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Physical Methods for the Identification of Irradiated Food
-Review-

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Abstract

The development of methods for the identification of irradiated foods helps enforce national and international regulations on labelling to ensure the consumer's free choice to buy irradiated or unirradiated foods, and the availability of such methods may assist the promotion of international trade in irradiated food products and help prevent abuse of the technology. A number of approaches to determine the physical, chemical, microbiological and biological changes that occur in foods treated with ionizing radiation have been studied. However no single method is universally applicable. Among physical measurements, the leading methods of identification are electron spin resonance (ESR) spectroscopy and thermoluminescence (TL). ESR is an established non-destructive method for the analysis of free radicals from their traps and TL is the emission of light from irradiated mineral extracts by heating. Viscosity of carbohydrate polymers by causing chain breaks by irradiation, measuring the impedance of potatoes and detection of gases produced radiolytically are promising techniques for identification purposes. Irradiated water-containing foods show significant supercooling when monitored with a differential scanning calorimeter (DSC), which can be applied to identifying irradiated ones.

Key words : ESR, TL, viscosity, supercooling

INTRODUCTION

Treatment of foods by ionizing radiation is considered as an important method to prevent food spoilage and food-borne diseases. It can prolong the shelf-life of products, used for the disinfestation, and the reduction of microbial contamination, reduce health hazards which might be caused by pathogenic microorganisms and prevent sprouting or delay ripening of fruits and vegetables (1).

Suitable techniques for the detection of irradiated food and the determination of the applied dose requirements are needed to guarantee proper customer information, to prevent misuse of the technology and to facilitate trade in irradiated foods. In connection with the ongoing world-wide application of food irradiation in the 1980's, identification of these food products for control to ensure safety health standards and proper trade regulations became very important (2).

Identification of irradiated food was discussed in detail in three international meetings. Two meetings were organized by the Commission of the European Communities: the first colloquium was held in Luxemburg in 1970, the second in Karlsruhe in 1973. A third meeting was organized by the WHO in cooperation with the German Federal Health Office (BGA) in Munich in 1986. Activities in the field of irradiated food detection have been intensified in the 1990's. Governments encouraged research on methods of identification, and international research activities were sponsored by the Bureau of Reference of the European Community (BCR) and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, known as the ADMIT (Analytical Detection Methods for Irradiation Treatment of Foods) program (3).

When food is treated with ionizing radiation, free radicals are formed. A radiation dose of 10 kGy generates 5×10^{-3} mol of free radicals per kg of food. Free radicals usually disappear extremely fast as they react with each other and with molecules of the food matrix, because of their high reactivity and mobility provided by the water present. However, the stability of organic radicals produced by irradiation are increased if the unpaired electron is incorporated into the complex polymeric system, as in peptides and polysaccharides, and is structurally isolated from the water (4).

A number of approaches to determine the physical,

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chemical, microbiological and biological changes that occur in foods treated with ionizing radiation have been studied. However no single method is universally applicable. Among physical measurements of radiation-specific radicals, the leading methods of identification are electron spin resonance (ESR) spectroscopy which detects radicals in the dry components of foods such as the bones of chicken meat and trout as well as the shells of pistachio nuts, and thermoluminescence (TL) techniques which detects irradiated spices and herbs, their mixtures and fruits and vegetables. The above tests require relatively sophisticated equipment or technical skills and are often time-consuming and costly. Relatively simple tests which could be used as a rapid screening system or confirmatory method are also available (5,6).

ELECTRON SPIN RESONANCE (ESR) SPECTROSCOPY

ESR is a spectroscopic method allowing the observation of unpaired electrons, especially free radicals (paramagnetic centers) induced by irradiation. The samples may be measured in powdered form or as bone fragments. The sample is placed in a standard ESR tube (7).

An intense external magnetic field produces a difference between the energy levels of the electron spins $m_s=+1/2$ and $m_s=-1/2$, leading to resonance absorption of an applied microwave beam in spectrometer. ESR spectra are conventionally displayed as the first derivative of the absorption with respect to the applied magnetic field and it is helpful to measure the field and frequency ratio (g values) for an identification of irradiated samples(Table 1). The radiation-induced ESR signal has a characteristic shape, and an improved dose quantification of individual sample is possible by serial addition of known doses to construct a calibration curve (8).

