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ANALYTICAL METHODS FOR POST-IRRADIATION DOSIMETRY OF FOODS

(Technical Report)

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Analytical methods for post-irradiation dosimetry of foods (Technical Report)

ABSTRACT

The trade and acceptance of foods treated with ionizing radiation, gamma radiation or x-rays, require appropriate means of control. A foolproof test to detect whether or not food has been irradiated, and eventually to quantify the amount of radiation, is vital to verify the labelling and enforce legislation. Such an assay also provides the information for avoiding repeated irradiations which are likely to degrade the food in terms of organoleptic acceptability and nutritional quality.

METHODS OF ANALYSIS

An ideal analytical method should measure a specific radiation effect which is proportional to the dose, and is unaffected by processing and storage conditions or by the length of time between irradiation treatment and analysis. Futhermore, the test should distinguish irradiated food even in the absence of a nonirradiated, control sample.

If properly carried out, the irradiation process is a remarkably gentle treatment and the changes induced are generally very small and nonspecific. As a matter of fact, in spite of the strenuous efforts driven by toxicological concerns, no unique radiolytic products have ever been isolated from foods irradiated within the limits of recommended doses. In addition, the physical differences and natural variability in food composition, make it unlikely that a universal postirradiation dosimetry method for all foods will be found.

The legislative and commercial interest, combined with the scientific challenge, has prompted extensive efforts to develop methods that permit the identification of irradiated foods (Raffi and Belliardo 1991, IAEA 1991).

A multitude of approaches have been evaluated. They can be classified in several groups based on detection of the following radiation-induced changes:

1. Radiolysis of lipids
2. Modification of amino acids
3. Modification of DNA
4. Modification of carbohydrates
5. Formation of free radicals
6. Release of hydrogen gas
7. Alterations of microbiological flora
8. Measurements of biological differences
9. Other physical measurements

1. Radiolysis of lipids

The radiolytic splitting of fat molecules does not occur randomly; two hydrocarbons are produced preferentially from each fatty acid. One, resulting from preferential cleavage at the carbon-carbon bond alpha to the carbonyl group, has a carbon atom less than the parent fatty acid. The other, has two carbons less than the parent fatty acid and an extra double bond, and results from cleavage at the carbon-carbon bond beta to the carbonyl group (Fig. 1).

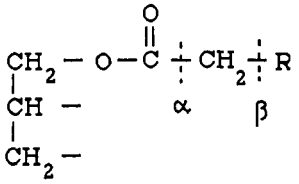

Fig. 1

These major radiolytic hydrocarbons reflect the severity of the irradiation treatment, since their production increases linearly with dose and temperature of irradiation. Indeed, these hydrocarbons resulting from specific cleavage of fatty acid residues in triglycerides may be used as indicators of irradiation in fatty foods. The presence of moisture or air during irradiation does not significantly alter the radiolytic pattern.

Experiments with ground pork indicate that these compounds are present in samples irradiated at doses as low as 1 kGy but absent in unirradiated or heated samples (Nawar and Balboni 1970, Vajdi and Nawar 1979, Vajdi and Merritt 1985, Meier and Biederman 1990). Volatile hydrocarbons and aldehydes were detected in irradiated chicken meat using on-line coupled liquid chromatography - gas chromatography (Meier et al. 1990) and in irradiated frog legs by a gas chromatographic method (Morehouse and Ku 1990). The estimations of the applied dose on frog legs of unknown origin, by gas chromatography of the hydrocarbons formed during radiolysis of lipids, were in good agreement with ESR measurements of free radicals trapped in the bone (Morehouse et al. 1991).

Irradiation of simple triglycerides yields also substituted cyclobutanones, with an alkyl group with as many carbon atoms as the parent fatty acid, at the ring position 2. 2-Dodecylcyclobutanone formed from palmitic acid, was suggested as a potential postirradiation marker for minced chicken meat. The concentration of 2-dodecylcyclobutanone produced by 5 kGy irradiation is approximately 0.2 μg/g fresh meat, five times higher than the estimated limit of detection (Stevenson et al. 1990). The compound was not detected in either raw or cooked nonirradiated minced chicken meat and it was detectable for 20 days postirradiation (Boyd et al. 1991).

