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INVITED REVIEW
RECENT ADVANCES IN THE DEVELOPMENT OF A DIAGNOSTIC TEST FOR IRRADIATED FOODSTUFFS

MARTIN GROOTVELD* and REETU JAIN

Department of Applied Chemistry and Life Sciences, Polytechnic of North London, Holloway Road, London N7 8DB, UK

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Recent advances in the development and application of diagnostic tests for irradiated foodstuffs are reviewed. Exposure of water, the major chemical constituent of most foodstuffs to a source of ionising radiation initially generates the highly reactive radical species $H^{\cdot}$, $^{\cdot}OH$ and $e^{-}(aq)$ which react very rapidly with a wide variety of biological molecules. The detection of foodstuffs subjected to irradiation processing requires the identification and/or quantification of 'unnatural' chemical species (i.e. those not usually formed by normal metabolic processes) produced by the attack of $^{\cdot}OH$ radical or $e^{-}(aq)$ on suitable 'target' molecules. Modern methods for the analysis of a series of these 'unnatural' products arising from the interaction of radiolytically-generated $^{\cdot}OH$ radical or $e^{-}(aq)$ with polyunsaturated fatty acids, DNA, aromatic compounds and other biologically important scavenger molecules are examined. It is concluded that the analytical test to be conducted is highly dependent on the nature of the foodstuff to be tested.

KEY WORDS: Food irradiation, hydroxyl radical, diene conjugation, lipid peroxidation, aromatic hydroxylation

INTRODUCTION

The irradiation of foodstuffs has long been employed as a process to destroy harmful bacteria and to increase the shelf-life of perishable commodities in over 30 countries worldwide. However, the process has recently aroused considerable concern for both medical and dietary reasons, a consequence of the apparent loss of essential nutrients (e.g. Vitamins C and E) that occurs during treatment with X-rays or γ-rays. Moreover, other undesirable chemical modifications of foodstuffs such as lipid peroxidation have also been implicated. Hence, for these reasons and to enforce legislation and allow consumer choice, there is an urgent requirement for a diagnostic analytical technique which is able to discriminate between irradiated and non-irradiated foodstuffs.

Since the irradiation of foodstuffs utilises relatively low levels of ionising radiation (up to 10 kGy), both the qualitative and quantitative chemical changes that occur are usually very small. Hence, the first problem for those involved in the development of a diagnostic test for the irradiation of foodstuffs is the requirement of a highly sensitive analytical technique which permits the quantification of very low concentrations of individual species. Fortunately, recent developments in chemical methods of analysis have led to the ability to measure concentrations of parts per billion and below.

With these considerations in mind, the task for the analytical chemist is one of deciding which particular chemical changes resulting from food irradiation are most

* Author to whom correspondence should be addressed.

likely to lead to an acceptable method of analysis. This problem is further complicated by cost restraints and by the fact that the analytical test to be conducted is likely to be highly dependent upon the nature of the foodstuff to be tested.

It should also be noted that the various tests for food irradiation that are currently being developed can be classified as being either quantitative or qualitative. A quantitative test is one in which a decrease or increase in the concentration of a chemical species present in a particular type of foodstuff is monitored and is only applicable if the difference between irradiated and non-irradiated samples observed is large enough to be declared highly statistically-significant. However, due to the existing wide variation in the chemical content of any type of foodstuff, it is clear that a quantitative diagnostic test will not have the advantages of a qualitative one. A qualitative diagnostic test is one that involves the identification of a new chemical species that is produced solely by the irradiation process and hence is a more desirable type of test, not only for its ability to give a 'yes or no' answer but also for any adverse toxicological properties that the new species monitored may possess.

PRINCIPLES

Exposure of water, the major chemical constituent of most foodstuffs, to a source of ionising radiation produces ionisation and electronic excitation within 10^{-16} second (equation (1))

$$2H_2O \rightarrow H_2O^+ + H_2O^* + e^- \quad (1)$$

where e^- represents an electron and H_2O^* a water molecule with excited electrons. The species H_2O^* undergoes homolytic fission in $10^{-14} - 10^{-13}$ second to yield hydrogen atoms and hydroxyl radicals (equation (2))

$$H_2O^* \rightarrow H^{\cdot} + {}^{\cdot}OH \quad (2)$$

Hydroxyl radicals are also produced from reaction of H_2O^+ within the same timescale (equation (3)).

$$H_2O^+ + H_2O \rightarrow H_3O^+ + {}^{\cdot}OH \quad (3)$$

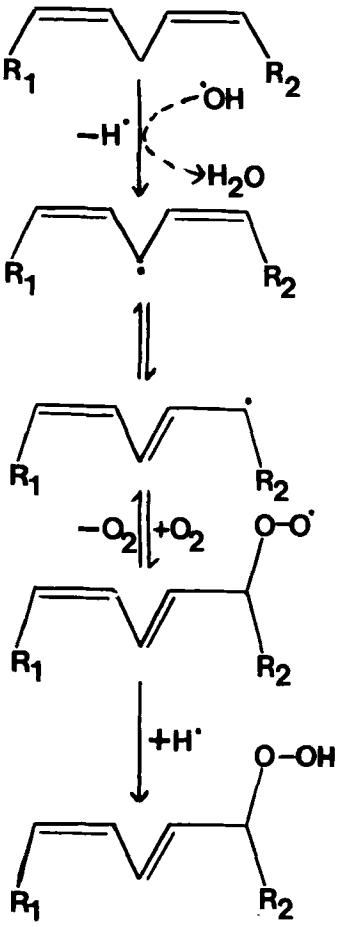
Within $10^{-12} - 10^{-11}$ second, the electrons become hydrated. Thus, the radical species that are initially generated when water is irradiated are $H^{\cdot}$, ${}^{\cdot}OH$ and e^-(aq.).[1-3] However, these react very rapidly with chemical constituents of foodstuffs (at diffusion-controlled rates) so that their half-lives are less than 10^{-9} second. Approximately 5×10^{-3} moles of these radicals are produced per kg of food when exposed to the maximum permitted dose of 10 kGy.

Hydrated electrons are very powerful reducing agents which initially react by addition to a large number of organic constituents of foodstuffs such as aromatic and carbonyl compounds which are located close to their site of formation. Similarly, the highly reactive hydroxyl radical (${}^{\cdot}OH$) will react at or close to its site of formation so that the nature (and extent) of chemical changes occurring upon irradiation of foodstuffs will largely depend on what its site of formation was, e.g. production of ${}^{\cdot}OH$ radical near to DNA could result in base modification or strand breakage. The reaction of ${}^{\cdot}OH$ with biological molecules present in foodstuffs usually produces secondary radicals of lower reactivity which are able to diffuse away from their site of formation to attack other molecules. Probably the best example of secondary

radical production is the interaction of 'OH with polyunsaturated fatty acids (PUFA's) to stimulate the process of lipid peroxidation.

LIPID PEROXIDATION

One approach to the development of a diagnostic test for food irradiation is the identification of intermediates in, and end-products of, the process of lipid peroxidation. Polyunsaturated fatty acids are particularly common constituents of many of todays foodstuffs, and their oxidative degradation leading to a rancid 'off' flavour has been extensively studied for many years.[4,5] They are particularly sensitive to oxidative damage due to the ready abstraction of an allylic hydrogen atom from their methylene ($-CH_2-$) carbon atoms by radical species of sufficient reactivity (the process is facilitated by the low bond dissociation energy of the methylene C–H bonds). Radiolytically-produced 'OH radical readily initiates the oxidative degradation of edible fats



Fatty acid with 2 double bonds (e.g. linoleic acid)

Conjugated diene

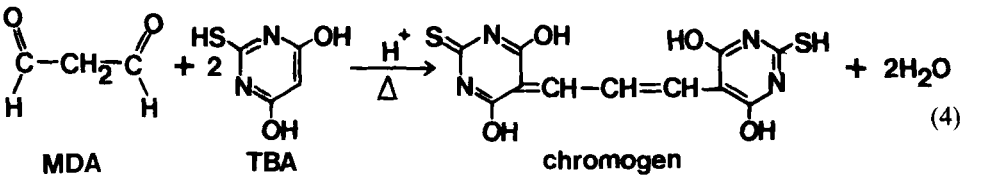
Peroxy radical which abstracts H· from another fatty acid in propagation step

conjugated lipid hydroperoxide

FIGURE 1 Autocatalytic chain reaction of lipid peroxidation.

and oils by abstracting an allylic hydrogen atom, with which it combines to form water (Figure 1). The carbon-centred radical so produced generates a conjugated diene species which reacts with dioxygen to produce a diene peroxy radical (ROO˙), which in turn abstracts a hydrogen atom from an adjacent polyunsaturated fatty acid to form a lipid hydroperoxide and cause an autocatalytic chain reaction. Although lipid hydroperoxides are relatively stable species at ambient temperature, their degradation to a wide variety of further so-called lipid peroxidation 'end-products' is catalysed by traces of redox active transition metal complexes, notably those of iron and copper.[6] These secondary and tertiary end-products consist of saturated and unsaturated aldehydes, di- and expoxyaldehydes, lactones, furans, ketones, oxo and hydroxy acids, and saturated and unsaturated hydrocarbons. The aldehydes produced are largely responsible for the rancid 'off' flavour of edible fats and oils. Identification and/or quantification of one or more of these end-products resulting from the ˙OH radical-initiated peroxidation process can be exploited to produce a suitable test (qualitative or quantitative) for food irradiation. However, in order to do this properly it must be stressed that it is absolutely essential to ensure that the end-products monitored do not readily arise in non-irradiated foodstuffs. Clearly, this is a very difficult task since factors such as the availability of catalytic trace metal ions and oxygen, temperature and exposure to light are all capable of promoting the oxidative degradation process.

