromas. Part of the desirable aroma present in potatoes, tomatoes and cucumbers is due to the action of lipoxygenase followed by enzyme catalysed transformation of the hydroperoxides. The action of hydroperoxide lyases and dehydrases, the consequent formation of secondary products and their physiological importance has been reviewed recently by Gardner.[18] The aroma and flavour deterioration of maize, green beans, peas and soybeans is attributed to the presence of lipoxygenase,[19] although the enzyme itself may only be directly responsible for the oxidation of polyunsaturated fatty acids by a free radical mechanism. Thus it is implied that the enzyme generated radicals may also react chemically with the important aroma compounds causing a loss of quality as well as producing grassy-beany off-flavours. During the mechanical development of wheat dough the enzyme catalysed oxidation of lipids increases the ability of doughs to withstand over mixing, since the peroxy radical product may oxidise the thiol groups of the intermolecular gluten network.[19] Oxygen is required for the manufacture of bread dough and Graveland *et al.*[20] have proposed that superoxide is both generated and responsible for the reduction of disulphide bonds. Recently we have shown that bovine superoxide dismutase added to wheat dough also delayed peak dough development indicating the involvement of superoxide and possibly other free radicals.[21]

Different lipoxygenases from various plant and animal species oxidise polyunsaturated fatty acids stereospecifically. The insertion of molecular oxygen is chiral and positionally specific.[19] For the oxidation of linoleic acid by plant lipoxygenases, the hydroperoxide group may be located at carbon-9 or carbon-13, depending on the type of lipoxygenase.

Soybean lipoxygenase type 1 (LOX-I)[22,19] is the most characterised enzyme and

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is widely considered as a model for other plant lipoxygenases. Type I enzymes have optimum activity at approximately pH 9, whereas type II enzymes are most active between pH 6.5 and 7. All of the soybean lipoxygenase isoenzymes have a molecular weight of approximately 100,000 and contain one atom of iron per molecule.

It is assumed that there is a common mode of action for all lipoxygenases in which iron is the electron acceptor. The substrate must contain a *cis-cis*-pentadiene moiety with an activated methylene group at ω8 located between the double bonds. It is suggested that the native enzyme exists as E-Fe^{II} and that activation is by a small amount of hydroperoxide naturally occurring in lipid substrates or by nanomole quantities of hydrogen peroxide to generate the E-Fe^{III} form. The enzyme iron in oxidation state III is believed to abstract stereo-specifically an electron from the activated methylene group of the substrate to form an enzyme-linked pentadienyl resonance stabilised radical with the Fe atom then reduced to oxidation state II. Addition of oxygen to pentadienyl radicals results in the formation of chiral and regio-specific hydroperoxy isomers. Theoretically such isomers are possible from each *cis*-1,4-pentadiene configuration (Figure 1).

Typically for the type I LOX acting on linoleic and linolenic acids hydrogen abstraction from ω8 is chiral and oxygenation occurs on the opposite side of the fatty acid chain and oxygen insertion is at ω6 to form 13(S)-peroxy radical. An

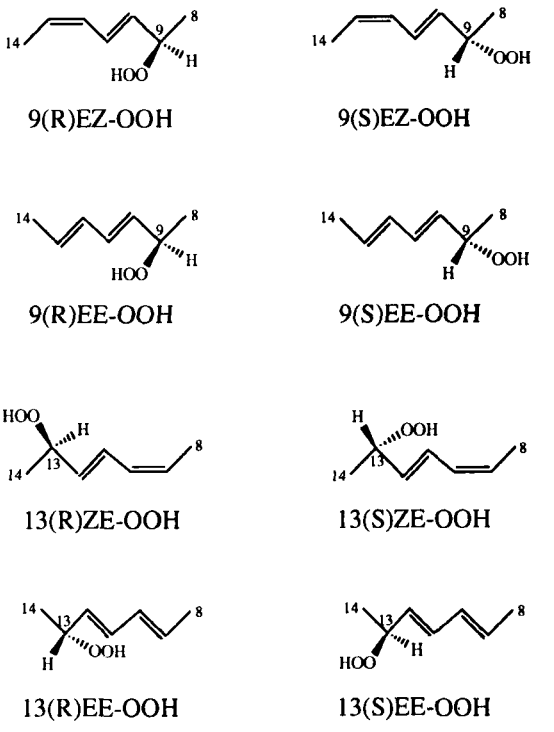

FIGURE 1 Chiral types of hydroperoxides produced by lipoxygenase-catalysed oxidation of linoleic acid. There are a total of eight intramolecular arrangements involving the optical isomers (R, S), geometrical isomers (cis,trans-ZE and trans, trans-EE dienes) and the positional isomers 9- and 13-hydroperoxides.

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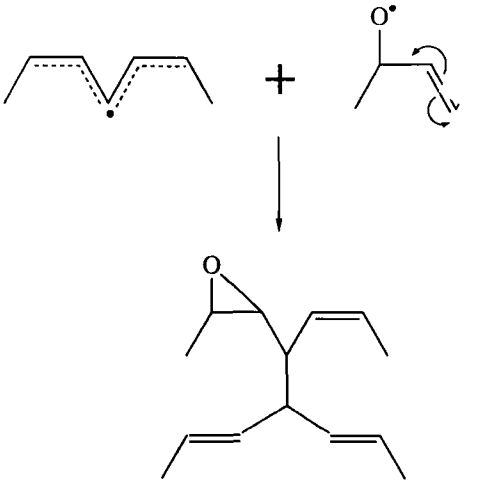
electron is then transferred from the reduced enzyme to form a peroxyl anion and the regenerated active Fe^{III}-LOX. In an "anaerobic" environment the E-Fe^{II}-pentadienyl radical complex is believed to dissociate to a pentadienyl radical which may then polymerise or propagate the formation of free radicals from other substrates. It has been proposed that the enzyme is recycled in oxygen-depleted systems by small amounts of hydroperoxides which reoxidise the Fe^{II}-LOX to Fe^{III}-LOX. Even in the anaerobic pathway the products might be oxygenated by interaction of the pentadienyl radical with small amounts of residual hydroperoxyl or lipoxyl radicals:



Soybean LOX-I forms the 13(S)-hydroperoxy-9(Z), 11 (E)-octadeca-dienoic acid from linoleic acid and the 13(S)-hydroperoxy 9(Z), ll(E), 15(Z)-octadecatrienoic acid from linolenic acid. The C-20 tetraenoic acid found in animal tissues is (surprisingly) oxidised to the respective 15(S)-hydroperoxytetraenoic acid. Furthermore a range of secondary products including dihydroperoxides and compounds normally considered to be of mammalian origin (leucotrienes and lipoxins) also arise from the oxidation of arachidonic acid catalysed by soybean and potato LOX.[18] For the type II enzymes Soybean LOX-II has been claimed to form the 9R-hydroperoxide, while maize germ lipoxygenase has been claimed to remove a hydrogen radical from the pro-R position of the $\omega 8$ carbon coupled to insertion of oxygen from the opposite side of the fatty acid chain to form the 9S-hydroperoxide.[23,18] Ketodienoic acids absorbing at 270–280 nm have also been shown to be formed during the oxygenation of arachidonic acid by human lipoxygenases. It has also been reported that keto compounds are formed during the oxygenation of linoleic acid by a pea lipoxy-genase.[23]

Microsomal cytochrome P450 enzymes (EC 1.14.14.1) may also catalyse lipid oxidation in foods from animal and microbial sources. Although there is controversy concerning the mechanism, it is considered to be free-radical mediated as the antioxidant compound butylated hydroxytoluene (BHT) causes complete inhibition.[24] Although a number of iron-oxygen complexes have been proposed as initiators,[25] the precise nature of the initiator is not known.

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Cooxidation

Lipoxygenases are used to bleach carotenoids by a cooxidation reaction in wheat flour during bread making. The cooxidation activity of lipoxygenases may be source-dependent; lipoxygenases in peas and beans have been claimed to have a high cooxidation activity.[26] For soybean and peas, the type II isoenzyme which has optimum activity at pH 6-7, as opposed to the type I isoenzymes with an optimum at pH 9, is more effective at co-oxidation.[27] Recently a high cooxidation activity has been reported for one of two chick pea type II isoenzymes[28] and oxidation by LOX in nonconventional media has been reported.[29] Purified tomato lipoxgenase has been reported to oxidise β-carotene faster than α-carotene and lutein, while lycopene (the main tomato pigment) remained unaffected.[30] Given the choice of natural substrates available and the occurrence of different isoenzymes in any given source it is not surprising that there have been few reports in the literature relating loss of carotenoids to lipoxygenase activity.

Lipoxygenases are known to catalyse the oxidation of carotenoids and chlorophyll by a free radical mechanism but still requiring the presence of a polyunsaturated fatty acid. It is possible that the enzyme is an integral part of the system for cooxidation of carotenoids through the involvement of an enzyme pentadienyl radical-complex. The cooxidation reaction may arise from abstraction of a hydrogen atom from the carotenoid resulting in the formation of a resonance stabilised radical, which would then combine with oxygen to produce carbonyl compounds.[31] Further products could arise by either decomposition of the radicals or condensation reactions to form dimers or higher polymers. However little is known about the chemistry of the degradation products.[30]

For the apparent associated oxidation of other substances, including thiol groups and inhibitors, it is possible that free radicals are first dissociated from the enzyme. Endogenous inhibitors of lipoxygenase in plant sources include chlorophyll, α-tocopherol and phenolic compounds. These substances could act as scavengers for released, or possibly enzyme-bound radicals. One potential mechanism involves the leakage of a peroxyl radical from the enzyme which can then attack the carotenoid, presumably at positions adjacent to double bonds. A second mechanism is that an enzyme-bound hydroperoxide is the oxidising species. A third mechanism which may operate is the generation of free-radicals in reactions catalysed by anaerobic cycling of lipoxy genase. Whatever the primary mechanism, the net effect generates carotenoid moieties containing a free radical centre which can then react with oxygen to cleave an adjacent double bond to give two carbonyl fragments. In addition to loss of carotenoid colour, such processes, if continued, lead to the formation of odourous molecules involved in flavour and off-flavour in foods. Our previous unpublished work with tomato LOX has indicated that cleavage can occur at a number of the double bonds in lycopene and that 6-methyl-5-hepten-2-one is one of the products.

Lipid Autoxidation

All foods that contain lipid are susceptible to oxidation, but dried foods, and those subjected to high temperatures during cooking/processing and subsequently stored are particularly affected.[32] Lipid oxidation products include aldehydes and especially malonaldehyde, fatty acid hydroperoxides, secondary degradation products and thermally altered oxidised lipids as well as cholesterol oxides. Addis and

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Warner[2] have emphasised the wealth of publications on the deleterious effects of oxidised lipids and have recommended a number of reviews on the health aspects of dietary lipid oxidation.