Radiation-induced oxidation of lipids can also, *a priori*, be a suitable reaction for detection of irradiated food because of the amplifying effect achieved by the chain character of this reaction. Indeed, the lipid hydroperoxides content has been proposed as an indicator of the irradiation of egg and milk powder and soya flour. The formation of hydroperoxides appears to depend only on the irradiation dose, and even after six months, the level was still higher than the background value of nonirradiated samples (Katusin-Razem et al. 1990). However, few parameters unrelated to irradiation, like temperature, exposure to light, the availability of oxygen and traces of catalytic metal ions may affect the extent of lipid oxidation. A recent study on cholesterol oxidation indicated that the change in the ratio of 7-oxocholesterol to the sum of cholesterol 5α, 6α–epoxide and 5β, 6β-epoxide may be a means of determining whether or not meat or other foods containing cholesterol have been subjected to ionizing radiation (Lakritz and Maerker 1989). However, results with beef indicated that the content of the cholesterol oxidation products was affected more by the origin of the sample than by irradiation (Zabielski 1989).

2. Modification of amino acids

This analytical approach is primarily aimed at foods like raw meat which are rich in protein and contain more than 50% water. When exposed to ionizing radiation, the hydroxyl radical generated radiolytically from water interacts with amino acid residues in proteins (Fig. 2).

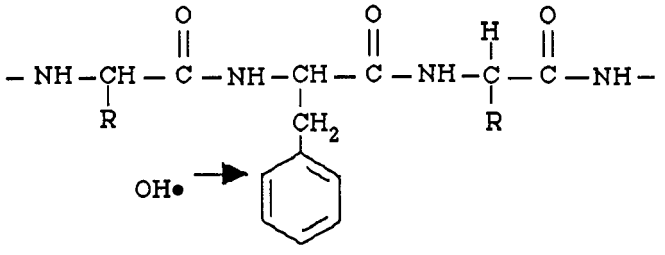

Fig. 2

2-Hydroxyphenylalanine (*o*-tyrosine) is not naturally incorporated into proteins and can serve as an internal dosimeter. The formation of this compound can be monitored, after drying and hydrolysis of the meat sample, by gas chromatography - mass spectrometry (Karam and Simic 1988) or by high performance liquid chromatography with fluorescence detection (Meier et al. 1989). Because the formation of *o*-tyrosine depends not only on the dose but also on the dose rate and the temperature during irradiation, it is not possible to determine the irradiation dose. It seems, however, that the major problem with *o*-tyrosine has been the variable levels of *o*-tyrosine found in unirradiated foods (Hart et al. 1988).

"Unnatural" hydroxylated aromatic compounds generated after irradiation, can also be separated from other phenolic constituents present in extracts of vegetable foodstuffs. Reverse-phase HPLC and an electrochemical detector equipped with a glassy carbon working electrode operating in the oxidation mode, were employed for this separation (Grootveld and Jain 1989b, Grootveld et al. 1990).

3. Modification of DNA

The effects of radiation on genetic material in general, and DNA in particular, have been thoroughly investigated because of their importance in radiation biology (von Sonntag 1987). DNA molecules are very sensitive to gamma radiation even at very low doses. Radiolytic products of purine, pyrimidine or sugar residues, as well as structural changes due to strand breaks or crosslinking between bases and protein, are easily generated. Since very sensitive techniques for detection of radiation induced lesions in nucleic acids are already available, and foods derived from living organisms, meat, fish, vegetables, always contain DNA as a trace constituent, the use of DNA modifications for detection is most promising. Thus, the

detection of the abnormal bases created in DNA by hydroxyl radicals, such 8-hydroxyguanine and 5-hydroxycytosine, have been suggested as a diagnostic test (Grootveld et al. 1990). These base products, however, need not be a result of irradiation. Hydroxyl radicals arise also in biochemical oxidations unrelated to radiation and may cause similar damage. Unfortunately, conformational changes, like strand breaks, are not specific to radiation, and can be a result of storage and processing conditions (freezing-thawing, etc.). Background levels of unirradiated food items, therefore, have to be checked carefully. There is a possibility that mitochondrial DNA is better protected from enzymatic reactions than cellular DNA, so its alteration might be more specific to irradiation.

Due to rapid progress in molecular biology and gene engineering, methods of analyzing nucleic acids are gaining sensitivity. It is therefore conceivable that if radiation-specific changes in DNA occur, it will be possible to develop an analytical detection procedure based on these changes.