The technique is a non-destructive, specific, rapid method with trained personnel and can detect very low doses, and simple and lower priced equipment is available with high sensitivity and reliability (e.g. Bruker EPR spectrometer) (9). Using a time constant and sweep rate appropriate for an ESR signal with a peak to peak linewidth of approximately 0.4 mT for bones or 0.8 mT for cellulose, the following ESR spectrometer settings are found to be satisfactory (8).

ESR provides an excellent method for the identification

Table 1. ESR spectrometer settings and calculation of the g value

- MICROWAVE RADIATION : frequency, 9.5 GHz, power 5 mW to 12.5 mW for bones, 0.4 mW for nuts, to 0.8 mW for paprika powder.
- MAGNETIC FIELD : center field, 342 mT for bones or 348 mT for cellulose, sweep width, 20.0 mT.
- SIGNAL CHANNEL : modulation frequency, 50 kHz or 100 kHz, modulation amplitude, 0.2 mT to 0.4 mT for bones, 0.4 mT to 1 mT for cellulose, time constant, 50 ms to 200 ms, sweep rate, 2.5 mT min^{-1} to 10 mT min^{-1} or accumulation of 3 to 5 spectra at greater sweep rate shorter time constant.
- RECEIVER GAIN : between about 1.0×10^4 and 1.0×10^6.
- TEMPERATURE : room temperature.

Irradiated samples containing bones show a typical asymmetric signal due to hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] free radicals having g values of 2.002 and 1.998. A low-intensity symmetrical signal having a g value of g_{symm}= 2.005 may be present in the ESR spectra.

Using the measured values obtained, the g value (g_{signal}) can be calculated using the following equation:

$$g_{signal} = \frac{71{,}448 \cdot \nu_{ESR}}{B}$$

where:

ν_{ESR} is the microwave frequency, in gigahertz (GHz);
B is the magnetic field (magnetic flux density), in millitesla (mT) (10 Gauss=10 Gs=1 mT).

The following values are found in bones:

g_{symm} = 2.005±0.001 (no proof of irradiation), peak-to-peak linewidth = ΔH~0.6 mT;
g_1 = 2.002±0.001 (irradiated);
g_2 = 1.998±0.001 (irradiated)

of irradiated foods containing bones or calcified cuticle, such as crustacea, some fruits, foods that contain shells, and several spices. As the irradiation dose increases, the number of free radicals trapped in the crystalline hydroxyapatite lattice of the bone increases, so it is feasible that the technique could be used to estimate the irradiation dose to all meat and fish species containing bones (Fig. 1) (10).

The shell spectrum is rather complex consisting of a number of radicals because of chitin and Mn^{2+} signals. However, it is possible to identify it with available software to isolate the signal induced by irradiation (11).

An ESR signal ascribed to a cellulosic free radical can be observed in solid parts of fruit (seeds, pips, stones, peel) or in dried fruit. The irradiated samples reveal ESR spectra consisting of a strong central signal, also present in unirradiated samples, and two relatively small signals from cellulose radical on both sides of the main signal,

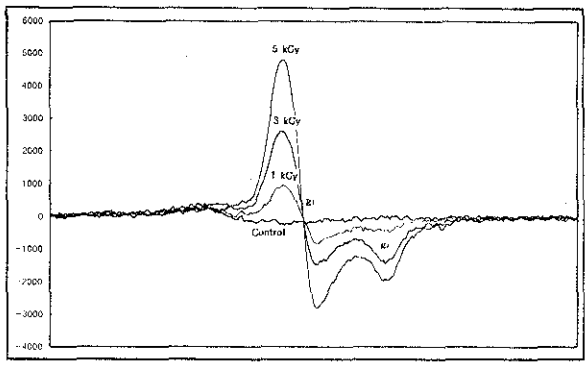

Fig. 1. Typical ESR spectrum of unirradiated and irradiated food samples.

only observed symptomatically in irradiated samples with a spacing of about 6.0 mT. This phenomenon can be extrapolated to many other vegetable products such as spices and dried fruits and vegetables (12).