The thiobarbituric acid (TBA) test has been frequently used for many years as an indicator of the peroxidation of edible fats and oils in various foodstuffs. This test involves the reaction of aldehydes in the sample with TBA under acidic conditions (equation (4)) to produce a pink-coloured chromogen which absorbs light strongly at a wavelength of 532 nm, enabling a simple spectrophotometric measurement of oxidative rancidity.[7]



(4)

Although malondialdehyde (MDA), an end-product of lipid peroxidation is thought to be the major contributor to the chromogen, it has now become clear that a number of other species such as sucrose, urea, proteins and other aldehydes also react with TBA to produce chromogens that absorb at a wavelength near to 532 nm.[8] Moreover, it should also be noted that since only a small amount (1–2%) of the lipid peroxidation end-products is actually MDA,[9,10] a large proportion of the chromogen produced in the TBA test is due to the further degradation of lipid peroxides when heated with acid in the presence of trace amounts of catalytic transition metal ions. Hence, due to its inherent lack of specificity and also the fact that a wide variety of non-irradiated food products give positive results even in the early stages of oxidative rancidity, the TBA test is of little or no practical use in determining whether or not a foodstuff has been irradiated.

One method which has yielded very promising results is the detection of conjugated dienes and diene hydroperoxides in extracts of irradiated foodstuffs. These species and the conjugated ketodienes arising from their degradation exhibit absorbance in the

ultraviolet region of the electromagnetic spectrum at various wavelengths in the range 230–265 nm.[11] However, although lipids are readily extracted from foodstuffs by a simple chloroform extraction process, direct measurement of absorbance in this wavelength region is of no fundamental use because the absorption maxima of the conjugated diene species appear as a poorly-defined shoulder superimposed on the high absorbance of other food sample components present in the lipid/chloroform extracts. One way in which the conjugated diene absorption maxima can be clearly identified is the observation of minima in the corresponding second-derivative spectra of the extracts (an absorption maximum in the conventional sense appears as an absorption minimum in the second-derivative spectrum).[12]

The second-derivative absorption spectrum of a cyclohexane solution of a commercial sample of corn oil which has been irradiated at a dose of 5.0 kGy exhibits clear differences to that of a corresponding non-irradiated sample (Figure 2(a)). The conjugated diene absorption maxima (appearing as minima in second-derivative spectra) located at 232 and 242 nm which are present only in the irradiated sample are conceivably attributable to *trans, trans* (*t, t*) – and *cis. trans* (*c, t*) – conjugated diene hydroperoxides respectively.[12] Hence, measurement of the minima centred at 232 and 242 nm in chloroform extracts of foodstuffs containing edible fats and oils may be characteristic of irradiation. In order to ensure that these minima observed in the second-derivative absorption spectra of irradiated samples are not also detectable in non-irradiated samples undergoing the process of lipid peroxidation that has been initiated by factors other than irradiation (artefactual peroxidation), second derivative absorption spectra of corn oil samples which were subjected to oxidation by air at 30°C for 20 hours were also obtained and compared with those of irradiated samples. Such a comparison is shown in Figure 2(b). Despite the appearance of several new minima in the spectrum of the heated sample (including one very close to 242 nm), it is clear that the signal present at 232 nm in the irradiated sample is absent, indicating that its detection may be of some use as a diagnostic test for food irradiation. These differences become more pronounced when the corn oil is irradiated at a dose of 10 kGy.

Although it may be argued that doses of γ-irradiation as high as 5 or 10 kGy are at the upper end of the levels of ionising radiation recommended for treating various foodstuffs, additional experiments have indicated that this methodology is also applicable at doses as low as 0.50 kGy. This can be explained by the autocatalytic nature of lipid peroxidation, i.e. only a small quantity of radiolytically-produced $^{\cdot}OH$ radical is required to trigger the process.[13] This potential diagnostic test is now in an advanced stage of development and has the advantages of being relatively simple to perform and cheap to operate (most public analyst laboratories already possess the necessary equipment).

Volatile hydrocarbon gases such as pentane and ethane are well known end-products of the lipid peroxidation process, and their measurement in foodstuffs may be indicative of irradiation. However, it should be pointed out that they are only minor end-products (e.g. about 2.0×10^{-3} mole of pentane produced per mole of lipid hydroperoxide formed) and their production is highly dependent upon the availability of catalytic transition metal ions which promote the degradation of lipid hydroperoxides.[14] Equations (5)–(7) indicate the mechanism for the formation of pentane from the reaction of iron(II) with a hydroperoxide located on the fifth carbon atom from the methyl end of a polyunsaturated fatty acid,

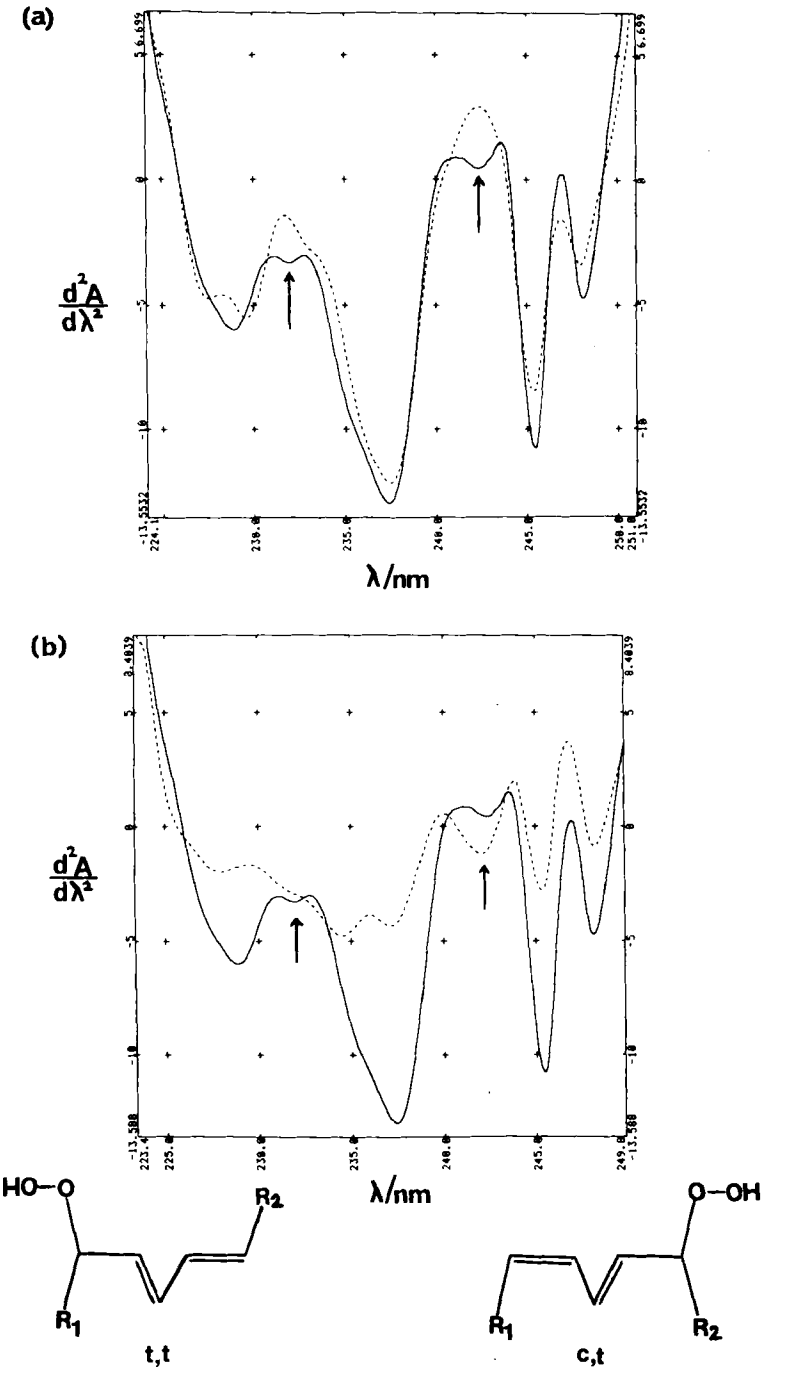
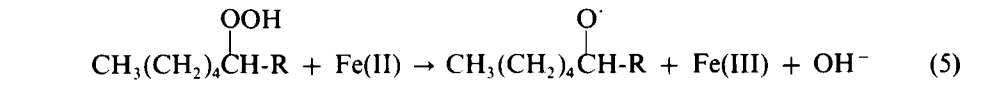
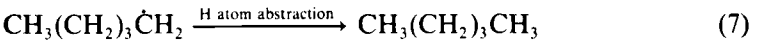


FIGURE 2 (a) Second-derivative absorption spectra of a cyclohexane solution of a commercial sample of corn oil untreated (– – –) and after irradiation at a dose of 5.0 kGy (–). (b) Second-derivative absorption spectra of a cyclohexane solution of a commercial sample of corn oil following oxidation by air at 30°C for 20 hours (– – –) and after irradiation at a dose of 5.0 kGy (–). The arrows indicate the minima located at 232 and 242 nm, attributable to *trans, trans* (*t, t*)- and *cis, trans* (*c, t*)- conjugated diene hydroperoxides respectively.[12]



$$CH_3(CH_2)_4\overset{OOH}{\overset{|}{C}}H\text{-}R + Fe(II) \rightarrow CH_3(CH_2)_4\overset{O^{\cdot}}{\overset{|}{C}}H\text{-}R + Fe(III) + OH^- \quad (5)$$

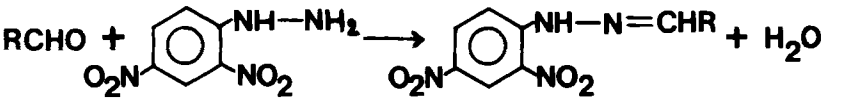
$$CH_3(CH_2)_4\overset{O^{\cdot}}{\overset{|}{C}}H\text{-}R \xrightarrow{\beta\text{-scission}} CH_3(CH_2)_3\dot{C}H_2 + RCHO \quad (6)$$



$$CH_3(CH_2)_3\dot{C}H_2 \xrightarrow{\text{H atom abstraction}} CH_3(CH_2)_3CH_3 \quad (7)$$

e.g. Arachidonic or linoleic acid. Similarly, ethane is formed from linolenic acid. These hydrogen gases are absorbed and concentrated by passage through silica gel at low temperatures. On desorption, they can be easily measured by gas chromatography, a technique which combines the ability to separate complex mixtures with high sensitivity. Despite these advantages, hydrocarbon measurement is at present an unattractive technique because of the necessity for control experiments conducted with non-irradiated foodstuffs undergoing oxidative rancidity. Moreover, the analytical system is further complicated by bacterial hydrocarbon gas production.

