COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Detection Methods for Irradiated Foods

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ABSTRACT: Proper control of irradiation processing of food is very critical to facilitate international trade of irradiated foods and to enhance consumer confidence, consumer choice, and safety. Analytical detection of radiation-processing of food is very important to implement quality control at all levels. An ideal detection method should measure a specific radiation effect, which is proportional to the dose and should not be affected by processing parameters and storage conditions or the length of time between irradiation processing and analysis. The detection of irradiated foods is mainly based on radiolysis of lipids, modification of amino acids, modification of DNA, modification of carbohydrates, formation of free radicals, release of hydrogen gas, alterations in microbial load, measurement of biological difference, and other physical methods.

Introduction

Irradiation has recently become one of the successful techniques to preserve food with minimum interruption to the functional, nutritional, and sensory properties of food products. This processing of food involves controlled application of energy from ionizing radiations such as gamma rays, X-rays, and electron beam for food preservation. Irradiation preserves the food by disrupting the biological processes that lead to decay of food quality. Radiation interacts with water and other biological molecules in a food system and produces various radiolytic products, which generally act as oxidizing agents and can cause several changes in the molecular structure of organic matter. Radiations also damage DNA molecules effectively, so living cells such as in microorganisms, insects, and gametes are prevented from reproduction, resulting in a preservative effect. Irradiation, like other processing techniques, results in physicochemical changes in food products.

The nature and extent of these changes depend on the kind of food subjected to irradiation and the irradiation dose. Irradiation involves exposing the food, either prepackaged or in bulk, to a predetermined level of ionization radiation. Almost 40 countries, including India, have approved the use of irradiation for over 100 food items, but in some countries it is prohibited. Table 1 shows the current uses of food irradiation by processing industries and institutional catering worldwide (Farkas 1988; Kilcast 1995). In recent years, the volume and number of irradiated food products introduced in the market have grown steadily. As a result, consumers and legislative authorities demand clear labeling of irradiated foods. Presently, the labels of irradiated food products indicate the treatment and purpose of irradiation.

The food irradiation policies vary from country to country. The U.S. Food and Drug Administration (FDA) regulates all aspects of irradiation, such as irradiation dose, product type, and labeling requirements. Foods permitted for irradiation under FDA's regulations are shown in Table 2 (Morehouse 2002). The U.S. Dept. of Agriculture (USDA) is responsible for the inspection and monitoring of irradiated meat and poultry products, as well as for the enforcement of FDA regulations related to these products. Since 1986, it has become mandatory that all irradiated products must carry the internationally accepted radiation symbol "radura." The development of analytical methods for correct identification of irradiated samples from nonirradiated samples has thus become important for upholding regulatory controls, checking compliance against labeling requirements, facilitating international trade, and reinforcing consumer confidence. Regulatory authorities in all countries are interested in having reliable methods to detect irradiated foods as well as estimation of dose.

Prior to 1980, limited work was carried out for the development of reliable detection methods for irradiated foods. After 1980, extensive research was undertaken which resulted in the development of a range of test methods that can be used to reliably determine the irradiation status of a wide variety of foods. During 1996, the European Committee for Standardization (CEN) adopted 5 European standards for detection of irradiation process in food commodities, EN-1784 to EN-1788, and in 2004 5 more validated standard methods, EN-13783, EN-1384, EN-14596, EN-13708, and EN-13751 came in to existence (Stewart 2001) (EN 13708:2001, 13551:2002, 13783:2001, 13784:2001, 14569:2004, 1784:2003, 1785:2003, 1786:1996, 1787:2000, 1788:2001, and 14569:2004). The methods used for the detection of irradiated foods are based on physical, chemical, biological, and microbiological changes in food products during irradiation, although these changes are minimal.

Physical methods

The physical methods measure the effects of the radiation-generated radicals or trapped electrons in the solids. These methods may either leave free radicals and electrons unchanged or stimulate some of the electrons and measure their absorbed radiation energy. They are practically involved with the radiation-generated defects by dissolution of the solid substances.

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Table 1—Some examples of current uses of food irradiation in different countries.

Region	Country	Food	Maximum dose (kGy)
The Americas	USA	Pork	1.0
		Poultry	3.0
	Canada	Potato	0.1
		Spices	10.0
	Brazil	Strawberries	3.0
		Fish	2.2
Europe	France	Camembert cheese	3.5
		Egg white	4.0
	The Netherlands	Frog-legs	5.0
		Dried fruits	1.0
	U.K.	Roots and tubers	0.2
		Shellfish	3.0
Asia and Europe	China	Garlic	0.1
		Rice	1.0
	Thailand	Mango	1.0
		Fermented sausage	4.0
	South Africa	Avocado	3.0
		Fruit juice	3.0

(Source: Farkas 1988; Kilcast 1995).

Table 2—Foods permitted for irradiation under FDA's regulations.

Food	Purpose	Dose (kGy) (maximum limit)
Fresh pork	Control Trichinella spiralis	0.3 to 1.0
Fresh food	Growth and maturation inhibition	1.0
Dry enzyme preparation	Microbial disinfection	10.0
Dry spices and seasoning	Microbial disinfection	30.0
Poultry	Pathogen control	3.0
Frozen meat (NASA)	Sterilization	44.0
Refrigerated meat	Pathogen control	4.5
Shell eggs	Pathogen control	3.0
Seeds for sprouting	Pathogen control	8.0

(Source: Morehouse 2002).