A dose estimation can be obtained by re-irradiating the sample repeatedly with a known dose and measuring the ESR signal intensity at each dose interval, thus generating an exponential dose-response curve. Extrapolation of the curve to the negative dose axis yields an estimate of the initial absorbed dose to the sample (13).

CHEMILUMINESCENCE (CL)

When irradiated foods are heated (thermoluminescence: TL) or stimulated by light (photostimulated luminescence: PSL) or dissolved in a liquid (lyoluminescence) with a chemical amplifier, luminol (chemiluminescence: CL), they emit light (14).

For the analytical investigation of CL, a dry substance (about 10~20 mg) is weighed into a disposable cuvette, a freshly prepared luminol-hemin solution is injected (about 0.2 ml), the CL response on dissolution being immediately measured by a sensitive light detector, and the signal being recorded during the first 5 sec., registered integral and maximum values (15).

While the CL method is very fast and easy to perform, it is only used to screen foods for irradiated products as extensive studies on spice products revealed large inter-sample variations.

THERMOLUMINESCENCE (TL)

The physical process of thermoluminescence is based

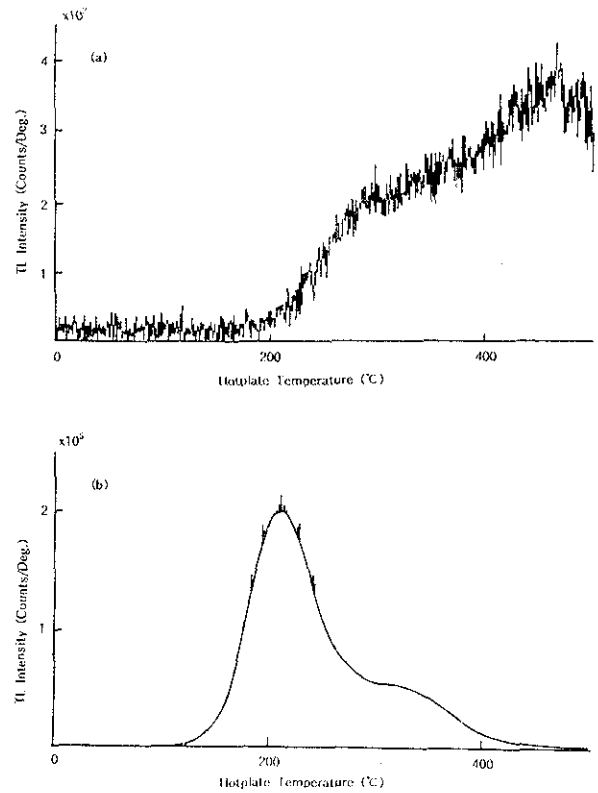

Fig. 2. Typical TL glow curve of unirradiated (a), and irradiated (b) samples.

on electrons transferred to an excited state by ionizing radiation and returning to a ground state emitting light when thermally stimulated. The TL signal is due to extraneous inorganic matter adherent to the sample surfaces. Simple sieving or separation of organic and inorganic components by centrifugation in high-density (2 g/ml) sodium polytungstate ($Na_5[H_2W_{12}O_{40}]xH_2O$) solution permits TL measurements to be carried out at the separated mineral phase (16). Grain sizes lower than 50 μm are preferred for a high reproducibility. For the determination of luminescent signals, sample carriers are placed on the planchet of a TL reader with a heating chamber which can be evacuated and flushed with an inert gas. The material (about 3~20 mg) is heated at a constant rate of 5~10°C/sec, up to a final temperature of 300~400°C, and the light emission is registered by a detector (17).

A graph of measured light output against temperature or time during heating is known as a glow curve(Fig. 2). The integral light intensity under the glow curve is measured, the reheated samples are used as the reference value, and the background black-body emission is subtracted. A second TL measurement of the same sample

after exposure to a known dose of radiation is necessary to normalize the TL response, since various amounts or types of silicate minerals (e.g. quartz, feldspar) exhibit variable TL intensities after irradiation. The TL glow ratio, thus obtained, is used to indicate the irradiation treatment of the food, since the population of irradiated samples yields higher TL glow ratios than that of unirradiated samples (6,7). Glow curve shape parameters offer additional evidence for identifying irradiated foods (18).