An alternative procedure involves the identification and quantification of carbonyl compounds which are also end-products of the lipid peroxidation process (equation (6)). These species include pentanal, hexanal, propan-2-one, hexan-2-one, malondialdehyde (see above), 4-hydroxynonenal and 4,5-dihydroxydecanal. Several of these carbonyl compounds have been shown to be cytotoxic,[13,15–17] and hence their determination is of major toxicological importance. These undesirable toxicological properties are understood to be attributable to their ability to inhibit protein synthesis, inactivate enzymes, cross-link proteins, prevent macrophage action and stimulate thrombin production *in vitro*.[18–23] These compounds can be directly determined by HPLC coupled with U.V. detection (by virtue of their relatively high absorbance in this region of the spectrum) or by gas chromatography following their extraction into a suitable organic solvent. Another methodology which is currently being developed employs their prior conversion to 2,4-dintrophenylhydrazone derivatives by reaction with 2,4-dintrophenylhydrazine in acid solution (equation (8)). The resulting 2,4- dinitrophenylhydrazones are then separated by HPLC and detected with a high level of sensitivity by electrochemical oxidation.[24]



(8)

Volatile hydrocarbons and aldehydes are also produced by the attack of radiolytically-generated aquated electrons on fatty acids (equation (9)).

$$e^-(aq). + R\overset{O}{\overset{\|}{C}}OCH_2\text{-} \rightarrow R\overset{O^-}{\overset{|}{C}}OCH_2\text{-} \rightarrow \text{-}\dot{C}H_2 + RCO_2^- \quad (9)$$

Determination of aldehydes with the same number of carbon atoms as the predominant fatty acid component and alkanes or alkenes with one or two carbon atoms less (by gas chromatography) may also be applicable as a diagnostic test.

It is interesting to note that the interaction of hydrated electrons with oxygen produces the superoxide anion radical (O_2^-) which can itself generate more hydroxyl radical in the presence of hydrogen peroxide (H_2O_2) *via* the transition metal-catalysed Haber-Weiss reaction,[25,26] illustrated for iron in equation (10).

$$O_2^- + H_2O_2 \xrightarrow[\text{catalyst}]{\text{Fe-complex}} O_2 + {}^{\cdot}OH + OH^- \quad (10)$$

Furthermore, it is well known that the iron-dependent breakdown of H_2O_2 also produces the hydroxyl radical according to equation (11), the so-called Fenton reaction,[27] which is also part of the overall Haber-Weiss reaction.

$$Fe^{2+}(aq.) + H_2O_2 \rightarrow Fe^{3+}(aq.) + {}^{\cdot}OH + OH^- \quad (11)$$

Due to a favourable decrease in the redox potential of the Fe(II)/Fe(III) couple and an increase in iron solubility some iron chelators (e.g. EDTA) are able to enhance the formation of ·OH radical in the Fenton reaction.[28] This is a very useful laboratory method for generating ·OH radical in aqueous solution, enabling studies of its damaging effects on certain molecules of biological importance, i.e. it is a (partial) chemical simulation of the irradiation process.

Finally, it is important to emphasize that most foodstuffs containing edible oils and fats also contain permitted antioxidants such as chemically-synthesised α-tocopherol (vitamin E), propyl 3,4,5-trihydroxybenzoate (propyl gallate) and 3,5-di-*tert*-butyl 4-hydroxytoluene (butylated hydroxytoluene) which have the ability to terminate the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals.[13] Hence, the application of all of these potential diagnostic tests for food irradiation will largely depend on the concentration of vitamin E or other synthetic antioxidants present in the foodstuff to be tested. However, conversion of these antioxidants to species with undesirable toxicological properties upon reaction with lipid peroxy or hydroxyl radicals has important medical implications.

DNA DAMAGE

DNA is an important target for attack by ·OH and other (less reactive) radicals, and the identification and quantification of 'unnatural' species produced in this way is currently being investigated as a means of telling whether foodstuffs have been irradiated. DNA is a relatively prevalent constituent available in a number of foodstuffs, for example it is present in calf thymus (2.3%), meat liver (0.2–0.4%) and fish (0.2%). Previous studies of the interaction of radiolytically-generated ·OH radical with DNA have shown that the sugar moiety 2-deoxyribose is degraded to malondialdehyde.[29] Although malondialdehyde can be readily determined spectrophotometrically following a simple colour reaction with thiobarbituric acid in acidic aqueous solution, or directly by high performance liquid chromatography (HPLC), its measurement is of no use since it is already present in many foodstuffs containing edible oils and fats as an end-product of the process of lipid peroxidation (see above). Chemical modification of the purine and pyrimidine base moieties of DNA, for example the hydroxylation of guanine and cytosine by ·OH radical to produce 8-hydroxyguanine and 5-hydroxycytosine respectively, shows much promise. The DNA, which is isolated from foodstuffs by hydroxyapatite chromatography, is initially subjected to digestion to the nucleoside level by treatment with a series of hydrolytic enzymes (DNAase I, endonuclease, phosphodiesterase and alkaline phos-

phatase), and the resulting hydroxylated nucleosides are then readily measured by HPLC coupled with electrochemical detection (ECD).[30] Although this methodology is very tedious, it has the added advantage that the parent nucleosides (e.g. deoxyguanosine or deoxycytidine) are not detectable by electrochemical oxidation.

Of major toxicological importance are the single and double-strand breakages that also occur when DNA is subjected to an $^{\cdot}OH$ radical flux.[31] This process may lead to immune reactions in subjects consuming irradiated foodstuffs of relatively high DNA content. A number of studies have shown that after its exposure to a superoxide ($O_2^{\cdot-}$)-generating source (hypoxanthine and xanthine oxidase), DNA becomes antigenic when injected into animals.[32] This has been postulated to be due to the superoxide-dependent production of DNA-damaging $^{\cdot}OH$ radicals in the presence of a transition metal ion catalyst (equation (10)).[33,34]

AROMATIC HYDROXYLATION

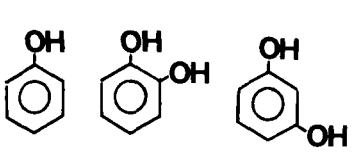
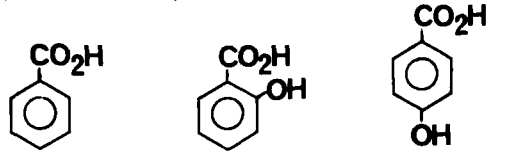
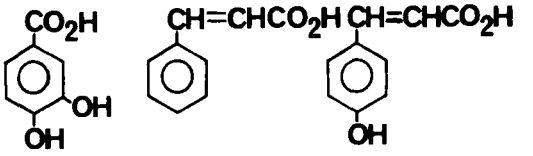
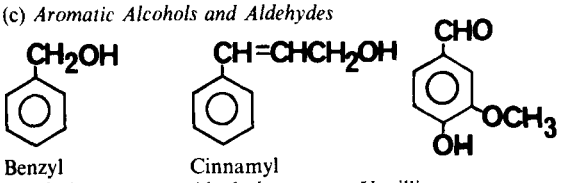
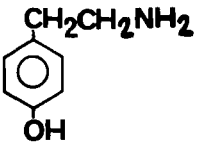
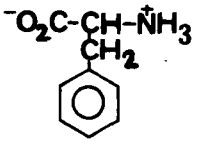
Of all the chemical constituents present in foodstuffs, aromatic compounds such as phenols and phenolic acids react extremely rapidly with $^{\cdot}OH$ radical to form a mixture of hydroxylated products.[35,36] Indeed, in medically-orientated studies, aromatic hydroxylation has been proposed as a method for measuring $^{\cdot}OH$ radical production both *in vitro*[28,37] and *in vivo*.[38]

If an aromatic compound reacts with $^{\cdot}OH$ radical to form a specific set of hydroxylated products that can be accurately identified and quantified in samples of foodstuffs, and one or more of these products are not identical to naturally occurring hydroxylated species i.e. not produced by normal metabolic processes, then the identification of these 'unnatural' products can be used to assess whether the sample has been irradiated. This is likely to be the case if the aromatic 'detector' molecule is present at the sites of $^{\cdot}OH$ radical generation at concentrations sufficient to compete with any other molecules that might scavenge $^{\cdot}OH$ radical.