4. Modifications of carbohydrates

Mono and polysaccharides are common components of various foodstuffs and are modified in specific patterns by radiolytically generated transients like hydroxyl radicals (den Drijver et al. 1986). Detection of oligosaccharide fragments derived from degradation of polysaccharides may have application as an analytical test for certain types of irradiated foods. For example, the detection of *N*-acetylglucosamine oligosaccharides derived from the fragmentation of chitin, if their formation is radiolytically specific, may potentially determine if prawns or shrimps have been irradiated. The detection can be achieved technically by high-field proton Hahn spin-echo NMR spectroscopy or alternative techniques (Grootveld and Jain 1989 a,b, Grootveld et al. 1990).

5. Detection of free radicals

The absorption of ionizing radiation by food molecules leads to formation of free radicals. Free radicals can be formed as a direct result of radiolysis (eq.1), dissociation of radical cations (eq.2), reactions between electrons and molecules (eq.3) and also as a consequence of ion-molecule reactions (eq.4):

$$RH \rightarrow R\cdot + H\cdot \qquad (1)$$

$$RH^+ + RH \rightarrow R\cdot + \dot{R}H_2^+ \qquad (2)$$

$$A + e^- \rightarrow A^- \qquad (3)$$

$$RX + e^- \rightarrow R\cdot + X^- \qquad (4)$$

In principle, the free radicals may reform the original bond, may react to form secondary radicals or may persist. The momentary concentration of free radicals depends on the nature of the material irradiated, the radiation dose, and the time interval between irradiation treatment and radical measurement.

If the process of radical formation occurs in a constrained matrix, such as dry polycrystalline or amorphous material, the free radicals are trapped and stabilized. Since the ultimate fate of the radicals depends on their ability to move into a position for bimolecular reaction, factors that alter their environment, such as water activity, predetermine the pathways for decay and hence, the lifetime. Indeed, the half life of free radicals produced in irradiated, solid foods decreased with an increase of the humidity in the system (Diehl 1972, Fritsch and Reymond 1970, O'Meara and Shaw 1957, Raffi and Agnel 1983, Uchiyama and Uchiyama 1979).

5a. Quantitation of free radicals by electron spin resonance (ESR)

Free radicals trapped in a solid matrix exhibit broad, poorly resolved, ESR signals which do not allow chemical assignments. Nevertheless, the ESR determination of total free radical content in dry materials, like spices, could serve as an indicative analytical tool to determine whether or not the food has been irradiated. The ESR signal intensity is approximately proportional to the total irradiation dose, between the background signal and the saturation dose. This analytical approach requires the analysis of a control, nonirradiated, sample because of variations in the background radical content. The free radicals in dry foods are not "unique radiolytic products". Nonirradiated spices, vanilla beans, heated cereal grains, contain stable paramagnetic species which apparently originate in the phenolic constituents of the vegetable material. The preirradiation free radical content of vegetable foods varies substantially, due presumably to the different processing conditions: grinding, exposure to sunlight, heat drying. Furthermore, the accuracy of this method is also affected by a gradual postirradiation loss of signal with storage time (Davidson and Forrester 1988, Troup et al. 1989, Yang et al. 1987).

Raffi et al. (1988) identified a signal in the ESR spectrum which was only present in the achenes of irradiated strawberries. Within the limits of commercial handling of strawberries, e.g. less than 20 days of storage at 5 ^{0}C, the ESR test was able to identify all samples irradiated by a dose of at least 1 kGy. Desrosiers and McLauglin (1989) observed an ESR signal in mango seeds which decayed after a few days.

Some hard, calcified tissues that occur in foods, like pork, chicken and cod bones or prawn cuticle, also stabilize free radicals (Desrosiers and Simic 1988, Desrosiers 1989, Raffi et al. 1989). The signal which is very long lived and even survives cooking, probably originates in the hydroxyapatite content of the bone (Dodd et al. 1988, 1989, Lea et al. 1988, Gray et al. 1989 a,b, 1990, Stevenson and Gray 1989a,b). The presence of such a signal can provide evidence of irradiation with doses as low as 200 Gy. Radiation absorbed dose given to such foods could be estimated by the additive re-irradiation of bone samples, followed by the back extrapolation of the EPR signal *vs.* dose fraction. This approach potentially eliminates the need for examination of non-irradiated samples (Desrosiers 1990, 1991).