In animal foods the most obvious effect of free radical mediated reactions is the production of off-flavours arising from the autoxidation of unsaturated fats. However the primary products of lipid oxidation, the hydroperoxides, do not modify the flavour or structure of the food to any significant extent, but, the secondary products i.e., aldehydes etc. are potent flavour modifiers responsible for objectionable flavours. Sometimes they modify the structure of the product.[33] The volatile aldehydes, ketones and hydrocarbons responsible for the off-flavours initially arise from hydroperoxy radicals formed during autoxidation. The formation of alkanes, aldehydes and alkenes can be explained in terms of the known mechanisms by which hydroperoxy and lipoxy radicals decompose, react intermolecularly and undergo rearrangement reactions. Consequently research on the origin of off-flavours in foods has aided the development of a mechanistic understanding of the various ways in which free radicals can interact with each other and with other food constituents. Indeed, knowledge of the mechanism of autoxidation preceded awareness of the existence of lipoxygenases and is still considered to be the major cause of oxidative rancidity in animal foods and possibly commercial fats and oils.

Lipid peroxidation is a free radical reaction in which initiation, propagation and termination phases are involved; each of these phases may be influenced by a variety of factors such as the structure of the lipid, the presence of antioxidants and the mechanism of initiation.[34] The presence of additional unsaturated bonds increases the susceptibility of fatty acids to autoxidation: the relative rate of autoxidation of oleic acid (18 : 1), linoleic acid (18 : 2) and linolenic acid (18 : 3) was reported[35] to be in the ratio 1 : 40–50 : 100 as indicated by oxygen uptakes and 1 : 12 : 25 on the basis of hydroperoxide production. Other polyunsaturated fatty acids with further double bonds (e.g., arachidonic acid (20 : 4) and eicosapentaenoic acid (20 : 5) and docosahexanoic acid (22 : 6) found mainly in fish oils are considerably less stable than linolenic acid. The rates of oxidation have been claimed to increase linearly with the number of double bonds.[36] With the increased number of double bonds their hydroperoxides are more likely to undergo rearrangement reactions. Due to the susceptibility to oxidation either through the mechanism of autoxidation or catalysis by lipoxygenases, the nutritionally important exploitation of fish oils for the manufacture of not only acceptable but desirable products poses a special challenge for the food industry.

Lipid oxidation is responsible for major changes in food acceptability even in foods with only a minor lipid component, such as cucumbers and melons. Lipoxgenase is probably responsible for the development of off-odour, loss of colour and flavour in legumes such as peas and green beans.[19] In fruits the initial products of lipoxygenase catalysed oxidation are converted to acids and alcohols which subsequently form esters characterising fruit flavours.[37] Polyunsaturated fatty acids (PUFA) are particularly susceptible to oxidation: it has been stated that the relationship between oxygen and unsaturated fatty acids is perhaps the most significant factor affecting the stability and nutritional value of many foods.[22] Many chemicals and enzyme systems in foods are able to reduce iron ions and so accelerate lipid peroxidation. Within processed foods, the treatments or addition of other ingredients may compound the problems of exposure to trace elements created through loss of cellular compartmentalisation. Changes in colour may be due to either the

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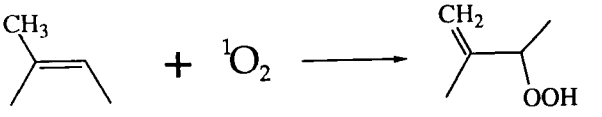
Maillard reactions between substances originating from lipids and proteins or to cooxidation of susceptible substances such as carotenoids and chlorophyll. Warmed over off-flavour in precooked meat poses a substantial problem and may be due to reactive free radicals. Changes to texture may arise through cross linking reactions of proteins and changes in nutritional value may occur through the loss of PUFA, especially through the loss of the n-3 essential fatty acids.

Transition metals play an important role in the initiation and propagation phases of lipid oxidation as they participate in the generation of hydroxyl radicals, in addition to their role as decomposers of lipid hydroperoxides. Several iron ion complexes, such as perferryl, ferryl or Fe^{2+}, $Fe^{3+} \rightarrow O_2$ complexes, have been claimed to initiate lipid oxidation,[39] although their ability to do this is as yet uncertain.[7] The hypervalent ferryl species containing FE(IV) is known to occur in the presence of hydrogen peroxide in catalase, peroxidase and cytochrome P450 and other haem proteins including myoglobin, all of which even in the denatured state, may initiate lipid oxidation. The possible role of hypervalent iron species and mixed metal oxygen complexes has recently been reviewed.[40] However it is not known whether hypervalent iron exists in denatured haem proteins or hydrolysed haem peptides and in some cases the rate of autoxidation may be much slower than lipoxygenase catalysed oxidation.

Light-induced lipid oxidation via the formation of free radicals resulting from light absorption first by other species (i.e., photo-sensitised oxidation) may affect food quality. For example, photo-sensitised oxidation processes which involve the formation of energised vitamins or colours, such as riboflavin (vitamin B_2, E101) or erythrosine (E127), may be responsible for the initiation of lipid peroxidation. There are two types of photo-sensitised reactions. Type 1 occurs when a photosensitised compound such as riboflavin abstracts the initial H atom from the lipid molecule and the oxidation proceeds as for autoxidation, excepting that the photosensitised reaction is not inhibited by antioxidants. A type 2 reaction occurs when molecular oxygen is activated to singlet oxygen ($^1O_2$). Its formation in foods is important where natural and added photo-sensitisers can generate singlet oxygen in microseconds. The stability including the colour and flavour of oils and oil based foods containing chlorophyll and carotenoids is susceptible to light induced oxidation, although this may be controlled by the use of suitable coloured packaging and many substances such as ascorbate, vitamin E and carotenoids act as chemical quenchers.

Singlet oxygen can react with carbon-carbon double bonds to yield hydroperoxides. Unlike ground state oxygen, singlet oxygen is not a radical species and it does not abstract hydrogen from reactive methylene groups or add to double bonds. However it will interact with double bonds to form an allylic hydro-peroxide in which the double bond is chemically shifted.[41]



The oxygen molecule is joined onto the end of the double bond and a new double bond is formed between the allylic position and the other end of what was previously the carbon-carbon double bond.[42] In addition to chemical combination it has been claimed that one mole of β-carotene can quench up to 1000 moles of $^1O_2$

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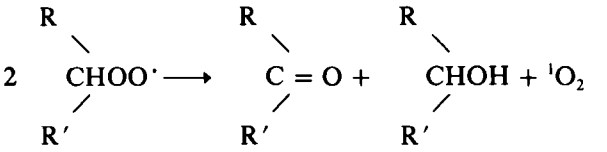
through the transfer of excitation energy.[43] One of the major ways in which $^1O_2$ is generated is through the exposure of photo-sensitive compounds such as chlorophyll, certain haem compounds and erythrosine to light.[44] In unsaturated lipids singlet $^1O_2$ oxygenation occurs at both ends of all the double bonds: that is at the ends of the 1,4-diene units as in autoxidation.[43] Autoxidation is typically accompanied by a weak chemilumin escence, some of which has been attributed to $^1O_2$ decay.[45] One of the main mechanisms may be the combination of peroxyl radicals, which yields a ketone, an alcohol and oxygen in the triplet ground state, or the singlet excited state.[46]



There have been proposals that $^1O_2$ generated from oxidising lipid may then contribute to the chain peroxidation reaction by further initiation, but these suggestions were based on the inhibition observed with scavengers and quenchers which lack specificity for $^1O_2$.[47] The singlet oxygen may induce oxidation of carotenoids present by an ene reaction to form allylic hydroperoxides,[47a] permitting cooxidation of carotenoids in the presence of lipoxy genase-generated peroxyl radicals. Although there is some evidence for the formation of $^1O_2$ during lipid peroxidation, whether it contribute significantly to the process has been questioned.[46,47]

Initiation of lipid oxidation involves the initial abstraction of a hydrogen atom from the lipid molecule to form a carbon-centred radical. This abstraction may be caused by irradiation or by interaction with other sufficiently reactive free radicals, or excited photochemicals. The direct reaction with an unsaturated fatty acid and oxygen to produce a free radical is endothermic (ΔH, 35 kcal) and is not significant due to the stability of triplet oxygen. However abstraction of an electron from an unsaturated fatty acid by a transition metal is exothermic (ΔH, -15 kcal) and therefore is an important process during autoxidation.

$$RH + M^{III} \longrightarrow R^{\cdot} + H^{+} + M^{II}$$

The hydroxyl radical possesses extreme reactivity (with typical rate constants $> 10^9\ M^{-1}\ s^{-1}$),[8] and therefore must be a significant initiator of lipid oxidation, whereas O_2^- is believed to be insufficiently reactive to abstract a hydrogen directly, although its protonated form, $HO_2^{\cdot}$ may be sufficiently reactive to abstract hydrogens from some fatty acids at a low rate.[48]

Free radicals ($R^{\cdot}$) which are sufficiently reactive can abstract a hydrogen atom from a methylene carbon of an unsaturated lipid (LH) to yield a fatty acid radical ($L^{\cdot}$):

$$LH + R^{\cdot} \longrightarrow L^{\cdot} + RH$$

The exact nature of $R^{\cdot}$ in foods may be difficult to determine because of its low concentration.[38] The removal of the hydrogen atom leaves behind an unpaired electron on the carbon atom to which it was originally attached. This carbon-centred radical rearranges to form a conjugated diene and generally such resonance stabilised radicals only abstract weakly bonded hydrogens.