There is a very wide range of aromatic compounds present in various foodstuffs (Table 1) e.g. foodstuffs containing or derived from plant tissues contain phenols such as catechol and phenolic acids such as salicylic and 4-hydroxybenzoic acids. The applicability of these species as suitable aromatic detector molecules for the irradiation of foodstuffs largely depends on the nature and extent of their chemical modification (hydroxylation and/or decarboxylation) following reaction with $^{\cdot}OH$ radical. For example, attack of $^{\cdot}OH$ radical upon salicylate (present in celery and lettuce) produces three products. The major products are 2,3-dihydroxybenzoate ($\sim 49\%$), and 2,5-dihydroxybenzoate ($\sim 40\%$) but a small quantity of catechol ($\sim 11\%$) is also formed by decarboxylation.[38] Although identification and quantification of 2,3- and 2,5-dihydroxybenzoate may serve as a useful qualitative diagnostic test for certain types of irradiated foodstuffs, measurement of catechol is of no practical use due to its known occurrence in a large number of foodstuffs of plant origin.

The resulting 'unnatural' hydroxylated aromatic species are readily separated from other phenolic constituents present in appropriate extracts of plant-derived foodstuffs by reversed-phase HPLC, and are detected and quantified by electrochemical oxidation at a glassy carbon working electrode. This technique has the necessary selectivity and sensitivity required to establish a suitable diagnostic test for the irradiation of fruits and vegetables. The methodology involves the prior isolation of acidic, neutral and basic phenolic species into one or more groups using extraction into organic

TABLE I
Examples of aromatic compounds occurring in various foodstuffs.

(a) *Phenols*		
 Phenol, Catechol, Resorcinol, Hydroquinone		present in the free state and as monoglucosides in plant-derived foodstuffs, e.g. tea
(b) *Aromatic Carboxylic Acids*		
 Benzoic Acid, Salicylic Acid, 4-Hydroxybenzoic Acid		present in plant tissues (e.g. strawberries, lettuce, celery, onions, potatoes, and coffee beans) as simple salts or as esters, the latter occurring in essential oils, gums and resins.
 3,4-Dihydroxy-benzoic Acid (Protocatechuic Acid), Cinnamic Acid, 4-Hydroxycinnamic Acid (p-Coumaric Acid)		
(c) *Aromatic Alcohols and Aldehydes*		
 Benzyl Alcohol, Cinnamyl Alcohol, Vanillin		present in the free state and as esters in foodstuffs or plant origin
(d) *Aromatic Amines*		
 Tyramine		present in large amounts in cheese and yeast extract
(e) *Aromatic Amino Acids*		
 Phenylalanine		naturally occurring amino acid that is present in proteins in a variety of meat and fish products. Small quantities are also present in the free state

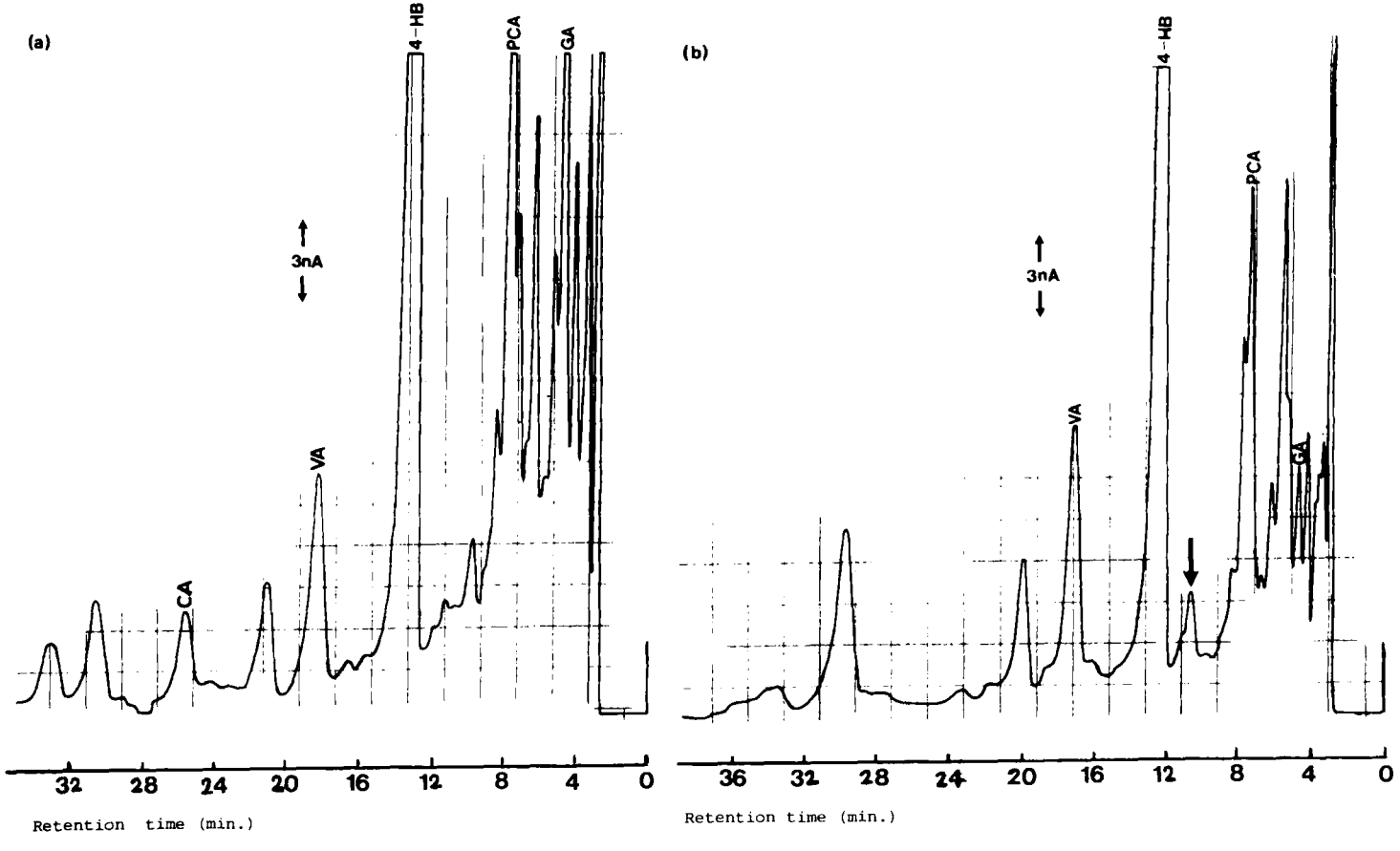


FIGURE 3 Reversed phase HPLC analysis of phenols and phenolic acids present in extracts of strawberry samples (a) before and (b) after irradiation at a dose of 5.0 kGy. Strawberry samples were homogenised and treated with sufficient HCl to adjust the pH to 2.0 and then extracted with two portions of HPLC-grade diethyl ether (ethoxyethane). The combined diethyl ether layers were then evaporated to dryness and the residue was dissolved in 0.10 mol dm^{-3} HCl prior to HPLC analysis. The electrochemical detector potential was + 0.80V. Further details of the HPLC procedure will be published elsewhere. Abbreviations: GA, gallic acid; PCA, protocatechuic acid; 4-HB, 4-hydroxybenzoic acid; VA, vanillic acid; CA, caffeic acid. The sharp vertical lines are injection spikes, and nA = nanoamps. The arrow in (b) indicates a new peak with a retention time of 10.48 min., conceivably attributable to a new 'unnatural' phenolic component produced solely by the irradiation process.

solvents such as diethyl ether or ethyl acetate at controlled pH. After removal of the organic solvent by evaporation to dryness, the extracts are reconstituted in a suitable aqueous medium and then subjected to analysis by reversed-phase HPLC. Initial identity assignments of the peaks in the resulting chromatograms are obtained by direct comparisons of retention time values of sample components with those of authentic compounds. However, it must be emphasized that in view of the large number of diethyl ether – or ethyl acetate-soluble species likely to be present in fruit or vegetable homogenates, a retention time is insufficient evidence to attribute an observed peak to a suspected component. The identity of putative peaks can be confirmed in three ways. Firstly, an aliquot of the reconstituted extract is 'spiked' with the suspected component at a concentration which is exactly equal to that estimated from its peak height or area in the chromatogram. If the identity of the putative peak is the same as that of the suspected component, then it will remain perfectly symmetrical and increase in height or area by a factor of 2. Secondly, the electrochemical detector can be employed to obtain a voltammetric characterisation of eluting species. Comparisons of the current-potential responses (plots of peak height or area versus electrochemical detector potential, known as hydrodynamic voltammograms) of sample components and authentic standards provides further confirmation of the initial identity assignment. Thirdly, the HPLC eluant composition can be varied (for example, by including different proportions of methanol or other solvent) to produce large changes in retention times. If a putative peak present in the chromatogram of the extract has the same identity as a suspected component, the variable retention times observed for it will always be identical to those given by an authentic standard.