Electron spin resonance spectroscopy (ESR). The ESR principle is based on the quantum theory and it detects the irradiation-produced long-lived paramagnetic active sites of the free radicals in the organic and inorganic complexes possessing a transition metal ion (Weil and others 2001). In free radicals and in other paramagnetic species, the unpaired electrons are trapped at different defects (vacancies and interstitials) of the crystal lattice. The other electrons may be trapped by anions of the crystal-forming anionic radicals with unpaired, paramagnetic electrons (Anderle 1997). These trapped electrons can exist in the native state, and in the presence of a magnetic field will align themselves in such a way that their magnetic moment is either parallel or antiparallel to the magnetic field. These 2 configurations are of unequal energy and the electron can be excited from a lower energy level to a higher energy level by the absorption of microwave energy. The electron has a spin that has angular momentum leading to magnetic moment. Consequently, the negative charges on the electron are spinning and constitute a circular electric current. An unpaired electron can move between the 2 energy levels by either absorbing or emitting electromagnetic radiation of energy, $\varepsilon = h\nu$, such that the resonance condition is $\varepsilon = h\nu$. In a magnetic field these electron spins are oriented "up spin" or "down spin," which is responsible for the circulating current. This current induces a magnetic field, causing the electron to experience a torque tending to align the magnetic moment with the field. Every electron has a magnetic moment and spin quantum number, $s = 1/2$, with magnetic components $m_s = +1/2$ and $m_s = -1/2$ (Figure 1). In the presence of an external magnetic field with strength B_0, the electron's magnetic moment aligns itself either parallel ($m_s = -1/2$) or antiparallel ($m_s = +1/2$) to the field, each alignment having a specific energy. At this point the unpaired electrons can move between their 2 spin states. Since there typically are more electrons in the lower state, due to the Maxwell–Boltzmann distribution, there is a net absorption of energy, and it is this absorption that is monitored and converted into a spectrum. In real systems, electrons are normally not solitary, but are associated with one or more atoms. The interaction of an external magnetic field with an electron spin depends upon the magnetic moment associated with the spin, and the nature of an isolated electron spin is such that 2, and only 2, orientations are possible. The application of the magnetic field then provides magnetic potential energy that splits the spin states by an amount proportional to the magnetic field. The ESR signal intensity is approximately proportional to the total irradiation dose between background signal and the saturation dose. This analytical approach requires simultaneous analysis of a control or nonirradiated food sample. The basic physical concepts of ESR are analogous to those of nuclear magnetic resonance (NMR). The ESR spectrometer (Figure 2) (Simovic 2004) consists of monochromatic microwave system, electromagnet, detector, wave-guide, and cavity. The monochromatic system contains a klystron/Gunn diode and Gunn oscillator as a source of microwave. The frequency used is about 9.1 to 9.7 GHz for the x-band cavity. The electromagnet is used to generate and modulate a uniform magnetic field of several thousand Gauss. A diode of crystal rectifier is used as a detector. A rectangular open-ended metallic tube known as wave-guide is used as a medium for electromagnetic wave propagation. A metallic-type cavity is used to keep the sample.

Application of ESR in food analysis

ESR has been accepted as a standard method (Committee Europe de Normalization [CEN]) in the EU Community. It has been applied for the detection of irradiation in a wide variety of foods. ESR has become increasingly popular all over the world, because of its nondestructive testing nature, specificity, rapidity, and simplicity to detect radicals in irradiated foods.

ESR has been utilized to detect the presence of radiation-induced free radicals in bone since the mid-1950s (Gordy and others 1955). Detection of irradiation treatment by this method is difficult in foods with high moisture content because free radicals produced during the irradiation process disappear very rapidly. In foods with bone, seeds, shells, and so on, having reduced moisture content radicals remain sufficiently stable and can be easily detected by ESR (Desrosiers and Simic 1988).

Onderdelinden and Strackee (1974) suggested that ESR spectroscopy had potential as a method for the detection of irradiated food containing bone. It has been shown that nonirradiated bone gave a weak broad signal, which increases in magnitude if bone is ground to a powder (Marino and Becker 1968). This method can be utilized in the meat industry. In radiation-treated food such as bone tissue, 2 prevailing types of paramagnetic species have been observed. One is derived from bone collagen and the other is due to structural defects in the crystalline fractions of minerals present in bone such as hydroxyapatite [$Ca_{10}(PO_4)_6$ $(OH)_2$] (Ostrowski

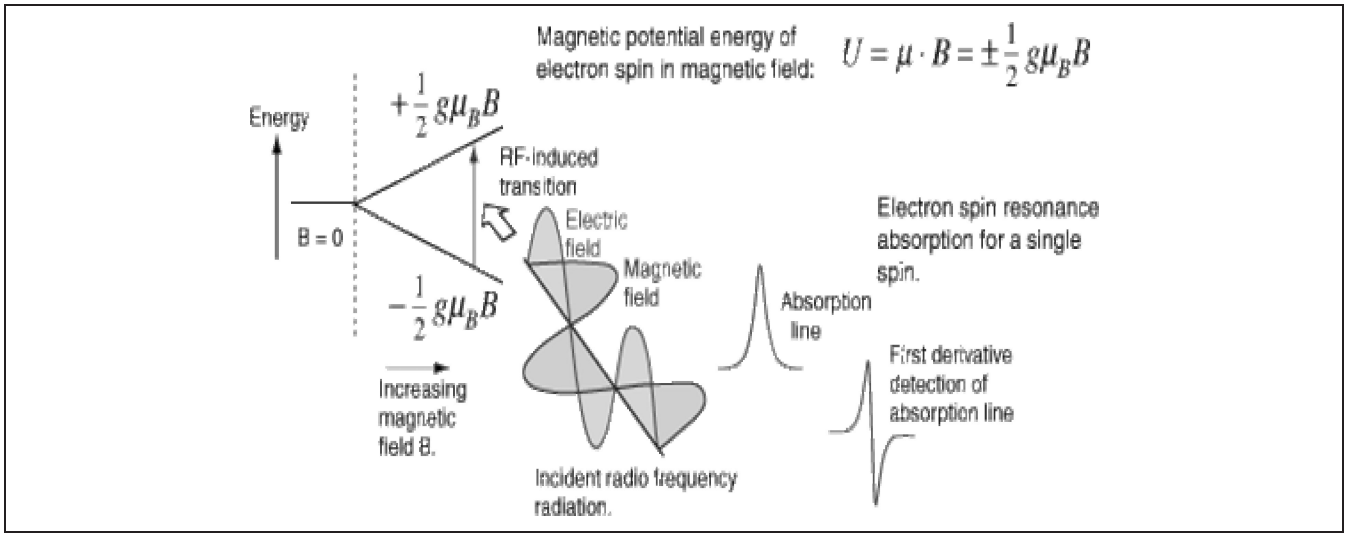

Figure 1—Energy level of electron in magnetic field (http://www.nationmaster.com/encyclopedia/Electron-spin-resonance).