5b. Lyoluminescence (chemiluminescence)

The term "lyoluminescence" applies to emission of light on dissolution of a solid in a liquid. One of the most common lyoluminescence effects is when solid samples of various substances irradiated with ionizing radiation are dissolved or brought in contact with a solvent, generally water. The total yield of emitted light increases with the administered radiation dose up to a saturation value. The ubiquity of lyoluminescence of irradiated solids is well known. Spices, dried foods like powdered milk and soups, cotton, cloth and paper generate light when placed in contact with a solvent (Ettinger and Puite 1982). Although one common mechanism is insufficient to explain this effect in such a broad variety of materials, there is a common denominator for the chemical basis of lyoluminescence - namely the production and subsequent reactions of free radicals. Radicals formed are trapped in the solid for relatively long times, particularly at lower temperatures. In a liquid medium, free radical reactions can take place and liberate enough energy for light emission in the short wavelength part of the visible spectrum. The lyoluminescence yield can be increased by adding chemiluminescent sensitizers such as the combination of luminol and hemin, adjusted to pH 10 - 11 (λ_{max} approx. 424 nm). Although the exact mechanism of activation of chemiluminescence of irradiated solids by luminol has not been thoroughly explained, the free radicals are the initiators of the sequential processes leading to intensely luminescent reactions.

A variety of spices, milk powder, whole onions and frozen chicken (particularly cartilage tissue rather than bone or meat), irradiated with doses up to 10 kGy showed lyoluminescence in a luminol solution (Bogl and Heide 1985). Likewise, gamma irradiated pepper could be detected (Sattar et al. 1987). In some foods, it was possible to identify the radiation treatment as late as several month after irradiation. The method is however plagued by problems generated by fading of the chemiluminescent response with storage time and by poor reproducibility.

5c. Thermoluminescence

Thermoluminescence, which is the heat-stimulated emission of light from irradiated solids, has also been evaluated as a method for identifying irradiated foods. This phenomenon is also most probably due to the presence of paramagnetic centers in the solid.

Irradiated herbs, spices and seasonings exposed to gamma ray doses from 1 to 20 kGy, emit light when heated from room temperature up to 400-500 ^{0}C with a liner heating rate of 6-8 ^{0}C s^{-1}, in a dry nitrogen atmosphere. Today, it is generally recognized that these thermoluminescence signals originate, at most, from the minute amount of mineral dust adhering to the sample surface (Goksu-Ogelman and Regulla 1989, Sanderson et al. 1989 a,b). Dating studies have shown that these minerals have thermoluminescence ability ranging from 10^3 to 10^6 photons s^{-1} mg^{-1} Gy^{-1} and therefore microgram quantities can easily yield observable signals. Sensitivity enhancements of 10^3 or more, and improved signal to blank ratios were recorded for measurements of the inorganic dust separated from the food. On the basis of isothermal decay experiments, the half-life time of the signals was estimated to about 5 years at room temperature. The measurements on separated mineral grains seem to allow reliable identification of irradiated spices with 90% confidence.

It can be expected that the use of more refined thermoluminescence evaluation methods able to resolve the different thermoluminescent components, and discriminate those without adequate dosimetric properties will result in a more reliable tool for detection and evaluation of irradiation doses in foods.

6. Release of hydrogen gas

The method is based on the fact that hydrogen gas is formed in organic substances irradiated with ionizing radiation (Dohmaru et al. 1989). Following gamma irradiation, black and white peppers were ground to powder in a ceramic gastight mill and the released gas was analyzed for hydrogen by gas chromatography. Identification of irradiated pepper with 10 kGy was possible up to 4 months after irradiation.