As an illustration of the merits of the HPLC/ECD approach, Figure 3 exhibits chromatograms of acidic diethyl ether extracts of strawberry samples (a) before and (b) after irradiation at a dose of 5 kGy. The peaks observed in the chromatogram

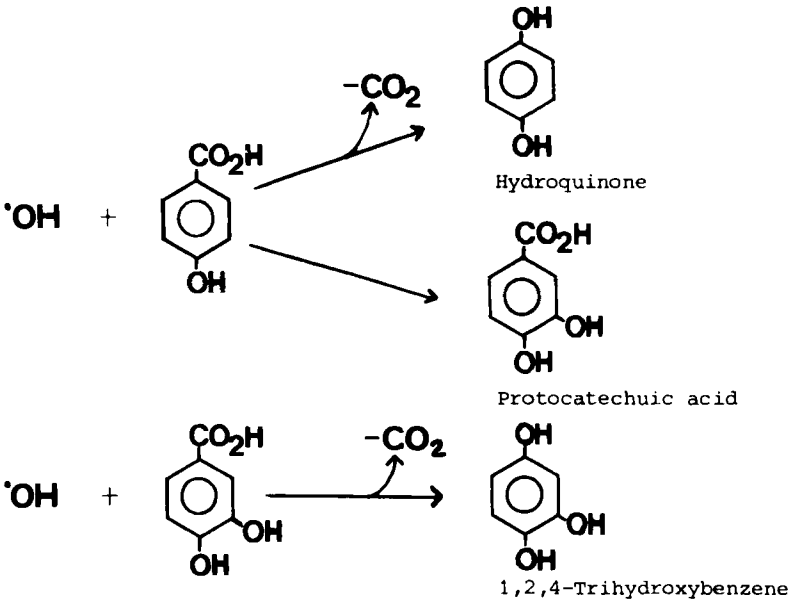


FIGURE 4(a) Products of the attack of 'OH radical on 4-Hydroxybenzoic and Protocatechuic acids.

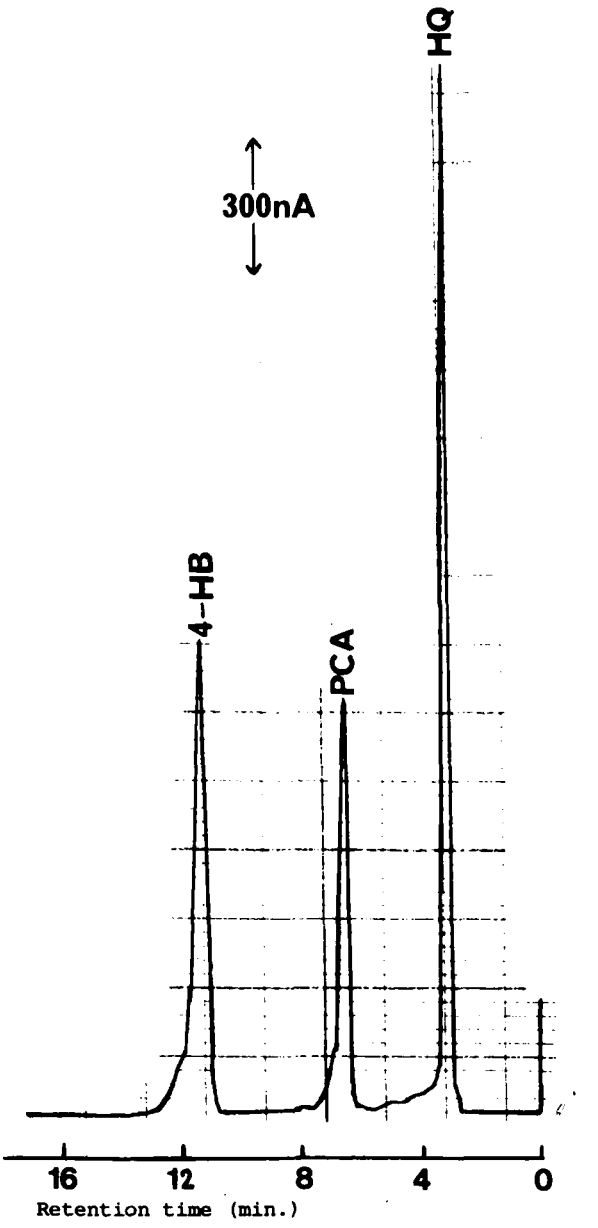


FIGURE 4(b) HPLC separation of the products formed during attack of ·OH radical upon 4-hydroxybenzoic acid on a reversed-phase column. The mobile phase was 93% (v/v) 30 mmol dm^{-3} sodium citrate/27.7 mmol dm^{-3} sodium acetate buffer, (pH 4.75) and 7% (v/v) methanol at a flow rate of 1.00 ml/min continuously sparged with helium. Detection of hydroquinone (HQ), protocatechuate (PCA) and unreacted 4-hydroxybenzoate (4-HB) was by electrochemical oxidation at + 0.86V. ·OH radical was generated by the Fenton reaction. The reaction mixture contained EDTA (1.00 mmol dm^{-3}), 4-hydroxybenzoate (1.00 mmol dm^{-3}), H_2O_2(3.30 mmol dm^{-3}) and iron(II) sulphate (1.00 mmol dm^{-3}) in aqueous solution at pH 7.00.

resulting from the non-irradiated sample are largely attributable to phenolic and phenolic acid components present in strawberries, e.g. gallic, protocatechuic, 4-hydroxybenzoic, vanillic, and caffeic acids. Following treatment with γ-irradiation, the intensities of a number of these peaks decrease substantially (i.e. those attributable to gallic and 4-hydroxybenzoic acids), indicative of the radiolytic depletion of phenolic acids which act as powerful scavengers of $^{\cdot}OH$ radical. Attack of $^{\cdot}OH$ radical on 4-hydroxybenzoic acid yields protocatechuic acid and hydroquinone, and its reaction with protocatechuic acid produces 1,2,4-trihydroxybenzene (Figure 4). Hydroquinone and 1,2,4-trihydroxybenzene are both formed by decarboxylation. Since gallic, protocatechuic and 4-hydroxybenzoic acids are all naturally-occurring in non-irradiated foodstuffs, the above complex interconversions are likely to lead to a marked variation in their effective concentrations with increasing doses of γ-irradiation. In accordance with this hypothesis, results obtained from the irradiation of strawberries at a variety of dosage levels (0, 0.5, 5.0 and 10.0 kGy) have demonstrated that the concentration of 4-hydroxybenzoic acid significantly decreases with increasing dose of γ-irradiation and the concentration of protocatechuic acid decreases significantly at a dose as low as 0.5 kGy but rises to higher concentrations with further increase in dosage level (5 and 10 kGy) due to its production from the hydroxylation of 4-hydroxybenzoic acid (Figure 4). However, the concentration of gallic acid decreases with increasing dosage from 0 to 10 kGy, indicating that it is attacked by $^{\cdot}OH$ radical (especially at the higher doses of γ-irradiation studied) to form other products.

As well as the quantitative differences observed between the two chromatograms shown in Figure 3, the chromatogram corresponding to the sample irradiated at 5.0 kGy exhibits new peaks e.g. one with a retention time of 10.48 minutes, demonstrating the qualitative value of this diagnostic test. As expected, chromatograms obtained from strawberry samples irradiated at the maximum permitted dose (10 kGy) exhibit additional new peaks which are conceivably attributable to 'unnatural' phenolic species. Experiments involving the identification of these new 'unnatural' compounds are currently in progress. Corresponding chromatograms obtained from strawberry samples irradiated at 0.5 kGy also exhibit both quantitative and qualitative differences to that of the non-irradiated (control) sample, suggesting that this technique is also applicable at low doses of γ-irradiation.

The cost of the analytical system used here enables the method to be readily accessible to public analyst laboratories. Since a large number of these laboratories are already equipped with HPLC equipment in routine use, it is only necessary for them to invest in a suitable electrochemical detection system (cost ca. £3,000) in order to be able to carry out this diagnostic test on a routine basis.

One compound which has been quite widely used as an aromatic detector is the naturally occurring amino acid phenylalanine present in the proteins of various meat products (Table 1). Attack of $^{\cdot}OH$ radical on phenylalanine produces three products: *ortho-*, *meta-* and *para-* tyrosine.[39] Based on the assumption that of these three products, only the *para-* isomer is a naturally occurring amino acid present in food residues, a test involving the measurement of the 'unnatural' amino acids *ortho-* and *meta-* tyrosine using gas chromatography-mass spectrometry[40] or HPLC with electrochemical detection[58] has been developed. Unfortunately, some *ortho-* and *meta-* tyrosine have been detected in non-irradiated food samples, but this is not totally unexpected due to the known production of these so called 'unnatural' species in animal tissues by the action of the enzyme tyrosine hydroxylase.[41] However, irradiated

foodstuffs contain significantly greater quantities of these isomers than the corresponding non-irradiated ones, and hence their detection and quantification may still have application as a quantitative type of diagnostic test.

EFFECTS OF IRRADIATION ON THE ESSENTIAL NUTRIENT CONTENT OF FOODSTUFFS

To date, there has been a growing number of reports available in the literature on the effects of ionising radiation on the vitamin content of various foodstuffs.[42–44] Some of these reports claim that the irradiation process severely depletes the content of vitamins A, B_1, B_2, B_3, B_6, B_{12}, C, D, E, K and folate in a large number of foodstuff classes, while others claim that there are no significant reductions in vitamin content. However, it is clear that the nature and extent of the vitamin loss is highly dependent upon (i) the dose given, (ii) the ability of the vitamin studied to act as a scavenger of radiolytically-produced $^{\cdot}OH$ radical (some vitamins react more rapidly with $^{\cdot}OH$ radical than others) and (iii) the chemical complexity of the foodstuff to be irradiated (for example, fruit juices are expected to lose more vitamin C (ascorbate) than fruits and vegetables on irradiation due to the relatively low content of other $^{\cdot}OH$ radical scavengers present). Since the most important examples of the damaging effects of irradiation on the vitamin content of foodstuffs involve chemical modifications of vitamins C and E, the following discussion is limited to these two nutrients.