Though TL analysis may be applied to detect irradiation of any food from which silicate minerals can be isolated, the TL method is suitable for the clear identification of irradiated spices and herbs. With the exception of seasoning mixtures containing salt which produced their own glow shape, all samples showed similar characteristics to those associated with feldspar and polymineral samples in TL dating. Many samples could be correctly identified as irradiated or unirradiated on the basis of signal strength alone. In mineral phases, TL sensitivity was enhanced by 10^3 or more and signal-to-blank ratios were improved (19). The TL procedure proved to be a good detection method, at least for herbs, spices, berries, mushrooms and seafood.

A European Standard for the detection of irradiation treatment of food by TL analysis of contaminating silicate minerals was formulated for spices, herbs, their mixtures and shrimps by the member states in 1996. The technique being simple, quick and requiring only small samples in the order of 100 mg or less can be regarded as a very sensitive physical method to identify irradiated foods available today (20).

PHOTO-STIMULATED LUMINESCENCE (PSL)

A luminescence signal could be obtained from quartz using optical rather than thermal stimulation. This process is known as optically stimulated or photostimulated luminescence (PSL). Samples such as herbs, spices and seasonings are introduced in disposable petri dishes, with no other preparation and the instrument produces a qualitative screening measurement in 15 seconds through a front panel display.

PSL is used in a larger number of applications than TL because of the comparative ease of measurement and the fact that PSL measures only the most light-sensitive portion of the luminescence signal from a sample (8). A novel low-cost PSL offers the advantage of direct and fast sample measurements of irradiated herbs and spices, as there is no need for sample preparation or reirradiation as required by TL. The TL and PSL methods are powerful techniques for the identification of irradiated food (20).

VISCOSITY MEASUREMENT

Ionizing radiation alters the rheological properties of macronutrients in food, resulting in the increased solubility of starch in water and decreased swelling power and viscosity which depends mainly on the composition and the amount of starch, pectins and cellulose. The ratio of viscosity to starch content is used for the identification of irradiated spices (21).

Measurement of viscosity is done at 20°C by preparing 300 g of a 10~20% spice suspension with a Brookfield viscosimeter and at the speed of 50.0 rpm. If necessary, 10 ml of 33% sodium hydroxide (NaOH) is added and the homogenate is heated to 90°C under occasional stirring to reach the gelatinization optimum. For each measurement, 30 g of heat gelatinised suspension is used. The time for the reading to stabilize is 30 seconds (22).

Other viscometers such as a Newport Rapid Visco Analyzer and a Brabender Rapid Amylogram can be used to study the viscosity. The damaged starch can be determined colorimetrically, the starch content by the gelatinization thermograms. A normalized parameter, such as (viscosity/starch amount) of a 10% suspension of pepper is better for detecting irradiation treatment than a viscosity value itself. The method offers a means for rapid detection of irradiated black pepper in a cost-effective way (23).

ELECTRICAL IMPEDANCE

Irradiation may cause changes in the electrical properties of foods. Membranes of living tissue, regardless of plant or animal origin, play a vital role for the selective transport of ions. Ions and charged molecules oscillate in response to the applied field. A consequence of the modification in cells caused by ionizing radiation is the decrease of the electric conductivity of irradiated tubers (24).

The proposed value to be used as an identifying parameter was the Z_{k50}/Z_{k5} ratio. Impedance ratio at 50 kHz to 5 kHz is the most favourable index. The impedance

at 5 kHz and 50 kHz is measured to determine the impedance ratio at 5 kHz to 50 kHz (Z_{5k}/Z_{50k}) of alternating current with the aid of two stainless-steel electrodes having 1 mm diameter, 10 mm long and 10 mm apart, with which an apical region of potato tuber is punctured (25).

The impedance ratio Z_{5k}/Z_{50k} is free from the influence of the type of the electrodes and the impedance ratio measured at the apical region shows the largest difference between unirradiated and irradiated potatoes, irrespective of potato cultivar and planting locality (26).