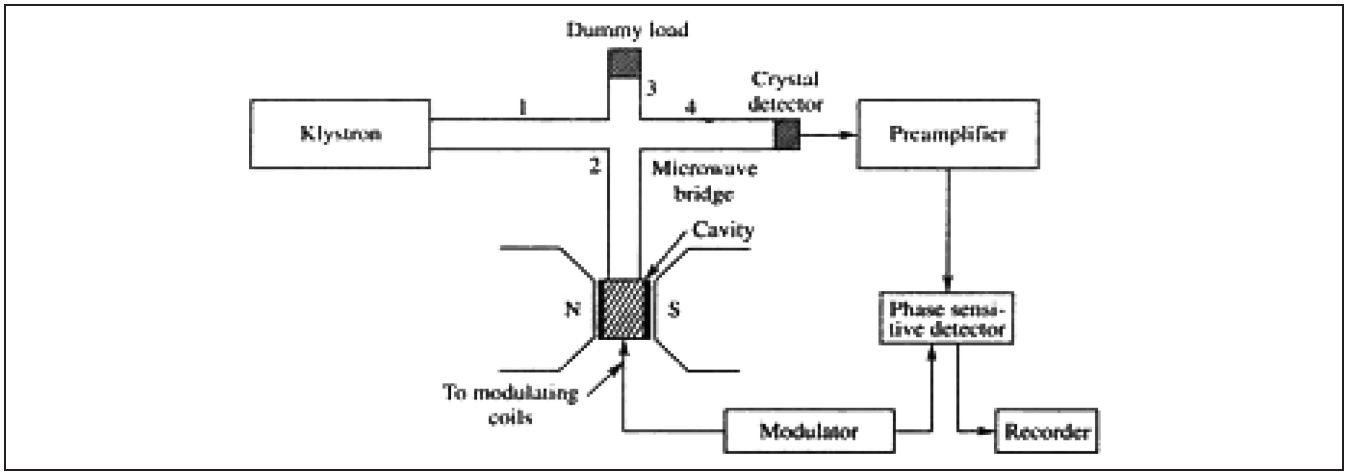

Figure 2—Block diagram of simple ESR spectrometer (adopted from Aruldhas 2006).

and others 1974; Sin and others 2005). The 1st paramagnetic species is slowly decayed by atmospheric oxygen, while the 2nd species is extremely stable at room temperature throughout years of storage. The characteristic signal generated during irradiation of bone is due to CO_3^{1-}, CO_3^{3-}, and CO_2^{1-} ions that are trapped in the hydroxyapatite matrix (Serway and Marshall 1967; Cevec and others 1972; Gray and Stevenson 1989). The signal patterns produced during irradiation in all bones are the same, thus it is evident that ESR can be used for qualitative detection of irradiated foods containing bone. Further, the intensity of the ESR signal increases linearly with the applied dose (Chawla and Thomas 2004) and the relationship still holds when corrected with standard ash, calcium, or phosphorus content. With chicken, the observed ESR signal intensity was found to be varying depending on the age of the chicken and which bone was monitored. These differences were assumed due to variation in the crystallinity of the bone (Ostrowski and others 1980; Glimcher 1984; Gray and others 1990). A comparison of the radiation-induced ESR signals for the bones of pork and salmon showed greater signal intensity, and subsequently, X-ray diffraction patterns showed that hydroxyapatite of pork bones was more crystalline than that of salmon bones (Goodman and others 1989).

Dodd and others (1985) and Goodman and others (1989) have demonstrated that ESR spectroscopy can also be used for irradiated crustaceans using signals induced in the exoskeleton or shell because they have very low moisture content. Desrosiers (1989) and Stewart and others (1994) reported that the aragonite or calcite minerals in shells were responsible for signal production. The ESR spectra produced by crustaceans are dependent on species and geographical origin of prawn and shrimp (Stewart and Kilpatrick 1997).

ESR spectroscopy can also be utilized for the identification of irradiated fruits and vegetables (Deighton and others 1993; Glidewell and others 1996). In high-moisture products like fruits and vegetables the radicals produced by irradiation are not stable; however, seeds, shell, skin, and so on having reduced moisture content can be used to detect irradiation treatment because the free radicals are relatively stable. Raffi and others (1988) first examined the ESR signal of strawberry seeds, and the signal increased with irradiation dose and was largely affected by water

content. This technique has also been used successfully for herbs, nuts, spices, and meat (Uchiyama and others 1990; Desrosiers 1991; Helle and Linke 1992; Raffi 1996). ESR spectroscopy is used for qualitative estimation because its signal and intensity are affected by a number of factors such as irradiation dose, sample site, irradiation temperature, and post-irradiation storage conditions (Stevenson and Gray 1995; Lee and others 2002).

Ukai (2004) identified the ion responsible for the ESR signal in black pepper. He reported that for the same pepper there were 4 distinct signals due to the presence of transition metal ions such as Fe^{3+}, Mn^{2+}, and organic free radicals from biochemical or radiation-induced reactions (Figure 3). The ESR spectrum of the nonirradiated pepper had shown 3 signals corresponding to Mn2+ ion, organic free radical, and Fe3+ ion. Upon irradiation, 2 new peaks were found at the symmetric positions on both sides of the organic free radical signal.

ESR can be utilized for the identification of irradiation treatment in soybean paste. Generation of signals in a protein-rich source is due to ions produced by decarboxylation and deamination of amino acids (Lee and others 2002). This technique can also be applied to packaging materials rich in cellulose (Helle and Linke 1992; Stevenson and Gray 1995).

It has been shown by various studies that ESR spectroscopy can be used for the detection of irradiation treatment in a wide variety of foods, packaging materials, and so on. This technique is rapid, specific, easy to perform, and can also be used for quantitative estimation. Although the cost is still substantial, the development of a desktop ESR spectrometer has significantly reduced the expenditure on necessary equipment and the method has become increasingly popular with food testing and control laboratories.

Luminescence techniques. It is well established that exposure to radioactivity causes things to glow in the dark. Luminescence is a genuine phenomenon, which has found applicability as a method for detecting the exposure of food commodities to irradiation. It can arise from the stimulation, either thermal or optical, of minerals that have been previously exposed to ionizing radiation. During exposure, radiation energy is accumulated and stored in the crystal lattice in the form of electrons that have been trapped at defect locations in the lattice. During stimulation, the trapped charge is released and, as a result, the luminescence signal becomes zero. Radiation-induced luminescence should be distinguished from other luminescence phenomena such as photoluminescence, phosphorescence, and others that are not dose-dependent and thus not relevant to dating or dosimetric application.

Thermoluminescence (TL). It is one of the well-known luminescent methods and represented as TL. The term thermoluminescence applies to the emission of light from irradiated solids based on the effect that a small portion of absorbed radiation energy stored at low temperature is emitted in the form of light when heated. When a substance exhibiting TL is exposed to ionizing radiation, electron hole pairs are produced which can move freely within the conduction and valance band, and some electrons or holes may become trapped at certain active sites in the material. These traps are provided by lattice defects or impurities, the fixation between the conduction and valance band is energetically metastable (Anderle 1997). These charge carriers can be captured again by traps or recombine in the luminescence center. They remain in this state until they acquire sufficient thermal energy to escape. As a material is heated, electrons are released from the trap and light is emitted as they recombine with holes. The intensity of the emitted light can be measured as a function of temperature, which is detected by a detector as a glow-curve, which is characteristic of the examined substance.