7. Measurements of microbiological alterations

The sensitivity of microorganisms to radiation depends on species and even on strain. Thus, Gram-negative bacteria are generally more sensitive than Gram-positive. A survival selection due to the different radioresistance of various microorganisms is expected in irradiated food. For example, the microflora on raw poultry meat shows a characteristic microbiological profile with significant numbers of Gram-negative bacteria, predominantly the genus *Pseudomonas*. By contrast, the flora which develop on a raw chicken after irradiation at a dose of 2.5 kGy are mostly Gram-positive bacteria and yeasts. The presence of Gram-negative bacterial cells, both live and dead, can be measured using the Limulus amoebocyte lysate (LAL) test which is specific to these organisms; the enumeration of the live cells can be achieved by counting colonies which grow on a selective agar. Taken together, a high LAL titre and a low Gram-negative bacterial count indicate irradiation treatment (McWeeny et al. 1991). A similar approach utilizes a comparison of an aerobic plate count with the Direct Epifluorescent Filter technique. The latter technique counts organisms differentially stained by acridine orange irrespective of viability, and the difference to aerobic plate count gives the number of organisms rendered non-viable by irradiation (Betts et al. 1988). The microbial characterization is not, however, confirmatory for irradiation since the shift in the microbial composition could be influenced by other factors, like the use of some preservatives, heat treatment, storage conditions, environmental and growing factors for fruits, etc. On the other hand, an advantage of the microbial method is that it provides additional information about the hygienic quality of the food.

The concomitant study of microbial profiles and enzyme activity patterns in fresh food (uncooked) has been proposed as a possible method to detect application of radiation. The conceptual basis of this approach is that enzymes are not inactivated by usual doses of radiation, remaining active during storage. The enzyme activity pattern of the treated food will be therefore very similar to that of the raw food, while the microbial profile will probably be different from the one expected in a raw food.

The radiation pasteurized fishery items are comparatively less susceptible to bacterial spoilage although the treatment does not hamper their ability to support bacterial growth. Thus the bacterial formation of volatiles (TVBN, TVA) which is much less in fish and shellfish irradiated at doses ranging from 1 to 5 kGy, was suggested as means to identify irradiated fish foods after intentional contamination with bacterial culture obtained from unirradiated fish samples (Alur et al 1991).

8. Measurements of biological alterations

Biological and histochemical measurements have been suggested for specific identification of irradiated potatoes (Thomas 1983) and onions (Thomas 1984). Some biological methods use the effect itself for which irradiation is used. Sprout inhibition of potatoes by irradiation is irreversible and may serve as a proof of irradiation, but the method is too slow for routine analysis (even if growth hormones are used to accelerate sprouting). The germination of half-embryo in irradiated grapefruits with doses over 0.15 kGy showed markedly reduced root growth and shoot elongation (Kawamura et al. 1989 a,b). Enzymatic changes in irradiated potatoes could be histochemically visualized for a few weeks with a tetrazolium stain (Jona and Fonda 1990).

9. Other physical measurements

Measurement of electrical conductivity seems to be a rapid method for identification of irradiated potatoes. Using this method, after storage for up to 6 months, unirradiated and irradiated potatoes could be differentiated and the applied dose estimated. For different potato varieties, an at least 75% probability was achieved in detecting radiation processing. The biological-physicochemical effect underlying the method has not been explained. Electrical conductivity measurements of other vegetables showed no consistent alteration by irradiation (Hayashi and Kawashima 1983). Electrical resistance measurements were suggested for identification of irradiated fish (Ehlermann 1972)

Viscosity measurements show some promise (Farkas et al. 1990) though the effects observed are not understood nor are they consistent from one product to another, i.e. in some materials the viscosity decreased after irradiation but increased in other materials. There is no correlation between the observed effects and the starch content of the food.

Differential scanning calorimetry revealed changes in initial freezing point or of heterogeneous nucleation temperature, for food products submitted to irradiation.

CONCLUSIONS

The lack of an absolute irradiation test is a paradoxical reality. On the one hand, the inability to detect irradiated food is the strongest proof that the food is safe; on the other, the absence of a surveillance test impedes acceptance of irradiation treatment.

Foodstuffs have different chemical compositions and physical states; the irradiation doses which can be applied, alone or combined with thermal treatments, vary in intensity according to the desired effect. Even within the same kind of food there are differences due to biological variability, growth conditions, state of maturity, or those caused by processing methods and storage. Therefore, it is unlikely that one method can

be universally applicable for all irradiated foods. It becomes more and more clear that only a combination of analytical methods can solve the problem of detection, both from scientific and practical points of view. A rapid screening method which is of low cost and relatively undemanding in skills and facilities should be followed by a more refined, reliable confirmatory test, even if it is more time consuming and demanding specialized skills and facilities. Modern methods of multicomponent analysis combined with multivariate statistical evaluation might be a solution to this complex problem.

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