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In aerobic conditions the lipid alkyl radical ($L^{\cdot}$) combines with molecular oxygen at an extremely rapid rate, the reaction is generally assumed to have very low activation energy.[22]

$$L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$$

As O_2 itself is a diradical the combination produces a peroxyl radical. The reactivity of the peroxyl radical is sufficient to remove a hydrogen atom from another fatty acid thus propagating a chain reaction. The peroxyl radical is the major chain propagating species which combines with the hydrogen atom abstracted to yield a lipid hydroperoxide (LOOH). It has been estimated that such a reaction sequence goes through 8–14 propagation cycles before termination[49] depending on the presence or otherwise of other constituents including antioxidants.

$$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot}$$

The hydroperoxide formed may participate in metal catalysed reactions in which alkoxyl and peroxyl radicals are generated by its decomposition.

$$LOOH + Fe^{2+} \longrightarrow LO^{\cdot} + OH^{-} + Fe^{3+}$$

$$LOOH + Fe^{3+} \longrightarrow LOO^{\cdot} + H^{+} + Fe^{2+}$$

The generation of radicals is therefore maintained, resulting in a chain reaction. The reductive pathway appears to be faster than the oxidative one[50] and the addition of reducing agents such as ascorbic acid or O_2^{-} can accelerate the reductive pathway. Although metal-independent reduction of lipid hydroperoxides is thermodynamically feasible, in general this occurs much more slowly than metal catalysed reduction. During food processing, that generally includes heat treatments and the addition of other ingredients, cellular disruption will increase exposure to transition metals and hence enhance free radical reactions. When the radical concentration is sufficiently high, radical-radical interactions may occur, yielding stable end products. This is the termination phase, which is dependent on many factors such as fatty acid composition, oxygen concentration and the presence of chain-breaking antioxidants. The combination of two peroxyl radicals is one example of an important termination reaction.[22]

$$LOO^{\cdot} + LOO^{\cdot} \longrightarrow LOOL + O_2$$

The potential range of oxidation products so formed is large and dependent on factors such as the nature and concentration of the initial substrate, the presence of pro-oxidants and the mechanisms by which they stimulate oxidation and the processing conditions.[22] The consequences of free radical reactions in foods which contain a very large number of potential substrates for interactions will be determined by the relative reaction rates or the various pathways taken by a radical.[51] Mixtures of products can include epoxyhydroxy and epoxyoxo-compounds, oxygenated and non-oxygenated dimers and possibly due to a hydro peroxidase activity ketodienes and ketodienoic acids. Epoxides are formed by intramolecular addition of oxygen across a double bond. The 13-hydroperoxide can rearrange to the 12,13-epoxide and the 9-hydroperoxide to the 9,10-epoxide.[52] This type of intramolecular rearrangement was detected.[53]

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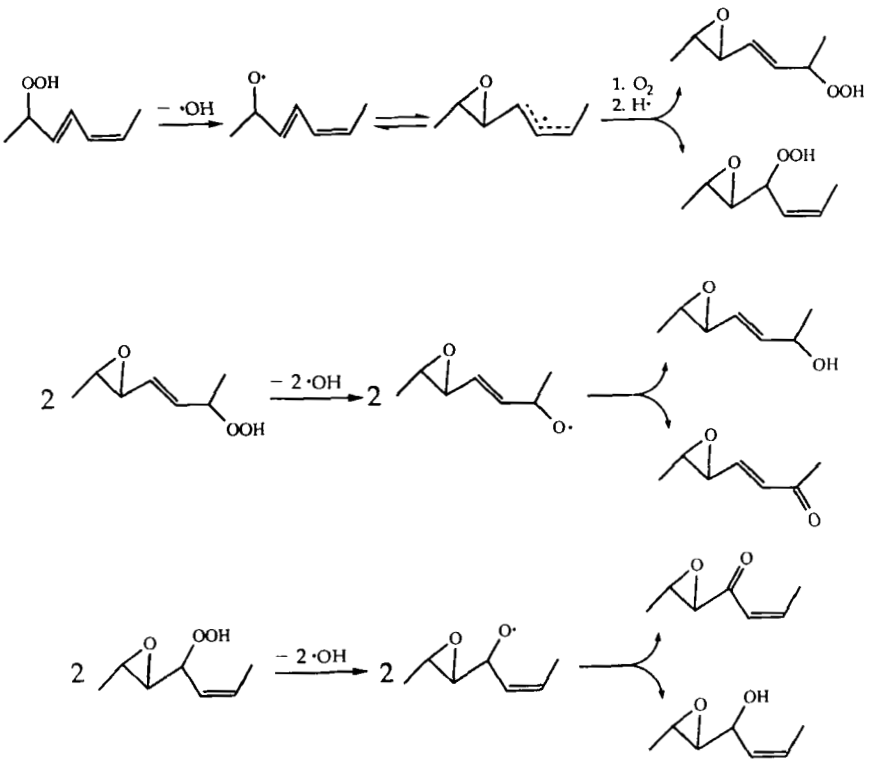
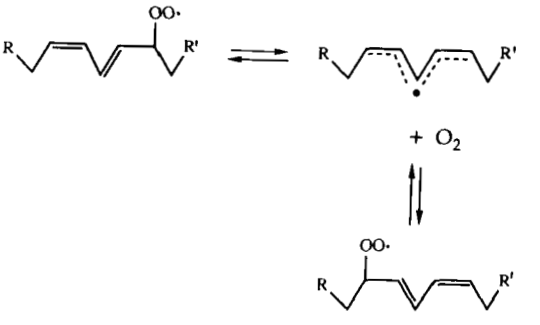


Also end products can be formed by combination with oxygen to produce epoxyhydroperoxides and disproportionation products such as epoxyhydroxy and epoxyoxo compounds.[52] In anaerobic systems the epoxy allylic radical may combine with radicals such as fatty acid diene radicals and α-tocopherol simiquinone radicals. Also, in the presence of thiols and disulphides, thiyl ($RS^{\cdot}$) and thiyl peroxyl radicals ($RSO_2^{\cdot}$) may be reactants. Glutathione present in both plant and animal foods reacts rapidly (rate constant $10^7\ M^{-1}s^{-1}$) to form the $GS^{\cdot}$ radical.[54]

Isomerisation of hydroperoxides of fatty acids can occur by rearrangement of the peroxy radical through the loss of oxygen and consequently the pentadiene radical.[43]



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In lipid hydroperoxides the hydroperoxyl group is next to one or more double bonds which increase rates of intramolecular rearrangements of peroxyl radicals. It seems that such reactions might be more likely in the longer chain fatty acids containing five and six double bonds found in pelagic fish, - mackerel, salmon, sardines etc. Lipid hydroperoxides are susceptible to homolysis of the O—O bond to form an oxyradical which may easily occur during cooking.

$$ROOH \longrightarrow RO^{\cdot} + {}^{\cdot}OH$$

This is essentially an irreversible reaction and once formed the oxyradicals are transformed into end products.[52] H-abstraction is the more important reaction of oxyradicals and this affords a further mechanism of cooxidation analogous to that brought about by peroxyl radicals. The mechanism of cooxidation is abstraction of H-radicals to yield another radical from substrates such as carotenoids, which may then be oxygenated to form carotenoid peroxy radicals. Retinol (vitamin A) and β-carotene, the main precursor of vitamin A, due to their location as fat soluble vitamins are especially susceptible to free radical induced co-oxidation initiated by either autoxidation or lipoxygenases.

Deoxygenation of peroxyl radicals can account for the formation of *trans,trans* isomers in mixtures of hydroperoxides. The sequence proposes a conformational change in the peroxyl radical, deoxgenation to form a new pentadienyl radical and reoxgenation at the end of the radical to form a new *trans, trans*-peroxyl radical to give the hydroperoxide on H addition.[43] Antioxidants that act as H-donors can interfere with such rearrangements by forming the *cis,trans*-hydroperoxide from the *cis,trans*-peroxyl radical. Thus antioxidants may not only inhibit oxidation but also channel O_2 to form certain isomers.

Cholesterol Oxides

Under oxidising conditions, cholesterol gives rise to a substantial number of products which are collectively known as cholesterol oxides. More than 70 oxidation products have been claimed.[55] Since the proposed role of cholesterol in coronary heart disease, the products of cholesterol oxidation have received an increasing amount of attention. Although the position is not yet clear the atherogenicity of cholesterol may be considered to be due to contamination by cholesterol oxidation products.[2] Oxidised products of cholesterol can be formed after exposure to air at elevated temperatures, light, ionising radiation,[56,57] free radical initiators or combinations of these factors.[58] Cholesterol and closely related plant sterols contain a hydroxyl group that is attached to the A ring and a ^{5}—double bond in the B ring. Oxidation is initiated by hydrogen abstraction at C-7 followed by attack by molecular oxygen to form two epimeric 7-hydroperoxides. These are thermally unstable and form 7-hydroxycholesterols and 7-ketocholesterol.[59]

Studies have concentrated on the oxidation of cholesterol in dried egg yolk which may frequently contain high levels,[60,61,57] comminuted beef,[62] and clarified butter fat or ghee.[58,63] Lebovics *et al.*[57] have produced data on the formation of cholesterol oxide in irradiated egg powder. This has led them to advise that the irradiation of whole-egg powder and powdered egg yolk be reduced because of the enhanced formation of cholesterol oxidation products.

Further the oxidation of sterols in foods may not be limited to cholesterol. Many plant foods including refined fats and oils contain significant amounts of

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β-sitosterol, stigmasterol, campesterol and brassica-sterol.[64] Exposure of plant based foods to heating during manufacture and/or long term storage at ambient temperatures may lead to oxidation of sitosterol, the major sterol in vegetable oils. The presence of these plant sterols and their oxidation products in foods complicates analysis for cholesterol oxides. An accumulation of sitosterol oxides may also be associated with undesirable biological effects similar to those of cholesterol oxidation.[65,2] Addis and Warner[2] argue that the weight of evidence suggests that oxysterols are far more atherogenic than their native sterol counterparts and that increased atherogenicity seen in the case of oxysterols is independent of increases in serum cholesterol. Interestingly Addis and Park[60] have suggested that food technology should focus on the prevention of lipid oxidation instead of cholesterol removal. Wasilchuk *et al.*[66] have developed a method for analysis of cholesterol oxidation products using deuterium labelled cholesterol and alternative methods using NMR are being developed for the detection of oxidised products formed during the processing and storage of foods such as the spray-dried egg yolk.[67]

Mechanisms of Free Radical Removal

Within freshly harvested foods there exists a range of defence mechanisms that inhibit or remove the intermediates during the reduction of oxygen and thus prevent oxidative damage. The formation of free radicals and active oxygen species may be prevented by suppressing the rates of initiating reactions. This can be brought about by chelators of metal ions and systems that prevent initiation by light or irradiation. Biological inhibitors are metalloenzymes such as superoxide dismutases (SOD) which scavenges O_2^- and catalases and peroxidases which remove H_2O_2 and hydroperoxides. Conversion of hydroperoxides into inactive species by reaction with reducing agents such as thiols and disulphides, thus preventing propagation, also brings about very effective inhibition. However by far the most well-known and most frequent method for the prevention of free radical generated oxidation in foods is through the use of antioxidants. These are defined for purposes of food legislation as substances that prolong the shelf life of foods by protecting against deterioration, such as rancidity and adverse colour changes caused by oxidation. Antioxidants may be indigenous or added during processing. The use of antioxidants is now common in food manufacture and those which are permitted in the UK are listed in the Antioxidants in Food Regulations 1978 (Statutory Instrument 1978 No 105). In addition to UK legislation, the European Union has drawn up proposals to harmonise legislation in Member States (Table 1). Without the addition of antioxidant compounds as ingredients, whether natural or synthetic, the shelf life of foods, especially those with a particularly high fat content and large surface area, such as cakes and biscuits would be severely limited. Along with microbial spoilage, the peroxidation of poly unsaturated fatty acids is considered to be a major factor limiting the shelf life of foods. According to Halliwell and Gutteridge[68] an antioxidant may be defined as "any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or inhibits oxidation of that substrate". Most food commodities contain significant amounts of lipid, including unsaturated fatty acids.