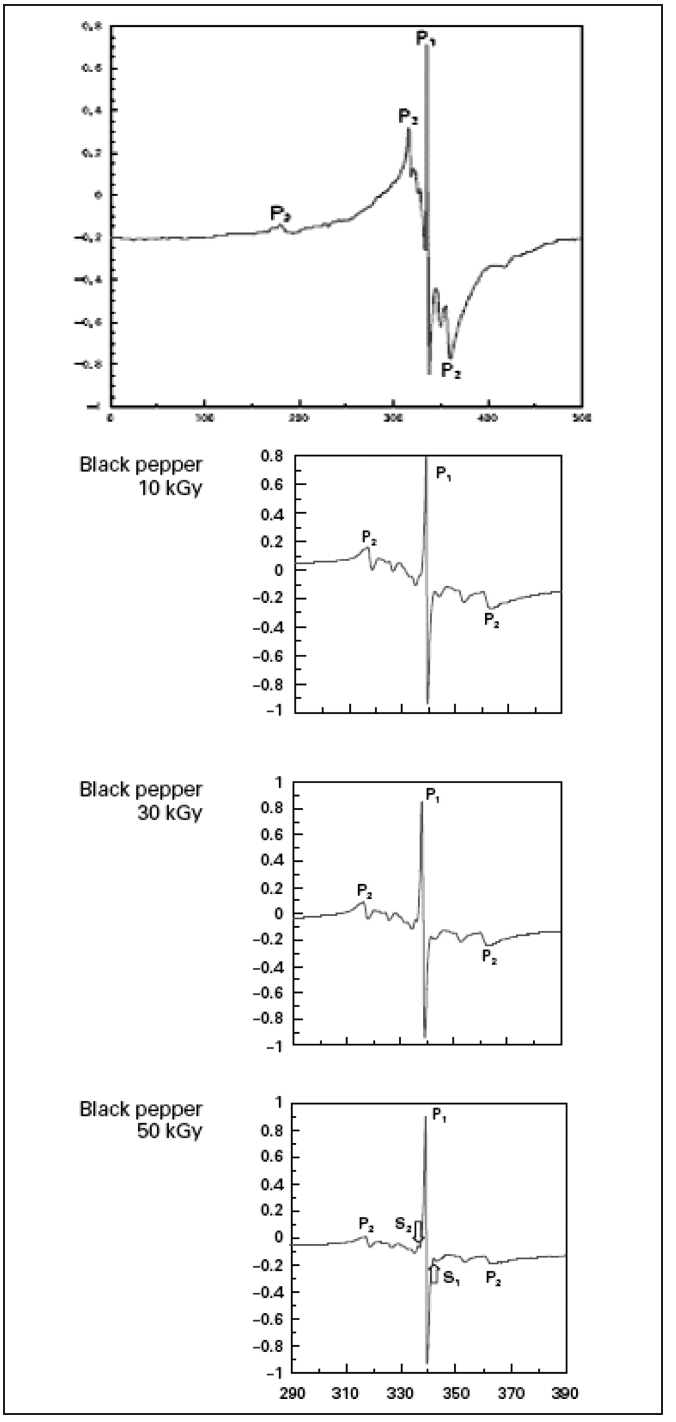

Figure 3—ESR curve for black pepper before irradiation (above) and after irradiation at different irradiation doses (below) (adopted from Ukai 2004).

Thermoluminescence equipment

Commercially available TL readers (Figure 4) comprise a heating bench, on which samples are placed, and a sensitive photon counter or photo multiplier tubes to measure and enhance the emitted TL light (Jensen 1997). The light emission is

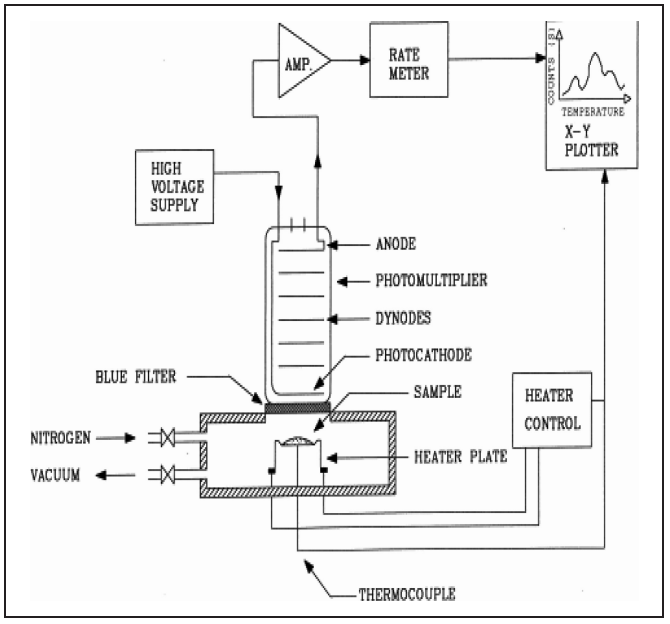

Figure 4—Schematic diagram of thermoluminescence reader system (adopted from Jensen 1997).

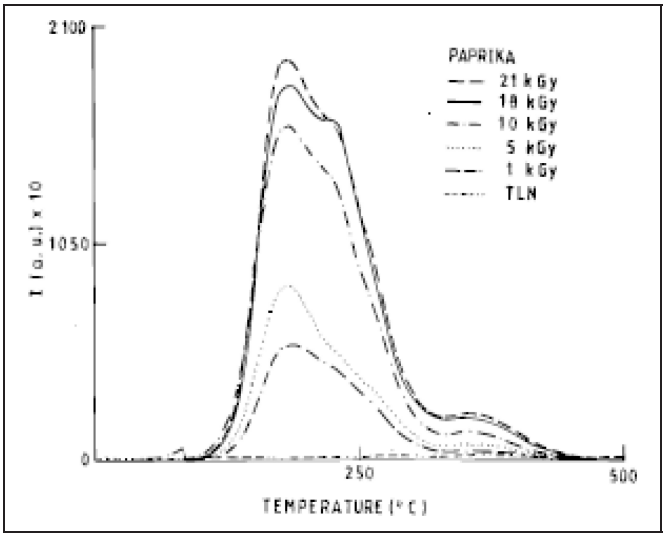

Figure 5—Thermoluminescence glow curve for paprika irradiated at different doses (1 to 21 kGy) (adopted from Correcher and others 1998).

dependent on the temperature and recorded as a glow curve. The 1st glow curve (Glow 1) is compared with a 2nd glow curve (Glow 2) obtained by a 2nd thermoluminescence measurement of the isolated minerals following their exposure to a defined radiation dose. This normalization procedure allows for differences in mineral composition. The comparison of size and shape of the 2 glow curves (plot of photon count versus temperature) reveals whether the sample from which the mineral particles were isolated has been irradiated or not (Delgado 1991). The TL glow ratio, which is the ratio of integrated TL intensities of Glow 1 to Glow 2, thus the Glow 1 area divided by the Glow 2 area, and evaluated over a defined temperature interval, is typically greater than 0.5 for irradiated and generally below 0.1 for nonirradiated samples. A prerequisite of the calculation of the TL glow ratio is that the area of Glow 2 evaluated over the defined temperature interval is 10 times higher than the minimum detectable integrated TL intensity level (MDL).