Ascorbate is an important naturally-occurring radical scavenger.[45] It is a very powerful electron donor (reducing agent) and reacts extremely rapidly with $^{\cdot}OH$ radical (for example, it reacts more than twice as fast as the aromatic amino acid phenylalanine) to form the semideydroascorbate radical. The resulting semidehydroascorbate radical then disproportionates to ascorbate and dehydroascorbate, the latter of which is unstable and breaks down to L-threonic and oxalic acids by a complex mechanism (Figure 5(a)). Since oxalate is quite toxic, the radiolytic depletion of ascorbate also has toxicological ramifications that may be of some significance. However, ascorbate may also play an important role in preventing carbon-centred radicals attacking other molecules to form species with undesirable toxicological properties. Ascorbate can also be consumed by its reaction with superoxide[46] (equation (12)) formed from the interaction of radiolytically-generated aquated electrons with oxygen.

$$\text{ascorbate} + O_2^- + H^+ \rightarrow \text{semidehydroascorbate radical} + H_2O_2 \qquad (12)$$

Ascorbate is readily determined in treated fruit and vegetable homogenates by HPLC coupled with detection by electrochemical oxidation. Since the vitamin can be detected at lower oxidation potentials than those required for other electrochemically-active components (e.g. phenolic acids) the assay has a high degree of selectivity. Figure 5(b) shows the facile HPLC/ECD determination of ascorbate in strawberry samples obtained before and after treatment with γ-irradiation, and Table 2 gives the ascorbate content of strawberry samples before and after irradiation at 5.0 and 10.0 kGy. From these data it is clear that irradiation at a dose of 5.0 kGy reduces the ascorbate content to 62% of its original level, and a dose of 10.0 kGy virtually wipes it out completely (< 1% remaining).

Since humans rely on the dietary intake of ascorbate from plant materials to aid the enzymes lysine hydroxylase and proline hydroxylase (required for the biosynthesis of

collagen) and the copper-containing enzyme dopamine-β-hydroxylase in its conversion of dopamine to noradrenalin,[45] its observed loss in foodstuffs following irradiation treatment is of much dietary significance.

Vitamin E (α-tocopherol) is a lipid-soluble species which has the ability to terminate the chain reaction of lipid peroxidation (triggered by the irradiation of PUFA's) by reacting with lipid peroxy radicals to form the relatively stable vitamin E radical.[47]

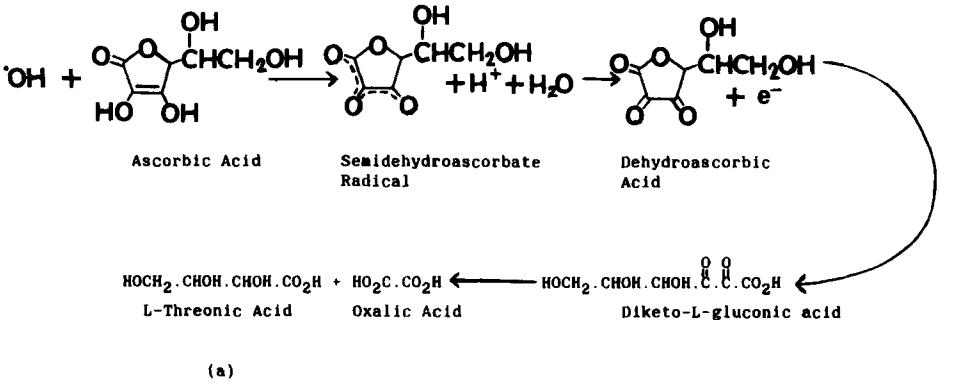


FIGURE 5(a) consumption of ascorbic acid (vitamin C) during irradiation of fruit and vegetable products.

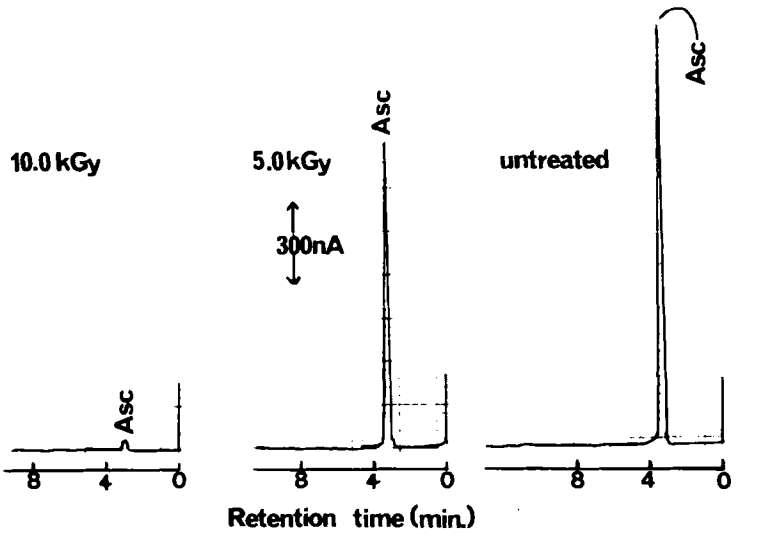


FIGURE 5(b) Reversed-phase HPLC determination of ascorbate in strawberry samples before and after treatment with γ-irradiation at doses of 5.0 and 10.0 kGy. The mobile phase was 90% (v/v) 30 mmol dm^{-3} sodium citrate/27.7 mmol dm^{-3} sodium acetate buffer (pH 4.75) and 10% (v/v) methanol at a flow rate of 1.00 ml/min continuously sparged with helium during elution. The electrochemical detector potential was + 0.40V. Asc = ascorbate.

TABLE 2
Variation in ascorbate content of strawberry samples with increasing doses of γ-irradiation.

Dose of γ-irradiation (kGy)	*Ascorbate content (μmoles/gram)*
0	0.88
5.0	0.54
10.0	0.008

The vitamin E radical is unable to abstract a hydrogen atom from another PUFA molecule and hence acts as a chain terminator (antioxidant) by blocking the autocatalytic process. Vitamin E is also able to react with superoxide, but the rate of this reaction is slow.[46] Various synthetic antioxidants (e.g. butylated hydroxytoluene, butylated hydroxyanisole, nordihydroguaiaretic acid and propyl gallate) are thought to act in the same way as vitamin E and, depending on the nature of the system studied, are sometimes more effective antioxidants.

Although not directly useful as diagnostic tests for the irradiation of foodstuffs, determination of vitamin contents in various irradiated samples provides a more complete understanding of the chemical modifications that occur during the irradiation process.

NON-INVASIVE SPECTROSCOPIC METHODS

The determination of conjugated dienes and diene hydroperoxides in extracts of irradiated foodstuffs by second-derivative absorption spectra (see above) could also be classified under this heading. A high level of success in the detection of irradiated foodstuffs has been achieved by the use of electron spin resonance (ESR),[48,49,59] a non-invasive spectroscopic technique that detects the presence of unpaired electrons in free radical species. Although the application of this technique is dependent upon the trapping of free radicals in hard, calcified parts of foodstuffs (e.g. chicken bone or prawn cuticle), it has been shown to be characteristic of irradiation treatment, even at doses as low as 0.2 or 0.3 kGy. The signal produced in the ESR spectrum following irradiation has a relatively long lifetime, and is still present after cooking. However, the potential of this test is limited not only by the observation that a number of non-irradiated foodstuffs already contain free radical species but also by the high cost of ESR equipment (£100,000–£200,000) and a requirement for specialist technical staff for its operation.

The recent development of high-field nuclear magnetic resonance (NMR) spectrometers with increased sensitivity, resolution and dynamic range has allowed the rapid study of complex mixtures of chemical species in fluids of biological interest. For example, high-field, high resolution ^{1}H NMR studies of human plasma, serum and urine have yielded much useful information.[50,51] Spin-echo pulse sequences are now frequently used to suppress broad overlapping resonances which arise from macromolecules present (e.g. proteins) so that the resulting NMR spectrum only contains well-resolved peaks attributable to low molecular mass metabolites and mobile portions of macromolecules at concentrations greater than ca. 1.0×10^{-4} mol dm^{-3}. The intense water signal is effectively removed from the spectrum by continuous secondary irradiation at the water frequency, and since the technique requires little or no pre-treatment of samples, it is largely non-invasive.