Although there is much concern about the toxicology of synthetic antioxidants, the potential hazard associated with the use of naturally occurring compounds as food additives, as an integral part of the diet, is largely unexplored and therefore the long term risk associated with their use is unknown. In addition to flavour and

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TABLE 1
Antioxidants Generally Permitted in Foodstuffs in European Union*

Serial number	Name
E300	L-Ascorbic acid
E301	Sodium L-Ascorbate
E302	Calcium L-Ascorbate
E304	Fatty acid esters of L-Ascorbic acid
E306	Extracts of natural origin rich in tocopherols
E307	Synthetic α-tocopherol
E308	Synthetic γ-tocopherol
E309	Synthetic δ-tocopherol
E310	Propyl gailate
E311	Octyl gallate
E312	Dodecyl gallate
E315	Erythorbic acid
E316	Sodium erythorbate
E320	Butylated hydroxyanisole
E321	Butylated hydroxytoluene
E1102	Glucose oxidase

*Taken from the Proposal for a Council Directive on Food Additives other than Colours and Sweeteners.

rheological modification, the arguments in favour of protection of manufactured food through addition of antioxidants are the health considerations related to the possible role of oxidised lipids in carcinogenesis, coronary heart disease and ageing.[33] Combinations of two or more types of antioxidant activity can result in a synergistic effect; many individual antioxidants have multiple modes of action and in addition to the action of the chain-breaking antioxidants, there are preventative antioxidant techniques. These include:

(i) the removal of oxygen using inert gas or vacuum packaging.
(ii) the binding of metals ions using chelating or sequestrating agents such as citric acid, amino acids or EDTA, and
(iii) the use of enzymes capable of catalysing the removal of reactive oxygen species eg., glucose oxidase, catalase, peroxidase and SOD, and the use of non-enzymic oxygen scavengers including ascorbic acid and ascorbyl palmitate.

Chemical antioxidants interrupt the autoxidation propagation reactions:

$$LO_2^{\cdot} + AOH \longrightarrow LO_2H + AO^{\cdot}$$

$$LO^{\cdot} + AOH \longrightarrow LOH + AO^{\cdot}$$

For effective antioxidants AOH, as typified by vitamin E, the radical product seems, due to resonance stabilisation, insufficiently reactive to rapidly abstract a hydrogen from other lipid molecules. The reaction may then be terminated by combination of antioxidant radicals. During the action of antioxidants a pathway to products may include several intermediate stages of radical reactions:

(1) radical combination where

$$AO^{\cdot} + AO^{\cdot} \longrightarrow AOOA$$

$$AO^{\cdot} + X^{\cdot} \longrightarrow AOX$$

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yields a new compound with paired electrons. For resonance stabilised radicals of antioxidants abstraction of a weakly bonded hydrogen is only possible and therefore these radicals prefer to combine to form dimers and further products.

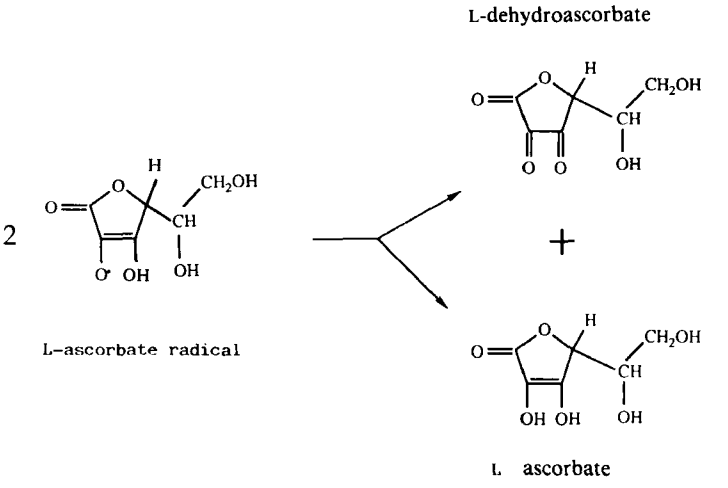
(2) oxidation-reduction by transition metals capable of effecting one electron transfer:

$$AO^{\cdot} + M(II) + H^{+} \longrightarrow AOH + M(III)$$

or

$$AO^{\cdot} + M(III) \longrightarrow AO + M(II)$$

(3) radical disproportionation as exemplified with ascorbate to yield oxidised and reduced products:



Ascorbic acid is a well known food antioxidant in aqueous environments. It reacts rapidly with O_2^- and very rapidly with $HO_2^{\cdot}$, to form a stable semidehydroascorbate radical, although in the presence of Fe^{3+} it may act as a pro-oxidant by reducing the Fe^{3+} which in the presence of H_2O_2, stimulates $^{\cdot}OH$ production.[8] The overall effect of ascorbate as a radical scavenger is likely to be variable being concentration dependent[68] as plant and animal tissues, and indeed organelles contain variable millimolar amounts of the vitamin. In postharvest and postmortem meats transition metals are likely to be released as cells and organelles break down and therefore may promote Fenton chemistry and consequently oxidation.

Other antioxidants, the "secondary antioxidants", act by decomposing peroxides by converting them into non-radical products. Examples are dilauryl thiopropionate and thiodi propionic acid. These two compounds are approved for food use in the USA, but not within the EU. At present the shelf life of products with a high fat content is extended through the use of synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, ascorbate and α-tocopherol. As indicated above the fate of these synthetic antioxidants is determined by the relative rates of radical-radical interactions, oxidative and reductive systems and disproportionation. The synthetic compounds act as primary

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antioxidants, or hydrogen donors. The chain reaction is halted by the low energy of the antioxidant radicals so formed.

There is a great deal of interest in the potential of naturally occurring compounds and in the use of food ingredients that possess antioxidant properties. Naturally occurring antioxidants include thiols, such as glutathione, a large number of phenolics, tocopherols and carotenoids. A wide range of possible biological antioxidants has been reviewed.[8] The search for natural antioxidants is also centred on the potential exploitation of plant phenolic compounds[69] and those found in the herb Rosemary. Herbs and spices are frequently used in food manufacture and offer an opportunity, as natural ingredients, for exploitation of their antioxidant properties.[70] Although *Rosemarinus officinalis* is the only spice extracted for use as an antioxidant many others are added as spices and the benefit of the antioxidant properties is in addition to their other use as flavouring agents. Carotenoid pigments are widely distributed in foods. Potential mechanisms of antioxidant action have been reviewed.[71] The carotenoid molecules such as carotene and lycopene have an extensive system of conjugated double bonds and can act as photo-protective agents which can use an energy transfer process to quench singlet oxygen and triplet photo-chemical sensitisers.

In lipid environments they may also have a chain-breaking antioxidant capability inhibiting free radical induced lipid peroxidation at low oxygen partial pressures. Molecules such as β-carotene are very susceptible to attack by alkoxyl and peroxyl radicals and there is experimental evidence that carotene reacts directly with peroxyl radicals involved in lipid oxidation:

$$\text{carotene} + \text{LOO}^{\cdot} \longrightarrow \text{carotene}^{\cdot}$$

The resonance-stabilized carbon centred radical (β-carotene$^{\cdot}$) may then be removed from the system in a termination reaction with another peroxyl to yield inactive products:

$$\text{carotene}^{\cdot} + \text{LOO}^{\cdot} \longrightarrow \text{inactive products}$$

The reactivity of β-carotene towards peroxyl radicals and the stability of the resulting carbon centred radical enables it to exert chain-breaking antioxidant activity at sufficiently low O_2 partial pressures. The stability of the carbon centred radical formed means that at low partial pressures of oxygen this form can predominate over the chain-carrying peroxyl form which is produced as the carbon-centred radical reacts reversibly with oxygen:

$$\beta\text{-cartoene}^{\cdot} + O_2 \rightleftharpoons \beta\text{-carotene} - \text{OO}^{\cdot}$$

The role of β-carotene and other carotenoids complements the role of vitamin E which is effective at higher oxygen pressures. Conn *et al.*[72] have used pulse radiolysis to show that β-carotene radical may transfer an electron to oxygen yielding a β-carotene superoxide complex. It is interesting to note that while the reversible electron transfer was not observed for β-carotene such a transfer was shown to occur with lycopene.

Tocopherols

Tocopherol (vitamin E) compounds occur in lipid components of both plant and animal tissues. They react with a wide range of organic peroxyl radicals, although beta-, gamma- and delta-tocopherols are less reactive than α-tocopherol. Burton

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and Ingold[73] have demonstrated that α-tocopherol is one of the most active *in vitro* chain-breaking antioxidants; it is also one of the best quenchers of singlet oxygen and reacts with O_2^- yielding a phenoxyl radical. Tocopherol is often used in conjunction with vitamin C as during reaction with lipid radicals it is oxidised and must therefore be regenerated for renewed antioxidant activity. The use of high concentrations of vitamin C to provide protection to vitamin E may be limited in some foods by the presence of transition metal ions and hydrogen peroxide, as the hydroxyl radical production would serve to increase lipid oxidation. Although lipid soluble antioxidants such as vitamin E are effective against lipid oxidation, when used in excess their action may not be entirely beneficial since their action can change the relative proportions of the peroxidatic products, resulting in undesirable taints.[38] Also, when considering using vitamin E for antioxidant action it should be remembered that although the deterioration of fats and oils is delayed by the inclusion of this type of antioxidant, there is also evidence to suggest that these antioxidants may be pro-oxidants in certain non-lipid systems.