This method is found suitable for food products such as herbs, spices, bulbs, tubers, vegetables, cereals, shellfish, and fruits containing silicate minerals. It is generally recognized that these thermoluminescence signals originate from the minute amount of mineral dust adhering to the sample surface (Sanderson and others 1989). This is one of the five Codex Alimentarius method commission standard methods approved by CAC and adopted as EN 1788 European standard (EN 1996).

The initial work using TL was reported for whole samples of spices, herbs, and dates (Sanderson and others 1989; Khan and Delincee 1995). The TL signal intensity is dependent on applied irradiation dose and temperature used during irradiation. Correcher and others (1998) reported that this method could also be used to discriminate between irradiated and nonirradiated paprika (Figure 5). They have reported that polymineral composition of the dust adhered to paprika was responsible for the luminescence (mainly quartz, feldspar, and calcite). Natural TL curves of nonirradiated samples showed 3 very-low-intensity peaks, while induced TL curves of irradiated paprika have shown 5 overlapping peaks. Thus, TL spectra revealed a very important difference in intensity and position of the peaks between irradiated and nonirradiated paprika.

The TL signal is long-lived and remains reliably greater in irradiated samples than in control samples over many months (Autio and Pinnioja 1990). However, the signal has been found to diminish with time after irradiation.

Atta and others (2001) studied irradiated chicken and fish using the TL method. The samples were irradiated by a ^{60}Co gamma-source at the absorbed doses of 1, 2, 3, 4, and 5 kGy. TL response of treated and untreated samples in the temperature range of 50 to 300 °C was measured using the TL reader with a temperature profile of 10 °C/s. The results showed that TL values increased with temperature and maximum signals were obtained at 195 °C in each case. It was also observed that the TL intensities were enhanced with the absorbed doses (1 to 5 kGy) and the increase was dependent on the absorbed dose. From this study it was concluded that the TL technique is a rapid, simple, and promising method for identifying chicken and fish treated with γ-irradiation. This method can be applied for the detection of irradiation process for any food from which silicate minerals can be isolated. Its detection limit and sensitivity depend on the quantity of minerals and the way of recovery from the irradiated sample as well as glow temperature interval selected for the analysis.

Photoluminescence (PL). This technique is analogous to thermoluminescence, except using light rather than heat to release the trapped energy while still retaining inherent sensitivity and specificity of luminescence methods (Sanderson 1990). The PL method may in principle be applied to detect irradiation of any foods, which contain mineral debris, especially silicate mineral and bioinorganic material such as calcite, which originate from shells or exoskeletons, or hydroxyapatite from bones or teeth. These materials store energy in charge carriers trapped at structural interstitial or impurity sites, and when exposed to ionizing radiation optical stimulation of minerals releases charge carriers. In PL measurement, whole samples or a mixture of organic and inorganic materials can be used. Sanderson (1991) proposed this method to resolve the practical limitations of TL methods. Thus it has overcome the need for full mineral separation and providing radiation-specific stimulation schemes appropriate for biogenic materials.

In a PL measurement system a high radiation-specific UV-visible luminescence signal is used which can be stimulated using infra-radiation sources. Photo-stimulated luminescence (PSL) arises as the result of energy transfer in the form of electromagnetic radiation from a stimulation source in the equipment to the sample and the subsequent emission of luminescence from the sample. Some components of irradiated foods store energy after the exposure to ionizing radiation as a result of trapping charge carriers at structural, interstitial, or impurity sites within a dielectric medium.

Subsequent stimulation with electromagnetic radiation can release trapped charge carriers, resulting in the emission of electromagnetic radiation during subsequent relaxation. Signals from small components of associated mineral debris can be found from most foods, particularly herbs, spices, and seasonings, but also fruits and vegetables. Whole samples of herbs and spices were stimulated by a range of visible and infra-radiation wavelengths (450 to 950 nm) and luminescence detected in the near UV (300 to 350 nm). For the mineral systems, IR (700 to 1000 nm)-stimulated PSL would appear to be of greatest interest to recent events such as food irradiation (Sanderson 1991). By avoiding the introduction of luminescence generated by the heating of materials, the use of PSL allows the procedure to be simpler by eliminating the separation of the mineral contamination from the sample. The PL sensitivity depends on the quantities and types of minerals present in the sample (EN 2002). In general, calibrated PSL measurements are recommended for shellfish with low mineral contents and "clean" spices (such as nutmeg and ground white or black pepper) to avoid false negative results. Optimum results are obtained from unblended products. Compound foods such as curry powders, and their blends, may contain debris with a range of PSL sensitivities. In such cases calibrated PSL may provide ambiguous results.

Signals below the lower threshold are generally associated with nonirradiated material, but can derive from low-sensitivity-irradiated materials (EN 1375:2002). Sanderson and others (1995, 1996) have developed a low-cost photo-stimulated luminescence (PSL) for high-sensitivity PLS measurements from food samples using the highly radiation-specific UV-visible luminescence signals that can be stimulated using infrared source. Results showed that over 90% of irradiated herbs and spices could be recognized without re-irradiation (Figure 6). There was a small overlap between high-sensitivity nonirradiated samples to the known dose, and re-reading the PLS signals allows the sensitivity of samples to be estimated. Thus 2 modes of operation may be employed: screening mode for negative or positive test and a second one involving calibrated mode to distinguish between low- and high-sensitivity samples. Sanderson and others (2003) conducted trials to validate photo-stimulated luminescence (PSL) detection of 5 species of irradiated shellfish (*Nephrops norvegicus*, mussels, black tiger prawns, brown shrimps, and king scallops). Analysis of each product and treatment was performed on both whole (including shell) and intestinal samples. The results for whole samples (including shell) confirmed that the method was able to distinguish between nonirradiated and irradiated samples, regardless of dose. Intestinal data have identified that the method is dependent on the quantity and sensitivity of grit present within the intestinal tract. They reported that calibration is required where only intestinal material is available. For whole samples with shell, screening alone is adequate. The results, while confirming the validity, also confirmed the absence of false positives. Therefore, this method has been adopted as a European standard method and by the Codex Alimentarius Commission for shellfish. Reliable PL readers are available for geological, paleontological, and archeological dating, but for food application it is yet to reach practical applicability.