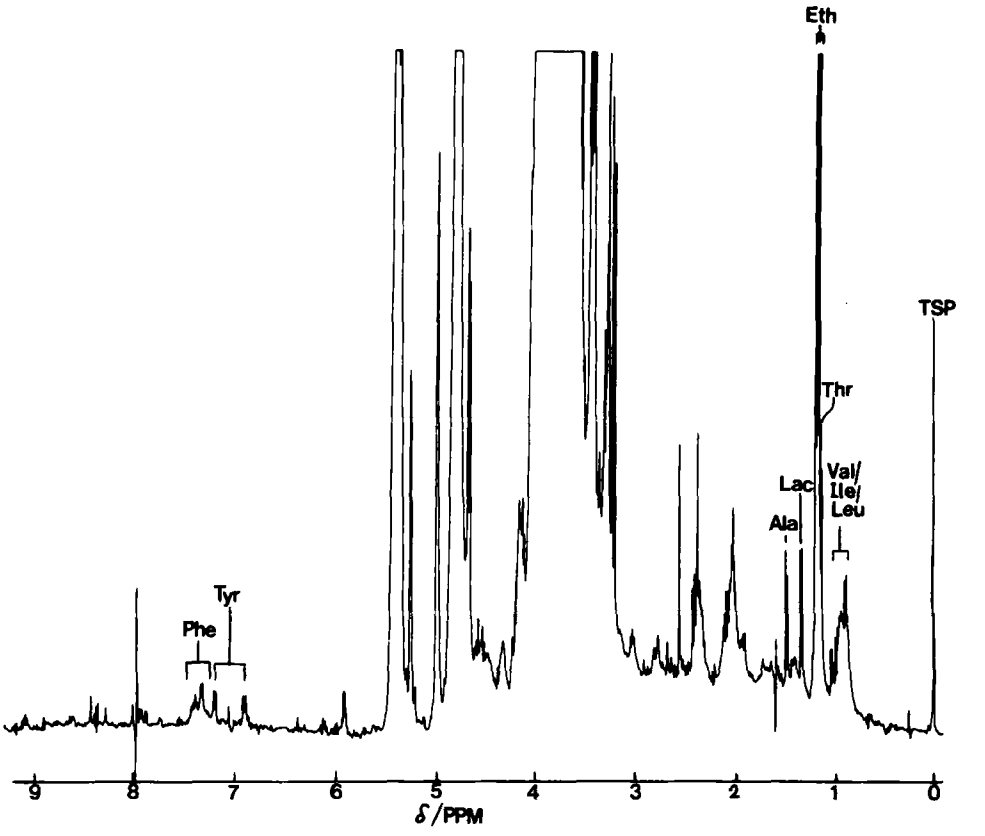


FIGURE 6 500 MHz ^{1}H NMR spectrum of a commercial sample of lager beer. The sample was previously lyophilized and redissolved in D_2O in order to avoid dynamic range problems posed by the large amounts of ethanol present. Chemical shift values were referenced to 3-(trimethylsilyl)- 1-[D-4]-propionate (TSP). A small quantity of residual ethanol remains in the samples after lyophilization. Assignment of peaks: Val, valine-CH_3's; Ile, isoleucine-CH_3; Leu, leucine-CH_3; Thr, threonine-CH_3; Ala, alanine-CH_3; Tyr, tyrosine and Phen, phenylalanine aromatic protons; Eth, ethanol-CH_3; Lac, lactate-CH_3.

Figure 6 exhibits the application of high-field, high-resolution ^{1}H NMR spectroscopy to the analysis of food products. A sample of non-irradiated lager beer (previously freeze-dried to remove the large quantities of ethanol present and reconstituted in deuterium oxide (D_2O)) exhibits resonances attributable to the amino acids valine, isoleucine, leucine, threonine, alanine, tyrosine and phenylalanine, and other metabolites such as lactate (Figure 6).

Although the sensitivity of this NMR technique is far lower than that obtained with other techniques such as HPLC/ECD, it has the potential to provide a complete 'picture' of chemical transformations occurring on irradiation of foodstuffs. Furthermore, its sensitivity can be increased by factors of 100-fold or greater by various pre-concentration techniques e.g. organic solvent or solid phase extraction. However, it should be noted that due to cost restrictions, the technique does not at present have prospects as a test for irradiated foodstuffs but, as illustrated here, it is a very useful and convenient tool for further research work in this area.

The non-destructive analysis of solids is an area of continuing interest for the analytical chemist. Reflectance spectroscopy, and more recently photoacoustic spectroscopy have been employed in the analysis of a wide range of food products (e.g. grains, oilseeds and raw meat).[52,53] The development of Fourier transform infra-red spectroscopy has provided a powerful asset in both these techniques.[54] Although the spectra obtained from food matrices are of a complex nature, other analytical methods such as those discussed above may be able to provide the criteria necessary for their interpretaton. Moreover, since these techniques are able to provide rapid, non-invasive and relatively sensitive analyses, they are suitable candidates for the development of simple instrumental analytical systems to detect chemical changes in foodstuffs resulting from the irradiation process.

It has been suggested that thermoluminescence may be a useful technique for the detection of irradiated spices.[55,57,60] This technique relies on the principle that species of electron excess and/or deficiency, or trapped free radicals emit light on heating. Despite having the necessary sensitivity, it can only be considered as a quantitative type of diagnostic test since non-irradiated foodstuffs also generate light on heating and it is not yet clear whether the large differences in thermoluminescence intensity observed between irradiated and non-irradiated samples will remain after long periods of storage. Similarly, peroxidising lipids can be detected by chemiluminescence.[56] The light emission observed here has been postulated to arise from the decay of excited carbonyl compounds (produced from the fragmentation of lipid peroxides) to the ground state, or from the formation of a species resembling singlet oxygen.

CONCLUSIONS

It is now recognised that no single diagnostic test will suffice for the wide range of foodstuffs which are currently subjected to irradiation. Although the major barrier to the establishment of suitable test systems in the UK appears to be cost, this problem can be alleviated by making full use of equipment which is already available in public analyst laboratories (e.g. HPLC). It is also clear that the nature and successful application of diagnostic tests for irradiated foodstuffs are subject to a number of other important factors. These include (i) specificity, (ii) the ability to perform the test in the absence of non-irradiated control sample, (iii) reference to a suitable substrate (e.g. $^{\cdot}OH$ radical scavenger) concentration (iv) the establishment of a critical threshold radiation dose at which the test is operable (i.e. defining the lowest radiation dosage level detectable), (v) the effect of interferences such as cooking and (vi) the influence of variables such as dose, dose rate, temperature, water content and oxygen concentration. Moreover, it should also be pointed out that in some cases various food packaging forms are also irradiated with the foodstuff, giving rise to the possibility of a number of 'unnatural' products detected in irradiated foodstuffs solely derived from packaging materials.

A major effort is required to evaluate the potential of the techniques discussed here.

Acknowledgements

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References

1. Spinks, J.W.T. and Woods, R.J. '*An introduction to Radiation Chemistry*', 2nd Ed., John Wiley and Sons, New York (1976).
2. Draganic, I.G. and Draganic, Z.D. '*The Radiation Chemistry of Water*', Academic Press, New York (1971).
3. Ebert, M., Keene, J.P., Swallow, A.J. and Baxendale, J.H. (Eds), '*Pulse Radiolysis*', Academic Press, London and New York (1985).
4. Allen, J.C. and Hamilton, R.J. (Eds), '*Rancidity in Foods*', Applied Science, Barking (1983).
5. Frankel, E.N. '*Recent Advances in the Chemistry of Rancidity of Fats*', Specialist Publication No 47, Royal Society of Chemistry, London (1984).
6. O'Brien, P.J. Intracellular Mechanisms for the Decomposition of Lipid Peroxide. Decomposition of a Lipid Peroxide by Metal Ions, Haem Compounds and Nucleophiles. *Can. J. Biochem.*, **47,** 485 (1969).
7. Nair, V. and Turner, G.A. The Thiobarbituric Acid Test for Lipid Peroxidation: Structure of the Adduct with Malondialdehyde Lipids, **19,** 804 (1984).
8. Marshall, P.J., Warso, M.A. and Lands, W.E.M. Selective Micro-Determination of Lipid Hydroperoxides. *Anal. Biochem.*, **145,** 192 (1985).
9. Sinnhuber, R.O., Yu, T.C. and Chang, Y.T. Characterisation of the Red Pigment Formed in the 2-Thiobarbituric Acid Determination of Oxidative Rancidity. *Food Res.*, **23,** 626 (1958).
10. Dahle, L.K., Hill, E.G. and Hollman, R.T. The TBA Reaction and the Autoxidation of Polyunsaturated Fatty Acid Methyl Esters. *Arch. Biochem. Biophys.*, **98,** 253 (1962).
11. Robards, K., Kerr, A.F. and Pastalides, E., Rancidity and its Measurement in Edible Oils and Snack Foods. *Review. Analyst*, **113,** 213 (1988), and references cited therein.
12. Corongui, F.P., Poli, G., Dianzani, M.U., Cheeseman, K.H. and Slater, T.F. Lipid Peroxidation and Molecular Damage to Polyunsaturated Fatty Acids in Rat Liver. Recognition of Two Classes of Hydroperoxides Formed under Conditions In Vivo. *Chem.-Biol. Interactions*, **59,** 147 (1986).
13. Slater, T.F. Free Radical Mechanisms in Tissue Injury. *Biochem. J.*, **222,** 1 (1984).
14. Tappel, A.L. and Dillard, C.J. In Vivo Lipid Peroxidation: Measurement via Exhaled Pentane and Protection by Vitamin E. *Fed. Proc.*, **40,** 174 (1981).
15. Benedetti, A., Comporti, M., Fulceri, R. and Esterbauer, H. Cytotoxic Aldehydes Originating from the Peroxidation of liver Microsomal Lipids. Identification of 4,5-Dihydroxydecanal. *Biochem. Biophys. Acta*, **792,** 172 (1984).
16. Cadenas, E., Muller, A., Brigelius, R., Esterbauer, H. and Sies, H. Effects of 4-Hydroxynonenal on Isolated Hepatocytes. Studies on Chemiluminescence Response, Alkane Production and Glutathione Status. *Biochem. J.*, **214,** 479 (1983).
17. Winkler, P., Lindner, W., Esterbauer E., Schauenstein, R.J., Schauer, R.J. and Khoschsorur, G.A. Detection of 4-Hydroxynonenal as a Product of Lipid Peroxidation in Native Ehrlich Ascites Tumour Cells. *Biochem. Biophys. Acta*, **796,** 232 (1984).
18. Gutteridge, J.M.C., Lamport, P. and Dormandy, T.L. The Anti-Bacterial Effect of Water-soluble Compounds from Autoxidising Linolenic Acid. *J. Med. Microbiol.*, **9,** 105 (1976).
19. Tappel, A.L. Lipid Peroxidation and Fluorescent Molecular Damage to Membranes. In Trump, B.J. and Arstila, A.V. (Eds), Pathobiology of Cell Membranes, Vol. I. Academic Press, New York, pp 145–170 (1975).
20. Turner, S.R., Campbell, J.A. and Lynn, W.S. Polymorphonuclear Leucocyte Chemotaxis Towards Oxidised Lipid Components of Cell Membranes. *J Exp. Med.* **141,** 1437 (1975).
21. Schauenstein, E., Esterbauer, H. and Zollner, H. Aldehydes in Biological Systems. Pion Press, London (1977).
22. Van Hinsbergh, V.W.M. LDL Cytotoxicity. The State of the Art. *Atherosclerosis*, **53,** 113 (1984).
23. Barrowcliffe, T.W., Gray, E., Kerry, P.J. and Gutteridge, J.M.C. Triglyceride-Rich Lipoproteins are Responsible for Thrombin Generation Induced by Lipid Peroxides. *Thromb. Haemostas.*, **52,** 7 (1984).
24. Grootveld, M. and Halliwell, B. Measurement of Allantoin and Uric Acid in Human Body Fluids. A Potential Index of Free Radical Reactions In Vivo? *Biochem. J.*, **243,** 803 (1987).
25. Halliwell, B. Superoxide-Dependent Formation of Hydroxyl Radicals in the Presence of Iron Chelates. Is it a Mechanism for Hydroxyl Radical Production in Biochemical Systems? *FEBS Lett.*, **92,** 321 (1978).
26. McCord, J.M. and Day, E.D. Superoxide-Dependent Production of Hydroxyl Radical Catalysed by an Iron-EDTA Complex. *FEBS Lett.*, **86,** 139 (1978).
27. Walling, C. The Nature of the Primary Oxidants in Oxidations Mediated by Metal Ions. In King, T.E. Mason, H.S. and Morrison, M. (Eds), Proc. 3rd Int. Symp. Oxidases Related Redox Systems.