Phenolics

Although there is a vast number of natural phenolic compounds in plants, which have been reported to exhibit predominantly chain-breaking antioxidant activity, only a few are used in isolated form in food products, eg., the antioxidant compound carnosol, found in the herb Rosemary. Coumaric and ferulic acids have little antioxidant activity whereas compounds possessing free unsubstituted hydroxyl groups, like caffeic and gallic acids, may be significant antioxidants. However, in foods these anti-oxidants are not continuously regenerated, in contrast to living systems where there exists a synergistic and co-operative inter-action between antioxidant scavengers during food storage and processing when these free radical scavengers are slowly lost. In addition to radical chain-breaking antioxidant activity, it has been claimed that some phenolic compounds are able to scavenge hydroxyl radicals,[74,75] superoxide radicals[76] and quench singlet oxygen.[77] However, although phenolic compounds, have iron chelating abilities, many are also capable of reducing Fe^{3+} to Fe^{2+},[78] and alternatively may therefore accelerate hydroperoxide breakdown and trigger Fenton chemistry[79] and so increase lipid oxidation by acting as pro-oxidants.

The concentration of flavonoids within plant cells is relatively high (often > 1 mM) and although not all may be available, perhaps due to their compartmentalisation, scavenging of O_2^- and other radicals is likely to be significant. There is now evidence that flavonoids are able to inhibit non-enzymic lipid oxidation,[80] lipoxygenase catalysed[81] and xanthine oxidase induced lipid oxidation,[82] as well as singlet oxygen induced bleaching of the carotenoid pigment, crocin.[83] Flavonoids exhibiting antioxidant activity have been identified and isolated from soya beans.[84] Flavonoids in soya beans may be present as iso-flavanoids with a rearranged C15 skeleton. The isoflavones exhibit less potent antioxidant activity than the flavones, flavanones and chalcones, since they lack the 3,4-dihydroxyphenyl structure which is significant in antioxidative action.

Compounds exhibiting antioxidant properties may be produced as a consequence of thermal processing during food manufacture,[33] for example, Maillard reaction products have been shown to provide protection from oxidative degradation of lipid. Protein hydrolysates, wood smoke and fermentation products, such as tempeh and miso can also act as process-induced inhibitors of lipid oxidation.[85] Bertelsen

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et al.[86] investigated the antioxidant activity of pea fibre in meat products and found that surface lipid oxidation and colour loss could be decreased. The use of proteins from plant sources as functional ingredients in manufactured food is an area of current interest amongst food scientists. Swanson[87] has pointed to the advantageous functional properties eg., improvement in colour, which may be accompanied by increased oxidative stability of food through such exploitation of proteinaceous material. A number of proteins have been shown to inhibit oxidation, eg. casein[88] and soya protein.[89] The antioxidant activity of proteins may be due to their radical scavenging or metal-binding activities. Oxidation of a tocopherol-free corn oil in a freeze dried model system containing carboxymethyl cellulose was found to be inhibited on addition of protein hydrolysates,[90] which lengthened the lag phase. Some amino acids are able to exert antioxidant activity, although the action is complex and dependent on a number of factors such as pH and temperature.[85]

Superoxide Dismutase

In 1954 Gershman *et al.*[91] proposed that the damaging effects of oxygen within the cellular environment were due to oxygen radicals, but the existence of oxygen radicals in a cellular environment was not widely accepted until 1969 when McCord and Fridovich[92] discovered the role of the enzyme SOD (EC 1.15.1.1). SOD is considered to be the first line of defence against oxygen toxicity as it converts O_2^- to H_2O_2 which may then be removed by peroxidases and catalase. It is commonly believed that O_2^- is the sole substrate for SOD, although Bors[93] and Bors *et al.* [94] have suggested that the Cu/Zn-SOD reacts with peroxyl radicals with rate constants comparable to those for the dismutation of O_2^-. Bors *et al.*[94] have recently suggested that SOD has unique properties at the protein surface having possibly evolved as a "general purpose radical scavenging protein". Nice and Robinson[69] have described an interaction of both bovine SOD and pea SOD with pea phenolic fraction to produce a high molecular weight thermostable antioxidant which is possibly a radical scavenging protein.

The various metalloforms of SOD have been found in a wide range of fresh food sources.[95] However the precise role of SOD in foods is not known, its influence on the preservation of fresh food quality is yet to be established, although it is likely that SOD is present in all fresh food sources. On harvest, a high antioxidant status in plant tissue, which may be specific to a particular cultivar, could make a positive contribution to the quality of seeds, fruits and tubers.[96] Our knowledge of the organisation of antioxidant defences, in particular species and inter-cultivar variations, and the potential for response to a variety of environmental conditions should be significant in plant breeding programmes.

Genetically modified plants may have a role in the control of fruit ripening and in the loss of quality due to peroxidase activity in harvested fruit and vegetables. Fruit ripening arises through complex biochemical changes which include lipid peroxidation, production of H_2O_2 and the deterioration of cell structures; as O_2^- is involved in many of the ripening changes it is likely that SOD has a significant role in the control of the ripening process. Wounding of plant tissue causes the releases of compounds such as ferredoxins, diphenols, pteridines and reduced flavins which easily autoxidise in the presence of oxygen to produce oxyradicals. *In vivo* fluxes in SOD activity in response to tissue wounding occur.[97] Sunscald injury in plants is also related to enhanced O_2^- production. SOD activity has been

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shown to increase during the ripening of tomatoes, supplementing the protective action of the carotenoids.[98]

O_2^- can oxidise nutrients such as ascorbate and vitamin E and so SOD may have a significant role in nutrient protection in fresh foods. In many highly-processed products SOD may have been destroyed as some indigenous sources may not be sufficiently thermostable: in addition food processing may remove SOD from its *in vivo* environment and thus it may be exposed to different pH values and ionic strengths. Graveland *et al.*[99] have suggested that O_2^- plays a role in dough rheology, possibly as a reductant of wheat gluten disulphide bonds during the mixing of the dough. However endogenous SOD may have a negative effect on dough development by scavenging O_2^-.[21] Non-enzymic oxidation is thought to occur throughout beer production and also in the finished product. It has been considered that since barley SOD is not thermostable the incorporation of a heat stable SOD, eg., purified from soya beans may be desirable.[100] A method of partial purification for the production of soya bean SOD has already been published[101] and other potential sources of heat stable SOD include yeast.[102] It should be noted that if SOD is used in beers and other pasteurised beverages to prevent staling, catalase may also be required to remove the H_2O_2 generated. Although SOD has been patented as a food antioxidant[103] the feasibility of using SOD as a natural food antioxidant is not yet known, since we understand so little about the function of the enzyme under the different conditions which exist in food products or during the processing of foods.

TABLE 2
Dose requirement in various applications of food irradiation

Purpose	Dose (kGy)[a]	Products
Low Dose (up to 1 kGy)		
(a) Inhibition of sprouting	0.05–0.15	Potatoes, onions, garlic, ginger-root, etc.
(b) Insection disinfestation and parasite disinfection	0.15–0.50	Cereals and pulses, fresh dried fruits, dried fish and meat, fresh pork, etc.
(c) Delay of physiological process (eg. ripening)	0.50–1.0	Fresh fruits and vegetables
Medium dose (1–10 kGy)		
(a) Extension of shelf life	1.0–3.0	Fresh fish, strawberries, etc.
(b) Elimination of spoilage and pathogenic micro-organisms	1.0–7.0	Fresh and frozen seafood, raw or frozen poultry and meat, etc.
(c) Improving technological properties of food	2.0–7.0	Grapes (increasing juice yield), dehydrated vegetables (reduced cooking time), etc.
High dose (10–50 kGy)[b]		
(a) Industrial sterilisation (in combination with mild heat)	30–50	Meat, poultry, seafood, prepared foods, sterilised hospital diets
(b) Decontamination of certain food additives and ingredients	10–50	Spices, enzyme preparations, natural gum, etc.

[a] Gy: gray – unit used to measure absorbed dose.
[b] Only used for special purposes.
Reproduced, with permission from WHO, from: *Food Irradiation: a technique for preserving and improving the safety of food.* Published by the World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations, Geneva, 1988, Table 1, p. 34.

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Food Irradiation

It is appropriate here to discuss irradiation of foods, firstly because free radicals are generated within the foodstuff and secondly because the process is increasingly receiving recognition and use world wide. Indeed its effectiveness depends on the generation of free radicals. Thus for reasons given earlier the use of irradiation of foods is therefore self-limiting. Food irradiation processes have been used in many countries for more than 35 years. Uses (see Table 2) include the delay of fruit ripening, elimination of pathogens and the product of shelf-stable sterile products and sterile ingredients. An expert committee under the auspices of the World Health Organisation, Food and Agriculture Organisation and International Atomic Energy Authority declared in 1980 that foods irradiated up to a dose of 10 kGy presented no toxicological hazard for human consumption.[104] The dose requirement for various applications of food irradiation are presented in Table 2.

The forms of ionising energy used for food irradiation include accelerated electrons, gamma rays and x-rays. Food irradiation is successful because the major component of foods is water, thus exposure of food to high energy ionising radiation results in the production of $OH^{\cdot}$ radicals which may cause damage to living cells where DNA seems particularly susceptible to disruption, by several processes including base hydroxylation. Thus irradiation is an effective technique for the disinfestation and decontamination of food. Formation of primary radical species from the radiolysis of water occurs via the following series of reactions (in which $H_2O^{\cdot}$ represents a water molecule in an excited state):[105]

$$H_2O \rightarrow H_2O^{*}$$

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

$$H_2O^{*} \longrightarrow {}^{\cdot}OH + H^{\cdot}$$

$$H_2O^{+} + H_2O \longrightarrow H_3O^{+} + {}^{\cdot}OH$$

Hydrated electrons produced during radiolysis

$$e^{-}_{aq} + O_2 \longrightarrow O_2^{-}$$

may reduce dissolved oxygen in water to superoxide, thus permitting production via the Fenton reaction of further $^{\cdot}OH$ radicals in the presence of transition metal ions. These $^{\cdot}OH$ radicals may in turn create organic peroxyl radicals through the reaction of generated organic free radicals with molecular oxygen.