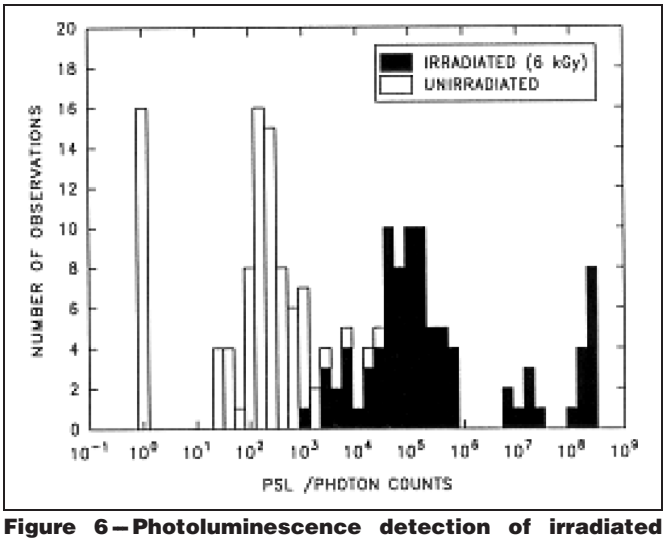

Figure 6—Photoluminescence detection of irradiated foods (adopted from Sanderson and others 1996).

Chemoluminescence (CL). It is very similar to thermoluminescence. It refers to emission of light on dissolution of a solid substance in liquid media. In this phenomenon, emission of electromagnetic radiation as light takes place when trapped energy is liberated by the addition of chemicals. Thus, it is not strictly a physical effect. Irradiated substances are dissolved in some solvent such as alkali halides in water or organic compounds like sugars, amino acids, and so on, resulting in emission of light.

Chemoluminescence usually involves the cleavage or fragmentation of the O–O bond of an organic peroxide compound. Peroxides, especially cyclic peroxides, are prevalent in light-emitting reactions because the relatively weak peroxide bond is easily cleavable and the resulting molecular reorganization liberates a large amount of energy (Lumigen 2002). This phenomenon can also be observed when irradiated aqueous acids attack calcium carbonate. Chemically enhanced chemoluminescence uses sensitizers, which give luminescence with the products formed by the reaction of radicals or electrons with a solvent during dissolution (Ettinger and Puite 1982). The most prominent chemoluminescence sensitizer is luminol (5-amino 2, 3-dihydro-1, 4-phthalazinedione), which gives a blue light when oxidized by hydrogen peroxide. There is no single mechanism responsible for chemoluminescence; it is mainly due to the production and subsequent reactions of free radicals. In the mid-1980s, when irradiation had reached a level of commercial applicability, chemoluminescence was investigated as a promising rapid and simple method for the detection of irradiated ground spices and milk powder (Bogl and Heide 1985). Several studies reported on chemoluminescence phenomena in fatty foods due to the generation of lipid peroxides. Such foods are not commercially used because samples are very sensitive toward ambient atmospheric moisture. Sometimes lipid peroxidation in spices, oils, or milk fat may lead to false positive results. Rosenthal (1993) reported that the total yield of emitted light during this phenomenon increases with the administration of radiation dose. The luminescence yield can also be increased by addition of sensitizers such as luminol or lumin, adjusted to pH 10 to 11 (λ_{max} 424 nm). Sattar and others (1987) reported on the uses of this phenomenon for irradiated pepper.

Viscosity measurement. The viscosity of foodstuffs is largely dependent on the content and structure of polysaccharides, proteins, gums, and so on, which may undergo alteration by

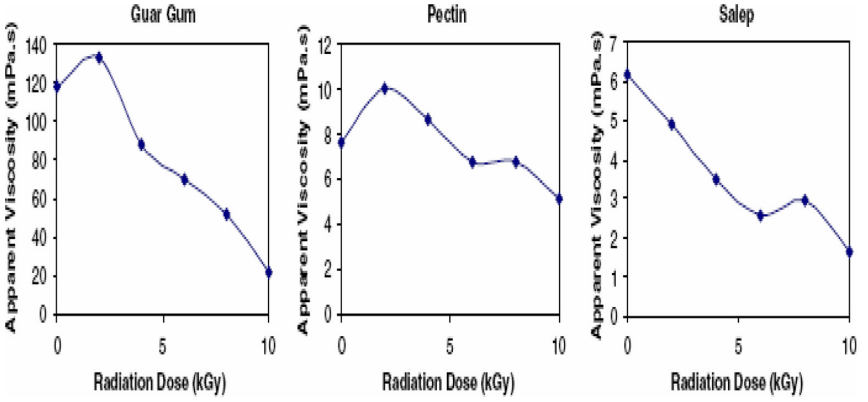

Figure 7—Effect of irradiation dose on apparent viscosity (adopted from Dogan and Kayacier 2004).

irradiation. The irradiation-induced change in these molecules is mainly chain-breaking or hydrolysis, resulting in changes in viscosity due to increased cross-linking or decreased chain length. The extent of change depends on the irradiation dose, temperature, water content, storage conditions, and atmosphere (Raffi and others 1977). Viscosity of homogenates and suspensions of biological materials in solvents like water depend on the extent of the penetration of solvent into cells, thus cell wall permeability is influenced by irradiation. In some materials viscosity increases after irradiation, whereas in others it decreases. Dogan and Kayacier (2004) reported that the viscosity and consistency of solutions decreased with increasing radiation dose in salep solution.

Dogan and others (2004) reported similar findings for different food hydrocolloids such as pectin, salep, and guar gum (Figure 7). Dwight and Kersten (1938) reported that the gelling capacity of pectin was decreased after irradiation.

It was observed that changes in viscosity occurred at 0.2 kGy in pectin, and for cellulose the dose was 10 kGy (Kertesz and others 1956). Mohr and Wichmann (1985) were the first to report on the use of viscosity as a marker for the irradiation process. Barabassy and others (1996) observed a decrease in viscosity in suspensions of black and white pepper, ginger, and nutmeg at an irradiation dose of 8 kGy. This method can generally be applied mainly for irradiated pepper (Hayashi 1996a,b). It can also be utilized for foods such as dried vegetable starches, fish, and other seafood (Farkas and others 1990). Hence, viscosity can be used at least as a screening method for the detection of irradiated spices, although it is influenced by shear rate, temperature, and the type of viscometer.