Pergamon Press, Oxford, pp. 85–98 (1982).
28. Grootveld, M. and Halliwell, B. An Aromatic Hydroxylation Assay for Hydroxyl Radicals utilising High Performance Liquid Chromatography (HPLC). Use to Investigate the Effect of EDTA on the Fenton Reaction. *Free Radical Res. Commun.*, **1**, 242 (1986).
29. Cheeseman, K.M., Beavis, A. and Esterbauer, H. Hydroxyl- Radical Induced Iron-catalysed Degradation of 2-Deoxyribose. Quantitative Determination of Malondialdehyde. *Biochem. J.*, **252,** 649 (1988).
30. Grootveld, M., Jain, R. and Winyard, P.G., unpublished work.
31. Mello Filho, A.C. and Meneghini, R. In Vivo Formation of Single-Strand Breaks in DNA is Mediated by the Haber-Weiss Reaction. *Biochem. Biophys. Acta,* **781,** 56 (1984).
32. Jansson, G. Formation of Antibodies to Native DNA in Rats After Administration of Native DNA Treated with the Xanthine – Xanthine Oxidase System. *Free Radical Res. Commun.*, **1,** 119 (1985).
33. Brawn, K. and Fridovoch, I. DNA Strand Scission by Enzymically Generated Oxygen Radicals. *Arch. Biochem. Biophys.*, **206,** 414 (1981).
34. Rowley, D.A. and Halliwell, B. DNA Damage by Superoxide-Generating Systems in Relation to the Mechanism of Action of the Anti-Tumour Antibiotic Adriamycin. *Biochim. Biophys. Acta,* **761,** 86 (1983).
35. Halliwell, B., Gutteridge, J.M.C. and Grootveld, M. Methods for the Measurement of Hydroxyl Radicals in Biochemical Systems. Deoxyribose Degradation and Aromatic Hydroxylation. *Methods Biochem. Anal.*, **33,** 59 (1989).
36. Halliwell, B. and Grootveld, M. The Measurement of Free Radical Reactions in Humans. Some Thoughts for Future Experimentation. *FEBS Lett.*, **213,** 1, 9 (1987).
37. Moorhouse, C.P., Halliwell, B., Grootveld, M. and Gutteridge, J.M.C., Cobalt(II) Ion as a Catalyst of Hydroxyl Radical and Possible 'Crypto-Hydroxyl' Radical Formation under Physiological Conditions. Differential Effects of Hydroxyl Radical Scavengers. *Biochem. Biophys. Acta,* **843,** 261 (1985).
38. Grootveld, M. and Halliwell, B. Aromatic Hydroxylation as a Potential Measure of Hydroxyl Radical Formation In Vivo. Identification of Hydroxylated Derivatives of Salicylate in Human Body Fluids. *Biochem. J.*, **237,** 499 (1986).
39. Kaur, H., Fagerheim, I., Grootveld, M., Puppo, A. and Halliwell, B. Aromatic Hydroxylation of Phenylalanine as an Assay for Hydroxyl Radicals: Application to Activated Human Neutrophils and to the Haem Protein Leghaemoglobin. *Anal. Biochem.*, **172,** 360 (1988).
40. Karam, L.R. and Simic, M.G. Methods for the Identification of Irradiated Chicken Meat. Presented at the WHO Working Group on Health Impact and Control of Irradiated Foods: Neuherberg, FRG, Nov. (1986).
41. Ishimitsu, S., Fujimoto, S. and Ohara, A. In Vivo Studies on the Formation of m-Tyrosine and *O*-Tyrosine from L-Phenylalanine in Rats. *Chem. Pharm. Bull.* **34(2),** 768 (1986).
42. Josephson, E.S. and Peterson, M.S. (Eds), Preservation of Food by Ionising Radiation (3 vols.) CRC Press., Florida, USA. Vol 1 (1982), Vol 2 and 3 (1983).
43. Elias, P.S. and Cohen, A.J. Recent Advances in Food Irradiation, Elsevier Biomedical Press (1983).
44. Elias, P.S. and Cohen, A.J. Radiation Chemistry of Major Food Components, Elsevier Biomedical Press (1983).
45. Seib, P.A. and Tolbert, B.M. (eds.), Ascorbic Acid: Chemistry, Metabolism and Uses. Advances in Chemistry, series 200, American Chemical Society, Washington DC, USA (1982).
46. Nishikimi, M. and Yagi, K. Oxidations of Ascorbic Acid and α-Tocopheral by Superoxide. In Hayaishi, O. and Asada, K. (Eds.), Biochemical and Medical Aspects of Active Oxygen, University of Tokyo Press, Tokyo pp 79–87 (1977).
47. Gutteridge, J.M.C. The Membrane Effects of Vitamin E, Cholesterol and their Acetates on Peroxidative Susceptibility. *Res. Commun. Chem. Pathol. Pharmacol.*, **22,** 563 (1978).
48. Dodd, N.J.F., Swallow, A.J. and Ley, F.J. Use of ESR to Identify Irradiated Food. *Radiat. Phys. Chem.*, **26,** 451 (1985).
49. Desrosiers, M.F. and Simic, M.G. Postirradiation Dosimetry of Meat by Electron Spin Resonance Spectroscopy of Bones. *J. Agric. Food Chem.*, **36,** 601 (1988).
50. Nicholson, J.K., Buckingham, M.J. and Sadler, P.J. High Resolution ^{1}H NMR Studies of Vertebrate Blood and Plasma. *Biochem. J.*, **211,** 605 (1983).
51. Nicholson, J.K., O'Flynn, M.P., Sadler, P.J., Macleod, A.F., Juul, S.M. and Sönksen, P.H. Proton-Nuclear-Magnetic-Resonance Studies of Serum, Plasma and Urine from Fasting Normal and Diabetic Subjects. *Biochem. J.*, **217,** 365 (1984).
52. Norris, K.H. Reflectance Spectroscopy. In Stewart, K.K. and Whitaker, J.R. (Eds.), Modern Methods of Food Analysis, AVI Publishing Co., Inc., Connecticut pp 167–185 (1984).
53. Hirschfeld, T. and Stark, E.W. In Charalambous, G. (Ed.), Analysis of Foods and Beverages: Modern

Techniques, Academic Press Inc., London pp 506–550 (1984).
54. Sanders, R.A., In Charalambous, G. (Ed.), Analysis of Foods and Beverages: Modern Techniques, Academic Press Inc., London pp 554–581 (1984).
55. Heide, L. and Bögl, K.W. The Identification of Irradiated Spices with Thermo- and Chemiluminescence Measurements, *Int. J Food Sci. Tech.*, **22,** (1987).
56. Boveris, A., Cadenas, E. and Chance, B. Ultraweak Chemiluminescence: a Sensitive Assay for Oxidative Radical Reactions. *Fed. Proc.*, **40,** 195 (1981).
57. Albrich, S., Stumpf, E., Heide, L. and Bögl, K.W. Chemiluminescent- and Thermoluminescent-Measurements to Identify Radiation-Treated Spices. A Comparison of the Two Methods. Report of the Institute for Radiation Hygiene of the Federal Health Office, ISH-HEFT 74, Neuherberg/Munich, F.R. Germany (1985).
58. Grootveld, M. and Jain, R. unpublished work.
59. Raffi, J.J. and Agnel, J.P. Influence of the Physical Structure of Irradiated Starches on their Electron Spin Resonance Spectra Kinetics, *J Phys. Chem.*, **87,** 2369 (1983).
60. Heide, L., Albrich, S., Mentele, E. and Bögl, K.W. Thermolumineszenz – und Chemilumineszenzmessungen als Routine – Methoden zur Identifizierung Strahlenbehandelter Gewürze. Untersuchungen zur Festlegung Von Grenzwerten für die Unterscheidung bestrahiter Proben. Report of the Institute for Radiation Hygiene of the Federal Health Office, ISH-HEFT 109, Neuherberg/Munich, F.R. Germany (1987).

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