At present the ability of $^{\cdot}OH$ radicals to initiate lipid oxidation chain reactions prevents the successful irradiation of foods with a high fat content, because of the undesirable flavours produced. This may be overcome in future by the inclusion of radical scavenging antioxidants such as SOD, although it should be remembered that the successful use of irradiation as a pasteurisation and sterilisation technique depends upon the disruption of DNA by oxyradicals. SOD has been found to exert a protective effect against irradiation in viruses, suspensions of bacteria, mammalian cell cultures and whole mice.[106] Chakraborti and Chatterjee[107] have demonstrated that irradiation of living plants resulted in an increase in the concentrations of the antioxidant enzymes SOD, catalase and peroxidase. Gee *et al.*[108] reported that whilst an irradiation dose of 5 kGy was found to cause extensive

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inhibition of alcohol dehydrogenase, the inhibition was enhanced by oxygen and lessened by SOD. Although SOD could be added to preserve the nutritional value of irradiated food products, it may provide protection for the pathogenic organisms.[109]

Irradiation of horseradish peroxidase using a ^{60}Co source was found to cause a loss of enzymic activity which was highest in the presence of oxygen saturated water, suggesting that hydroxyl and peroxyl radicals were responsible for the loss of activity.[110] The inactivation was probably the result of secondary attack on peroxidase by free radicals produced from water during the irradiation process. The accumulation of phenolic components in irradiated citrus fruits was considered to be due to enhanced activity of the enzyme phenylalanine ammonia lyase.[111] Increases in peroxidatic activity a few weeks after irradiation of citrus fruits have also been reported.[112] The major objective of irradiation of climacteric fruits is extension of post harvest storage life by slowing ripening and subsequent senescence through inhibition of biosynthesis of the key enzymes involved.

For low doses of irradiation (< 1 kGy) any loss of nutrients may be considered to be of little consequence. In the range 1–10 kGy, some vitamin losses may occur. As with other preservation techniques, in food irradiation there is a trade-off between the desirable aspects, eg. pasteurisation, control of pests, delay of sprouting in vegetables, ripening of fruit, and the extension of shelf life, with the potential loss of micro nutrients, especially vitamins. Whilst riboflavin, niacin and vitamin D concentrations may be largely unaffected by irradiation at low doses, vitamins A, B, C, E and K may be more sensitive.[104] The lack of precise quantitative data for such losses at first sight seems absurd and open to criticism. However to the analyst the acquisition of accurate meaningful data for the loss of small amounts of nutrients from a wide range of highly variable complex food materials derived from different cultivars is more than a considerable challenge. Individual reports can only provide indications of the likely expected losses of trace nutrients in the selected samples.

Detection of Irradiated Foods

Irrespective of the method of food irradiation, it is considered a cold process, and at appropriate levels it does not alter the physical appearance of the food and therefore there is suspicion amongst consumers about control procedures, the possible abuse of excessive irradiation to reduce an unacceptable microbial load and the ability of authorities to enforce labelling legislation. It has proved difficult to devise simple and reliable tests which may be routinely used to exclusively detect irradiated food products, and although no particular method is suitable for all foods, several techniques are being developed for a range of food commodities; the main techniques are electron spin resonance (ESR) spectroscopy, thermoluminescence and the detection of products from irradiated lipids. Reviews of these techniques has been compiled,[113,114] which conclude that there will not be a single test for detecting irradiated foods, but more likely a combination of techniques will be used.

The thermoluminescence (TL) method relies on the distinction between the levels of light emitted when irradiated and non-irradiated food samples are heated from ambient temperature to below combustion. The technique has been considered as suitable for herbs and spices and vegetables where the mineral debris adhering to the food, which is responsible for the signal, may be separated and re-irradiated. Goksu-Ogelman and Regulla[115] found that dust samples emitted a significant TL

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signal only if prepared from irradiated rather than unirradiated spices. The use of chemiluminescence may also be considered as a detection technique for irradiated spices and other foods, although strong chemiluminesence signals are thought to be associated with thermally induced radicals. Thus unknown heat treatment may result in false positives.[114]

The ESR spectroscopy technique relies upon the trapping of free radicals formed in food during the irradiation process. Generally, upon irradiation of food, the free radicals which are generated are short lived, but some radicals are trapped in the hard, dry components of the food where the rigid structure of the matrix inhibits radicals reacting with each other, or with food components in the wet portion of the food. The technique has been applied to foods such as fish, shellfish, meat, fruit and nuts where stable radiation induced free radicals may be detected in the hard matrices (eg., bone, shell, pips, stones etc) by ESR spectroscopy of a suitable sample. At present, the ESR technique is mainly qualitative as a number of factors may influence the intensity of the radiation-induced signal, for example, the method of sample preparation, the irradiation dose, the length and temperature of storage and post-irradiation processing. There is also some evidence to suggest that free radical composition and thus ESR signals may depend on other biological factors such as the age and part of the carcass sampled and be species dependent. Studies using the cuticle of Norway lobster[116] have indicated that whilst the ESR signal intensity increases with irradiation dose, the signal decreases slowly on storage; this factor would need to be considered if a quantitative measurement was required following the irradiation treatment. A collaborative European inter-laboratory trial on the use of ESR for the identification of irradiated food[117] indicated that qualitative results on irradiated beef bones, dried grapes and papaya were encouraging although less conclusive results were obtained for trout bones, sardine scales and pistachio nut shells. All the laboratories involved in the study were able to distinguish between chicken bones irradiated at 1–3 and 7–10 kGy.

The detection of volatile compounds from irradiated lipid-containing foods involves the measurement of appropriate markers such as tetradecene, hexadecadiene and heptadecene since the concentration of these compounds is known to increase with an increase in the irradiation dose applied. Although the dose response relationship may be exploited in the future, at present the technique is only qualitative as factors such as the effect of processing and the stability of long-chain hydrocarbons are not well known. The technique has been applied to high fat foods such as chicken. 2-Alkylcyclobutanones are a series of cyclic compounds formed from their parent fatty acids on irradiation. One such radiolytic compound which acts as a marker for irradiation in chicken meat is 2-dodecylcyclobutanone formed from palmitic acid. This cyclic compound has been detected in meat irradiated at doses < 10kGy,[118,119] but was not detectable in unirradiated food. The quantity produced has been shown to increase linearly with irradiation doses up to 10 kGy in fresh chicken meat[120] and up to 60 kGy in frozen chicken meat.[121]

Another approach to the detection of foods which have been irradiated involves analysis of mitochondrial DNA contained within the food, to detect strand breaks which will indicate that irradiation has been used. This technique may be suitable for the wide range of non-fatty foods lacking the suitable characteristics for the ESR spectroscopy or luminescence measurements. In addition some techniques have been focused on investigating irradiation-generated modified DNA bases. Products such as 8-hydroxyguanine and thymine glycol can act as markers and provide an index of oxidative attack on DNA.[25] Gray and Mower[122] have proposed that in fruit

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such as the papaya which has a high concentration of mono- and disaccharides, the derivatives from reactions between irradiation-generated ·OH radicals and these simple sugars, eg., malonaldehyde, may be of interest as post-irradiation detection markers. The sugars are thought to act as quenching agents, preventing alternative ·OH reactions when papaya juice is exposed to gamma rays. Mower and co-workers[123] suggest that secondary irradiation generated products from the reaction of ·OH with food substances may be minimal in the papaya fruit, although the scavenging effect of this fruit juice has not been observed to the same extent in other fruits.

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References

1. K.F. Gey, P. Paska, P. Jordan and U.K. Moser (1991) Inverse correlation between plasma vitamin E and mortality from ischaemic heart disease in cross-cultural epidemiology. *American Journal of Clinical Nutrition*, **53**, 326S-334S.
2. P.B. Addis and G.J. Warner (1991) The potential health aspects of lipid oxidation products in foods. In *Free Radicals and Food Additives* (Eds. O.I. Aruoma and B. Halliwell), Taylor and Francis, London, 77-119.
3. L.A. Piche, H.H. Draper and P.D. Cote (1988) Malondialdehyde excretion by subjects consuming cod-liver oil vs a concentrate of n-3 fatty acids. *Lipids*, **23**, 370-371.
4. R.L. Willson (1987) Vitamin, selenium, zinc and copper interactions in free radical protection against ill-placed iron. *Proceedings of the Nutrition Society*, **46**, 27-34.
5. B. Halliwell (1984) Superoxide dismutase and the superoxide theory of oxygen toxicity. A critical appraisal. In *Copper Proteins and Copper Enzymes, Vol. II* (Ed. R. Lontie), CRC Press, Boca Raton, Florida, 63-102.
6. R.A. Larson (1988) The antioxidants of higher plants. *Phyto-chemistry*, **27**, 969-978.
7. O.I. Aruoma, B. Halliwell, B.M. Hoey and J. Butler (1989) The antioxidation of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypo chlorous acid. *Free Radicals in Biology and Medicine*, **6**, 593-597.
8. B. Halliwell (1990) How to characterise a biological anti-oxidant. *Free Radical Research Communications*, **9**, 1-32.
9. J.M. McCord (1979) Superoxide dismutases; occurrence, structure, function and evolution. In *Isozymes: Current Topics in Biological and Medical Research*, **3**, 1-21.
10. T.F. Slater, K.M. Cheeseman, M.J. Davies, K. Proudfoot and W. Zin (1987) Free radical mechanisms in relation to tissue injury. *Proceedings of the Nutrition Society*, **46**, 1-12.
11. B. Halliwell and J.M.C. Gutteridge (1990) Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology*, **186**, 1-85.
12. D.A. Rowley and B. Halliwell (1983) Formation of hydroxyl radicals from hydrogen peroxide and iron salts by superoxide-dependent and ascorbate-dependent mechanisms - relevance to the pathology of rheumatoid disease. *Clinical Science*, **64**, 649-653.

12a. N. Hogg, V.M. Darley-Usmar and M.T. Wilson (1992) Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. *Biochemical Journal*, **281**, 419-424.

13. J.M.C. Gutteridge and G.J. Quinlan (19,86) Carminic acid-promoted oxygen radical damage to lipid and carbohydrate. *Food Additives and Contaminants*, **3**, 289-293.
14. E. Cadenas (1989) Biochemistry of oxygen toxicity. *Annual Reviews in Biochemistry*, **58**, 79-110.
15. B. Chance (1952) Effect of pH upon the reaction kinetics of the enzyme-substrate compounds of catalase. *Journal of Biological Chemistry*, **194**, 471-481.
16. B. Halliwell (1977) Generation of hydrogen peroxide, superoxide and hydroxyl radicals during the oxidation of dihydroxyfumaric acid by peroxidase. *Biochemical Journal*, **163**, 441-448.
17. D.S. Robinson (1991) Peroxidases and catalases in foods. In: *Oxidative Enzymes in Foods* (Eds. D.S. Robinson and N.A.M. Eskin), Elsevier Applied Science, London, 1-47.
18. H.W. Gardner (1991) Recent investigations into the lipoxygenase pathways of plants. *Biochimica Biophysica Acta*, **1084**, 221-239.
19. J.R. Whitaker (1991) Lipoxygenases. In: *Oxidative Enzymes in Foods* (eds. D.S. Robinson and N.A.M. Eskin), Elsevier Applied Science, London, 175-215.
20. A. Graveland, P. Bosveld, W.J. Lichtendonk and J.H.E. Moonen (1984) Isolation and characterisation of (3-methoxy-4-hydroxyphenyl)-B-cellotrioside from wheat flour; a substance involved in the reduction of disulphide-linked glutenin aggregates. *Journal of Cereal Science*, **2**, 65-72.