Electrical impedance measurement. The membrane of living tissues has selective permeability to transport ions. The membrane properties can be changed by changing ion concentration. This tool can be used for the detection of irradiation for food samples having tissue. Schertz (1973) had used impedance measurements for irradiated potato with the help of electrodes and passing an alternating current (AC). It is reported that conductivity for irradiated potato was higher than nonirradiated potato. Hayashi and Kawashima (1983) reported that conductivity measurement of other vegetables showed no consistent alteration by irradiation. Ehlermann (1972) suggested the use of resistance measurement for the identification of irradiated fish. Hayashi and others (1996) reported that detection of irradiated samples was best at 22 to 25 °C with 1 ma AC current and 5:50 kHz impedance magnitude ratio. It was also reported that magnitude ratio appears to be dependent on the cultivar of the tuber and hence knowledge of cultivar is required.

Other physical methods. There are other physical methods suggested by a number of researchers for irradiation detection, but they have limited application. Such methods include measurement of changes in initial freezing point or heterogeneous nucleation temperature after irradiation. Dubini and others (1991) successfully used differential scanning calorimetry to study the heterogeneous nucleation temperature in irradiated chicken breasts. The damage caused by ionization radiation to plant cell walls can lead to a number of changes in the bulk properties of a food sample including eutectic effects (Dubini and others 1990). Degradation of volatile oils, lipids, carotenoids, and starches by ionizing radiation can be indicated in the near-infrared wavelength region by analyzing the changes in the reflectance spectrum caused by radiation (Barabassy and others 1996). These changes in reflectance spectra are relatively permanent and can be observed as a result of excited molecules in the absorption peak. These changes generally depend on the radiation dose and the time after irradiation. Near-infrared spectroscopy has an identification limit of approximately 3 to 4 kGy.

Chemical methods

Changes in lipids. The lipid (fat) portion of food consists of triglycerides (TGs). In TGs fatty acid moieties break away mainly in the α and β positions, with respect to the TG carbonyl groups, resulting in the respective C_{n-1}: 1 and C_{n-2}: 1 hydrocarbon, alkyl–polyenes, on irradiation. TGs on irradiation produce normal aldehydes and a 2-alkyl cyclobutane and other products such as n-alkane and n-alkene, lactones, ketones, esters, and other low-molecular-weight hydrocarbons (Stewart 2001). The formation of these major radiolytic products reflects the severity of irradiation treatment since their production increases linearly with dose and temperature of irradiation. The composition of products formed by irradiation of lipids and lipid-containing foods can be predicted to a certain degree if the fatty acid composition is known (LeTellier and Nawar 1972).

Radiolytic products were present in samples irradiated at a dose as low as 1 kGy but absent in nonirradiated or heated samples and these compounds can be determined by HPLC, GC-MS, and other analytical tools. GC-MS is used for the detection of volatile hydrocarbons and aldehydes in irradiated chicken meat. Morehouse and others (1991) reported that the application of GC for the estimation of irradiation dose was in good agreement with ESR measurement of free radicals tapped in the bone. Radiation-induced oxidation of lipids can also be a suitable reaction for irradiation detection because of the amplified effect by the chain character of the types of reactions. The hydroperoxide content

has been proposed as an indicator for egg and milk powders and soya flour.

The content of hydroperoxides depends on the dose of irradiation, and the level was still higher than nonirradiated samples even after storage of 6 mo (Katusin-Razem and others 1990). Stevenson and others (1993) reported on the suitability of GC-MS application for the determination of radiation-generated changes in chicken and prawn meat. The oxidation of cholesterol by irradiation can be utilized as an indicator of irradiation. Schulzki and others (1995a,b) reported on the use of an online-coupled liquid chromatography-gas chromatography (LC-GC) and LC-LC-GC for the detection of hydrocarbons produced by irradiation. This technique was found to be very efficient even at a lower detection limit and thus was applied to fish oil, fat from mango kernel, and avocado flesh, as well as fat extracted from sponge cake containing irradiated liquid egg.

In addition to these compounds, 2-alkylcyclobutanones produced from fatty acids during irradiation can also act as radiation marker, as well as be usable for semi-quantitative dosimetry. Handel and Nawar (1981) have isolated 2-dodecyclobutanone (2-DCB) from synthetic phospholipids irradiated at 50 kGy and used as radiation marker. For lipids and hydrocarbons, solvent extraction with hexane and followed by conventional GC-MS analysis of isolated fat extract was used for semi-quantitative dosimetry. Supercritical fluid extraction (SCF) techniques can also be utilized for the extraction of the radiolytic fraction such as 2-DCB (Rahman and others 1996). Tewfik (2008) used direct solvent extraction to obtain cyclobutanone and concluded that this method is promising, rapid, simple, and robust for the analysis of irradiated lipid-rich foods. It was confirmed that 2-DCB could be utilized as a tool for irradiated food samples, because it was not detected in either raw or cooked nonirradiated chicken meat, but found only in irradiated samples (Boyd and others 1991). 2-DCB is reported to persist for at least 20 d in irradiated meat stored at 4 °C and its concentration increased with increased radiation dose. Crone and others (1992) also reported that 2-DCB is very stable and detectable in chicken meat that had been irradiated with γ-rays and electron beams 12 to 13 y earlier.

Another cyclobutanone, namely, 2-tetradecylcyclobutanone (2-TCB) is also found in irradiated foods. Similar to 2-DCB the concentration of 2-TCB formed in chicken meat was increased with increasing dose but its concentration was found to be lower than 2-DCB. The presence of 2-DCB and 2-TCB in irradiated liquid whole egg was reported by Stevenson and others (1993). For the detection of 2-DCB, enzyme-linked immunosorbent assay (ELISA) was developed for the detection of 2-DCB (Elliott and others 1995; Nolan and others 1998). Initially, polyclonal antibodies were raised to a cyclobutanone derivative with a side chain length of 10-carbons and incorporated into ELISA. These polyclonal antibodies were shown to be capable of detecting cyclobutanones in chicken meat irradiated at commercial doses. Hence, ELISA can also be useful for rapid, and simple, and on-site screening of irradiated foods.

A number of research studies suggested that lipid degradation could be widely utilized to detect irradiated foods containing fat that include meat, fish, shrimp, cheese, and sponge cake prepared with irradiated liquid egg (Morehouse and others 1991; Morehouse and Ku 1992; Bergaentzle and others 1994a; Schulzki and others 1995a,b; Villavicencio and others 1997). Recent studies have demonstrated that these irradiation markers can also be identified in food containing other irradiated ingredients. These methods are even adopted as European Standard (EN 1785:2003). Similar to hydrocarbon methods, the cyclobutanones can also be used as irradiation tool for any fat-rich food product.