GAS EVOLUTION

The measurement of gases produced radiolytically and trapped within the food is a promising screening test in the detection of irradiated food. Hydrogen (H_2) produced by irradiation is highly diffusible and its rapid loss from foods may render it less useful as a long term marker for irradiation (27). However, a novel gas sensing module warrants investigation. Irradiated pepper is evaluated by hydrogen detectors. The probe consists of a palladium-coated field-effect transistor and is adequate to monitor the head space of irradiated food, especially offering a test for irradiated frozen food in which positive detection gives conclusive evidence of irradiation (28).

For experiment, the sample is rapidly thawed in a microwave oven (15 sec. at 700 Watt) and about 3~4 ml of head-space is drawn off into a syringe. The H_2 and CO concentrations are measured in a single analysis using a Gas Chromatograph with a 72×1/8 inch column packed with a 5A molecular sieve at 100°C. Samples to be measured are injected into a 1 ml sample loop and dried via passage through 1/16 in. tubing immersed in dry ice-ethanol at 70°C. The carrier gas is zero air, free of H_2 and CO. The limits of detection are about 50 ppb and 20 ppb by volume, respectively (29).

Detection of trapped radiolytic H_2 and, particularly, CO can be a rapid (less than 5 min per sample) test for poultry over the dose range relevant commercially and for long storage periods at temperatures up to -15°C. However, its usefulness may be restricted to a limited number of frozen and possibly dry foods (30).

The use of multiple gas sensors increases the reliability of the test, which is cheap, rapid and easy to perform.

SUPERCOOLING

Ice nucleation temperature depends on many factors such as cooling rate, number of potential nucleation sites within the sample and nature of the contact surfaces. Irradiation modifies the number or sizes of the nucleation centers in water-containing biological tissue. This could happen through sorption of the products of radiolysis (e.g. free radicals) on ice nucleation sites, rendering them inactive. This phenomenon can be applied to detect high water-containing foods which are difficult with other methods (31).

The freezing and thawing onset temperatures of the foods are measured using a differential scanning calorimeter (DSC) with a low temperature cooling accessory. Subsamples of 5~10 mg are excised from the 100 g samples using scalpel and tweezers. The DSC subsamples are encapsulated between two aluminium pans using a sample press. The weighing and encapsulating takes approx. 40 seconds. At the instant of nucleation, samples (visible under a thin layer of silicone oil) increase their light reflectance markedly and an ordinary video camera can record these changes (32).

Two successive DSC scans cooling from 5 to -55°C and heating from -55 to 5°C are made on each subsample with 5°C/min of scanning rate, -100°C of DSC block temperature and nitrogen gas purge. Each scan is analysed using software, yielding the supercooling temperature (onset of freezing) and the onset of thawing. The onset temperatures are defined as the intersection of the tangent to the transition peak at its steepest point with the baseline (31).

The measured supercooling temperatures are subject to a large stochastic variation and the data for irradiated and unirradiated foods overlap considerably. Frozen, irradiated cod, mushrooms and chicken flesh show significantly greater supercooling. However there is high variability between samples and a possible dependence on spoilage which requires further investigation for devising a practical method of detection of irradiated foods. Increasing the sample volume would reduce the variance. Due to the stochastic variation in nucleation temperatures, multiple measurements are needed to detect irradiated foods. The ice nucleation method is potentially an inexpensive screening test for irradiated foods (1).

WETABILITY

Since rehydration of dry foodstuffs is frequently improved by irradiation, few experiments with the wetability of spices are carried out. The wetability of pepper is measured by dipping a layer of pepper of defined thickness, positioned on a sieve, into water, estimating the time after which the total layer of pepper is moistened. The time needed to moisten the pepper was reduced from 120 sec for the unirradiated sample to 95 sec for the irradiated pepper. However, the effect of reducing time is not pronounced enough to enable identification (17).

CONCLUSION

Free radicals induced by irradiation remain stable and persist for longer periods in dry foods for instance spices and herbs, dehydrated vegetables, and cereals and in parts drying foods, such as bone in meat and fish, shell of mollusks or crustacea, or stones and seeds in fruits, and may be used for identification by direct ESR method and indirect TL technique, but not on wet material. The other physical methods reviewed such as supercooling hold great potential as identification methods of high water-containing foods, thus enhancing consumer's confidence.

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