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21. D.S. Robinson, S.M. Lawlor, P.J. Frazier, N.W.R. Daniels and J.K. Donnelly (1994) Wheat superoxide dismutase isoenzymes. (in preparation).
22. H.W.-S. Chan (1987) The mechanism of autoxidation. In *Autoxidation of unsaturated lipids* (Ed. H.W.-S. Chan), Academic Press, London, 1-16.
23. H. Kuhn, R. Weisner, J. Rathmann and T. Schewe (1991) Formation of ketodienoic fatty acids by pure pea lipoxygenase -1. *Eicosanoids*, **4**, 9-14.
24. J.A. Buege and S.D. Aust (1978) Microsomal lipid peroxidation. *Methods in Enzymology*, **52**, 302-310.
25. O.I. Aruoma (1991) Pro-oxidant properties: An important consideration for food additives and/or nutrient components. In *Free Radicals and Free Additives* (Eds. O.I. Aruoma and B. Halliwell), Taylor and Francis, London, 173-192.
26. W. Grosch, G. Laskawy and F. Weber (1976) Formation of volatile carbonyl-compounds and cooxidation of β-carotene by lipoxygenase from wheat, potato, flax and beans. *Journal of Agricultural and Food Chemistry*, **24**, 456-459.
27. F. Weber, G. Laskawy and W. Grosch (1974) Cooxidation of carotene and crocin by the soybean lipoxygenase isoenzymes. *z. Lebensmittel-unter-Forsch*, **155**, 142-150.
28. L.C. Sanz, A.G. Perez and J.M. Olias (1992) Purification and catalytic properties of chickpea lipoxygenases. *Phytochemistry*, **31**, 2967-2972.
29. C. Pourplanche, V. Larreta Garde and D. Thomas (1993) β-Carotene cooxidation by soybean lipoxygenase type 1 in non-conventional media. *Sciences de Aliments*, **13**, 275-283.
30. M. Cabibel and J. Nicolas (1991) Partial purification, some properties and *in vitro* cooxidation of some carotenoid pigments. *Sciences de Aliments*, **11**, 277-290.
31. B.P. Klein, D. King and S. Grossman (1985) Cooxidation reactions of lipoxygenase in plant systems. *Advances in Free Radical Biology and Medicine*, **1**, 309-343.
32. P.B. Addis (1986) Occurrence of lipid oxidation products in foods. *Food Chemistry and Toxicology*, **24**, 1021-1030.
33. J. Loliger (1991) The use of antioxidants in foods. In *Free Radicals and Food Additives* (Eds. O.I. Aruoma and B. Halliwell), Taylor and Francis, London, 121-150.
34. S.D. Aust (1985) Lipid peroxidation. In: *Handbook of Methods for Oxygen Research* (Ed. R.A. Greenwald), CRC Press, Boca Raton, Florida, 203-297.
35. C.H. Lea (1952) Methods for determining peroxide lipids. *Journal of the Science of Food and Agriculture*, **3**, 586-594.
36. J.P. Cosgrove, D.F. Church and W.A. Pryor (1987) The kinetics of autoxidation of polyunsaturated fatty acids. *Lipids*, **22**, 299-304.
37. R. Croteau (1978) Biosynthesis of flavor components: Volatile carbonyl compounds and monoterpenoids. In *Post-harvest Biology and Biotechnology* (Eds. H.O. Hultin and M. Milner), Food and -Nutrition Press, Westport, Conn., 400-432.
38. H.W.-S. Chan (1987). Oxygen free radicals in food. *Proceedings of the Nutrition Society*, **46**, 35-41.
39. G. Minotti and S.D. Aust (1987) Superoxide-dependent redox cycling of citrate-Fe^{3+}: evidence for a superoxide dismutase-like activity. *Archives of Biochemistry and Biophysics*, **253**, 257-267.
40. K.M. Schaich (1992) Metals and lipid oxidation. Contemporary issues. *Lipids*, **27**, 209-218.
41. G. Scott (1993) Initiators, prooxidants and sensitisers. In *Atmospheric oxidation and antioxidant*, Vol. 1. (Ed. G. Scott), Elsevier, Netherlands, 83-119.
42. H.W.-S. Chan and D.T. Coxon (1987). Lipid hydroperoxides. In *Autoxidation of unsaturated lipids* (Ed. H.W.-S. Chan), Academic Press, London, 17-50.
43. C.S. Foote and R.W. Denny (1968) Chemistry of singlet oxygen-quenching by beta-carotene. *Journal of the American Chemical Society*, **90**, 6233.
44. M. Korycka-Dahl and T. Richardson (1980) Initiation of oxidative changes in foods. *Journal of Dairy Science*, **63**, 1181-1198.
45. E. Cadenas and H. Sies (1984) Low-level chemiluminescence as an indicator of singlet molecular oxygen in biological systems. *Methods in Enzymology*, **105**, 221-231.
46. H.W. Gardner (1987) Reactions of hydroperoxide - products of high molecular weight. In *Autoxidation of unsaturated lipids* (Ed. H.W.-S. Chan), Academic Press, London, 51-93.
47. A.H. Ding and P.C. Chan (1984) Singlet oxygen in copper-catalysed lipid peroxidation in erythrocyte membranes. *Lipids*, **19**, 278-284.
47a. W.A. Pryor and C.K. Govindan (1981) Decomposition of triphenyl phosphite Ozonide in the presence of spin traps. *Journal of Organic Chemistry*, **46**, 4679-4682.
48. B.H. Bielski, R.L. Arudi and M.W. Sutherland (1983) A study of the reactivity of $HO_2^{\cdot}/O_2^{-}$ with unsaturated fatty acids. *Journal of Biological Chemistry*, **284**, 4759-4761.
49. G.S. Wu, R.A. Stein and J.F. Mead (1978) Autoxidation of fatty acid monolayers absorbed on

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silica-gel. 3. Effects of saturated fatty acids and cholesterol. *Lipids*, **13**, 517-524.
50. B. Halliwell (1991) The biological toxicity of free radicals and other reactive oxygen species. In *Free Radicals and Food Additives*, (Eds. O.I. Aruoma and B. Halliwell), Taylor and Francis, London, 37-57.
51. N.A. Porter, L.S. Lehman, B.A. Weber and K.J. Smith (1981) Unified mechanism for polyunsaturated fatty acid autoxidation. Competition of peroxy radical hydrogen atom abstraction, β-scission and cyclization. *Journal of the American Oil Chemists' Society*, **103**, 6447-6455.
52. H.W. Gardner (1987) Reactions of hydroperoxides - products of high molecular weight. In *Biomimetic and Bloorganic Chemistry II (Topics in Current Chemistry 132)*, (Eds. F. Vogtle and E. Weber), Springer, Berlin, 1-61.
53. T. Galliard (1975) Degradation of plant lipids by hydrolytic and oxidative enzymes. In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (Eds. T. Galliard and E.I. Mercer), Academic Press, London/New York, 319-357.
54. R.C. Fahey (1988) Proteation of DNA by thiols. *Pharmacology and Therapeutics*, **39**, 101-108.
55. J. Nourooz-Hadez (1990) Determination of the autoxidation products from free or total cholesterol: a new multistep enrichment methodology including enzymatic release of esterified cholesterol. *Journal of Agricultural and Food Chemistry*, **38**, 1667.
56. G. Maerker and K.C. Jones (1992) Gamma-irradiation of individual cholesterol oxidation-products. *Journal of the American Oil Chemists' Society*, **69**, 451-455.
57. V.K. Lebovics, O. Gaal, L. Somogyi and J. Farkas (1992) Cholesterol oxides in gamma-irradiated spray-dried egg powder. *Journal of the Science of Food and Agriculture*, **60**, 251-254.
58. C.R. Prasad and R. Subramanian (1992) Qualitative and comparative-studies of cholesterol oxides in commercial and home-made Indian ghees. *Food Chemistry*, **45**, 71-73.
59. G. Maerker (1987) Cholesterol autoxidation. Current status. *Journal of the Americal Oil Chemists' Society*, **64**, 388-392.
60. P.B. Addis and S.W. Park (1989) In *Food Toxicology. A perspective on the relative risks* (Eds. S.L. Taylor and R.A. Scanlan), Marcel Dekker, New York, 297-330.
61. J.N. Morgan and D.J. Armstrong (1992) Quantification of cholesterol oxidation-products in egg yolk powder spray-dried with direct heating. *Journal of Food Science*, **57**(1), 43.
62. M. Mahadeo, N. Unklesbay, A.D. Clarke, K. Unklesbay and M. Ellersieck (1992) Moist and dry convective heating and chilled storage of restructured beef roasts - effect on cholesterol and cholesterol oxide content. *Journal of Food Science and Technology - Lebensmittel-Wissenschaft and Tech nologie*, **25**, 412-416.
63. N. Kumar and O.P. Singhal (1992) Effect of processing conditions on the oxidation of cholesterol in ghee. *Journal of the Science of Food and Agriculture*, **58**, 267-273.
64. D.S. Robinson (1987) *Food: Biochemistry and Nutritional Value*, Longman Scientific and Technical, Harlow.
65. J. Nourooz-Hadeh and L.A. Appelqvist (1992) Isolation of quantitative-determination of sterol oxides in plant-based foods - soybean oil and wheat flour. *Journal of the American Oil Chemists Society*, **69**, 288-293.
66. B.A. Wasilchuk, P.W. Lequesne and P. Vouros (1992) Monitoring cholesterol autoxidation processes using multi-deuteriated cholesterol. *Journal of Analytical Chemistry*, **64**, 1077-1087.
67. A. Fontana, F. Antoniazzi, G. Cimino, G. Mazza, E. Trivellone and B. Zanone (1992) High-resolution NMR detection of cholesterol oxides in spray-dried egg yolk. *Journal of Food Science*, **57**, 869-879.
68. B. Halliwell and J.M.C. Gutteridge (1989) *Free Radicals in Biology and Medicine*, 2nd edition, Clarendon Press, Oxford.
69. D.J. Nice and D.S. Robinson (1993) Co-purification of a heat-stable antioxidant with the superoxide dismutase activity from dried peas. *Food Chemistry*, **48**, 353-357.
70. J. Loliger (1989) Natural antioxidants. In *Rancidity in foods*, 2nd edition (eds. J.C. Allen and R.J. Hamilton), Elsevier Applied Science, London, 105-124.
71. G.W. Burton (1989) Antioxidant action of carotenoids. *Journal of Nutrition*, **119**, 109-111.
72. P.F. Conn, C. Lambert, E.J. Land, W. Schalch and T.G. Truscott (1992) Carotene-oxygen radical interactions. *Free Radical Research Communications*, **16**, 401-408.
73. G.W. Burton and K.U. Ingold (1981) Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants *in vitro*. *Journal of the American Chemical Society*, **103**, 6472-6477.
74. H. Chimi, J. Cillard, P. Cillard and M. Rahmani (1991) Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. *Journal of the American Oil Chemists Society*, **68**, 307-312.
75. S.F. Husain, J. Cillard and P. Cillard (1987) Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry*, **26**, 2489-2491.

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76. J. Baumann, G. Wurm and F.V. Bruchhausen (1980) Prostaglandin synthase inhibition by flavonoids and phenolic compounds in relation to their superoxide scavenging properties. *Archives Pharmacy*, **313**, 330–337.
77. Y. Sorata, U. Takahama and M. Kimura (1984) Protective effect of quercetin and rutin on photosensitised lysis of human erythrocytes in the presence of hematoporphyrin. *Biochimica Biophysica Acta*, **799**, 313–317.
78. M.J. Laughton, B. Halliwell, P.J. Evans and J.B.S. Hoult (1989) Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myricetin. Effects on lipid peroxidation, hydroxyl radical generation and bleomycin-dependent damage to DNA. *Biochemical Pharmacology*, **38**, 2859–2865.
79. A.K. Srivastava and G. Padmanaban (1987) Gossypol-mediated DNA degradation. *Biochemical and Biophysical Research Communications*, **146**, 1515–1522.
80. J. Torel, J. Cillard and P. Cillard (1986) Antioxidant activity of flavonoids and reactivity with peroxyl radical. *Phytochemistry*, **25**, 383–385.
81. U. Takahama (1985) Inhibition of lipoxygenase dependent lipid peroxidation by quercetin: mechanism of antioxidative function. *Phytochemistry*, **24**, 1443–1446.
82. M. Iio, Y. Matsumoto, N. Takaki and M. Fukumoto (1985) Inhibition of xanthine oxidase by flavonoids. *Agricultural and Biological Chemistry*, **49**, 2173–2176.
83. U. Takahama, R.J. Youngman and E.F. Elstner (1984) Transformation of quercetin by singlet oxygen generated by photo-sensitised reaction. *Photobiochemistry and Photobiophysics*, **7**, 175–181.
84. P.A. Hammerschmidt and D.E. Pratt (1978) Phenolic antioxidants of dried soybeans. *Journal of Food Science*, **43**, 556–559.
85. C.E. Eriksson (1982) Lipid oxidation catalysts and inhibitors in raw materials and processed foods. *Food Chemistry*, **9**, 3–19.
86. G. Bertelsen, A. Ohlen and L.H. Skibsted (1991) Pea fibre as a source of natural antioxidants in frozen minced beef. Lipid oxidation and colour stability during retail display. *z. Lebensmittel-Unterschang Forsuch*, **192**, 319–322.
87. B.G. Swanson (1990) Pea and lentil extraction and functionality. *Journal of the American Oil Chemists' Society*, **67**, 276–280.
88. M.J. Taylor and T. Richardson (1980) Antioxidant activity of skim milk: effect of heat and resultant sulfhydryl groups. *Journal of Dairy Science*, **63**, 11783–11795.
89. D.E. Pratt (1972) Water-soluble antioxidant activity in soybeans. *Journal of Food Science*, **37**, 322–323.
90. S.J. Bishop and A.S. Henick (1972) Antioxidant effect of protein hydrolyzates in a freeze-dried model system. *Journal of Food Science*, **37**, 873–875.
91. R. Gershman, D.L. Gilbert, S.W. Nye, P. Dwyer and W.O. Fenn (1954) Oxygen poisoning and x-irradiation: A mechanism in common. *Science*, **119**, 623–626.
92. J.M. McCord and I. Fridovich (1969). Superoxide dismutase. An enzymatic function of erythrocuprein (haemocuprien). *Journal of Biological Chemistry*, **244**, 6049–6055.
93. W. Bors (1989) An expanded function for superoxide dismutase. In *Programme of Abstracts for Fifth Conference on Superoxide Dismutase*, Department of Physical Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel, 45.
94. W. Bors, G. Czapski and M. Saran (1991) An expanded function for superoxide dismutase. *Free Radical Research Communications*, **12–13**, 411–417.
95. J.K. Donnelly, K.M. McLellan, J.L. Walker and D.S. Robinson (1989) Superoxide dismutases in foods. A Review. *Food Chemistry*, **33**, 243–270.
96. Y.Y. Lesham, A.H. Halevy and C. Frenkel (1986) Processes and control of plant senescence. *Developments in Crop Science*, **8**, Elsevier, Amsterdam, 162–199.
97. J.A. Olsen and C.R. Cook (1987) Superoxide dismutase in wounded etiolated pea seedlings. *Phytochemistry*, **26**, 71–73.
98. H.D. Rabinowitch, D. Sklan and P. Budowski (1982) Photo-oxidative damage in the ripening tomato fruit; protective role of superoxide dismutase. *Physiologia Plantarum*, **54**, 369–374.
99. A. Graveland, P. Bosveld, W.J. Lichtendonk and J.H.E. Moonen (1985) In *Proceedings of the 2nd International Workshop on Gluten Proteins* (Eds. A. Graveland and J.H.E. Moonen), TNO, The Netherlands, pp. 39.
100. C.W. Bamforth (1983) Superoxide dismutase in barley. *Journal of the Institute of Brewing*, **89**, 420–423.
101. S.P. Clarkson, P.J. Large and D.W. Bamforth (1989) Purification of a cyanide-sensitive superoxide dismutase from soya beans: A food-compatible enzyme preparation. *Journal of the Science of Food and Agriculture*, **38**, 87–97.
102. H. Lingnert, G. Akesson and C.E. Eriksson (1989) Antioxidative effect of superoxide dismutase

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from *Saccharomyces cerevisiae* in model systems. *Journal of Agricultural and Food Chemistry*, **37**, 23-28.
103. A.M. Michelson and J. Monod (1975) Superoxide dismutases and their applications as oxidation inhibitors. *US Pat. No. 3 920 521* November, 18.
104. WHO/FAO (1988) *Food Irradiation. A technique for preserving and improving the safety of food.* World Health Organisation, Geneva.
105. B.A. Goodman, D.B. McPhail and D.M.L. Duthie (1989) Resonance Spectroscopy of some irradiated foodstuffs. *Journal of the Science of Food and Agriculture*, **47**, 101-111.
106. I. Fridovich (1978) The biology of oxygen radicals. *Science*, **201**, 875-880.
107. M. Chakraborti and G.C. Chatterjee (1983). Effect of gamma-radiation of the activities of superoxide dismutase, catalase and peroxidase on the germinating wheat grain (*Triticum aestivum*). *Life Science Advances*, **2**, 11-14.
108. C.A. Gee, K.J. Kittridge and R.L. Willson (1985) Peroxy free radicals, enzymes and radiation damage: Sensitisation by oxygen and protection by superoxide dismutase and antioxidants. *British Journal of Radiology*, **58**, 251-156.
109. S. Schwimmer (1981) Other roles of enzyme action on phenols as determinants of food colour quality. *Source Book of Enzymology*, AVI Publishing Co Inc., Westport, Conn. 284-297.
110. D. Metodiewa, L. Gebicka and S. Bachman (1987) The radiation-induced inactivation and post-irradiation effects of horseradish peroxidase solution. *Journal of Radio-analytical and Nuclear Chemistry Letters*, **119**, 87-93.
111. J. Riov, S.P. Monselise and R. Goren (1972) Stimulation of phenolic biosynthesis in citrus fruit peel by gamma-radiation. *Radiation Research Review*, **3**, 417-427.
112. S.P. Monselise, J. Riov and R.S. Kahan (1969) Some changes in enzymatic activities of citrus peel tissues after fruit irradiation. In *Enzymological aspects of food irradiation*, International Atomic Energy Agency, Vienna, 71-81.
113. M.H. Stevenson (1992) Progress in the identification of irradiated foods. *Trends in Food Science and Technology*, **3**, 257-262.
114. S.M. Glidewell, N. Deighton, B.A. Goodman and J.R. Hillman (1983) Detection of irradiated food: A review. *Journal of the Science of Food and Agriculture*, **61**, 281-300.
115. H.Y. Goksu-Ogelman and D.F. Regulla (1989) Detection of irradiated food. (Letter) *Nature*, **340**, 23.
116. E.M. Stewart, M.H. Stevenson and R. Gray (1993) The effect of irradiation dose and storage time on the ESR signal in the cuticle of different components of the oxoskeleton of Norway lobster (*Nephrops norvegious*). *Applied Radiation and Isotopes*, **44**, 433-437.
117. J. Raffi, M.H. Stevenson, M. Kent, J.M. Thiery and J.-J. Belliardo (1992) European intercomparison on electron spin resonance identification of irradiated foodstuffs. *International Journal of Food Science and Technology*, **27**, 111-124.
118. M.H. Stevenson, A.V.J. Crone and J.T.G. Hamilton (1990) Irradiation detection. (Letter) *Nature*, **344**, 203-204.
119. D.R. Boyd, A.V.J. Crone, J.T.G. Hamilton, M.V. Hand, M.H. Stevenson and P.J. Stevenson (1991) Synthesis, characterisation and potential use of 2-dodecyclcyclobutanone as a marker for irradiated chicken. *Journal of Agricultural and Food Chemistry*, **39**, 789-792.
120. A.V.J. Crone, J.T.G. Hamilton and M.H. Stevenson (1992a) Effect of storage and cooking on the close response of 2-dodecyl-cyclobutanone, a potential marker for irradiated chicken. *Journal of the Science of Food and Agriculture*, **58**, 249-252.
121. A.V.J. Crone, J.T.G. Hamilton and M.H. Stevenson (1992b) Detection of 2-dodecylcyclobutanone in radiation-sterilised chicken meat stored for several years. *International Journal of Food Science and Technology*, **27**, 691-696.
122. J. Gray and H.F. Mower (1991) The role of simple carbohydrates in the suppression of hydroxyl free radicals in gamma-irradiated papaya juice. *Food Chemistry*, **41**, 293-301.
123. E.J. Webman, G. Edlin and H.F. Mower (1989) Free radical scavanging activity of papaya juice. *International Journal of Radiation Biology*, **55**, 347-351.

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