Ortho-tyrosine. Most food proteins contain aromatic amino acids such as phenylalanine, tyrosine, and so on. These aromatic amino acids react with hydroxyl radicals formed during the radiolysis of water and form ortho- and meta-tyrosine. These isomers of tyrosine are not naturally present. Identification of o-tyrosine isomer is easier than other forms of isomers using chromatography. Karam and Simic (1988a,b) reported the use of o-tyrosine as a marker for irradiation process. Miyahara and others (2002) compared this method with ESR in irradiated boned chicken and found that method is well correlated with ESR. They reported that low detection limits are 0.5 and 10 kGy for the ESR method and *o*-tyrosine determination method, respectively. However, the upper limits are 40 and 60 kGy, respectively. The time needed for analysis is 25 to 30 h for both methods. Meier and others (1990) reported the use of this method for determination of irradiation treatment in chicken.

Offermanns and McDoughall (1991) have used HPLC for the estimation of o-tyrosine in chicken meat and reported that there was a linear relationship between irradiation dose and yield of o-tyrosine. But some researchers reported the presence of o-tyrosine in nonirradiated samples of food in lesser quantities. This method has one drawback in that it is very difficult to find out the differences between o-tyrosine formed during irradiation processing and naturally occurring o-tyrosine. So this method is not commonly used as a test method and extensive collaborative testing has not been carried out for validating this method.

Estimation of gas evolution. Estimation of gases, such as carbon monoxide, hydrogen sulfide, hydrogen, ammonia, and others from food can also be utilized for the detection of irradiation processing. Furuta and others (1992) had used the amount of carbon monoxide evolved from irradiated frozen chicken and other animal products. In that study, microwave heating was used to expel the trapped gas and analyzing the expelled gas in the headspace of the products using GC. Hitchcock (2000) reported that when irradiated calcium carbonate or irradiated eggshell from domesticated hens is dissolved in acid, characteristic traces of molecular hydrogen are released along with the carbon dioxide. The determination of this hydrogen from the shells of eggs offers a reliable, rapid, and robust method for the detection of prior irradiation. Being based on an electronic sensor incorporated into a simple headspace analyzer, it is particularly useful as a cheap on-site screening procedure. Roberts and others (1996) reported similar promising results for frozen chicken meat and shrimp. Delincee (1993) reported on the use of an electrochemical sensor for the estimation of the carbon monoxide content in gas released from irradiated food. Hitchcock (1993) invented a hydrogen-specific electronic sensor for the estimation of gases from irradiated food. This sensor is based on aqueous solutions of hydrogen and on irradiated food. It is reported that it could be applied to chicken meat and eggshell dosed at 0.1 to 0.8 kGy. In that study, an electronic sensor was incorporated into the headspace of a sample. It is a very simple, inexpensive, and an on-site procedure. This technique did not give any false results, but sometimes it was unable to detect hydrogen in a sample. Therefore, it has limited uses only in frozen food products because of rapid diffusion of gases. Delincee (1996a) introduced the use of multiple gas sensors to increase the reliability of this method. By using several gas sensors a "gas fingerprint" for different products can be established, but more work is needed to establish the background values for various foods. This can then be used as a screening method. For a confirmatory test, irradiated samples should also be analyzed by other validated method.

Other chemical methods. A number of other chemical methods have been evaluated for the testing of irradiated foods (Delincee 1998). These methods are based on the chemical changes in foods induced by the irradiation process. Irradiated

egg can be identified immunochemically by the specific detection of radiation-induced degradation fragments of egg white protein. Proteins and peptide antigens can generally be detected by immunoblotting using specific antibodies, even in the presence of other coexisting proteins and peptides (Kume and others 1994). Electrophoresis of egg white showed that the partial fragmentation of egg white protein was induced by irradiation at 10 kGy. It has been suggested that this test would be applicable not only for the detection of irradiated egg, but also for foods containing irradiated egg white.

DNA methods

DNA comet assay. It is well known that DNA is the major cellular target for ionizing radiation. The radiation-induced DNA damage is responsible for inactivation of microorganisms, inhibition of growth, and other lethal effects. Therefore, it is logical to investigate whether radiation damage to DNA in food can be utilized as a means of detecting ionization treatment. It is reported that there are 3 types of major changes that take place in DNA by radiation, namely double-strand breaks, single-strand breaks, and base damage (von Sonntag 1987). Hydroxylation subsequent to attack by hydroxyl radicals is the prime cause of these changes, which may be observed and quantified using various techniques. One such technique is electrophoresis. This technique facilitates analysis of DNA leakage from single cells or nuclei extracted from food materials and embedded in agrose gel. In an irradiated sample, fragmented DNA will leak out from nuclei during electrophoresis forming a tail in the direction of the anode. Cells from nonirradiated samples appear as nuclei with no or only slight tails. This method has been applied successfully to poultry meat, beef, pork, chevon, and mutton, and also foods of vegetable origin such as almond, fig, lentil, soy bean, strawberries, grapefruit, linseed, and so on (Cerda and others 1993, 1997; Delincee 1996b, 1998). Cerda (1998) has successfully demonstrated the detection of irradiated frozen meat using the DNA comet assay method. The results showed that while irradiated cells had comets with long tails, nonirradiated cells showed no tail or very short ones, and the shape of comets depended on the irradiation doses (Figure 8). Chung and others (2004) also reported a similar result for irradiated ostrich meat with doses of 1 to 10 kGy. The irradiated ostrich meat showed comets with long tails, whereas nonirradiated samples with intact cells showed only slight comets.

Khan and others (2002) made an attempt to identify irradiated spices using micro gel electrophoresis of single cells or nuclei (DNA comet assay). After electrophoresis, radiation-damaged DNA appeared as a comet, whereas in nonirradiated spices round or conical spots appeared. Shape, length, and intensity of comets were also dose dependent. This method was found to be successful for spices. Alvarez and others (2007) carried out the DNA comet assay for irradiated onions and concluded that this method is a sensitive and quick technique for the qualitative detection of irradiated onions. Verbeek and others (2007) have used an automated image-analyzing system to measure DNA comets. This system allows the discrimination between irradiated and nonirradiated food as well as the set-up of standard dose–response curves, and gives sufficiently accurate dose estimation.

Agarose electrophoresis of mitochondrial DNA. Marchioni and Hasselmann (1991) reported that identification of fragmented DNA strands caused by irradiation is not possible in products like meat because of strong enzymatic degradation of DNA during storage. Therefore, identification of radiation-specific breakage of DNA was investigated by the isolation of irradiated DNA from cells with enzymatically ruptured DNA (Marchioni and others 1992). Since mitochondrial DNA (mDNA) is protected from enzymatic reactions due to the presence of mitochondrial walls, it is not protected from radiation. Therefore, it is assumed that mDNA breakage is specific to radiation. In foods of animal origin, mDNA is of low molecular weight (approximately 16 base pairs) and normally in super-coiled forms. Due to irradiation super-coiled forms relax into circular DNA and then linear DNA. These 3 forms can be easily separated by agarose gel electrophoresis.

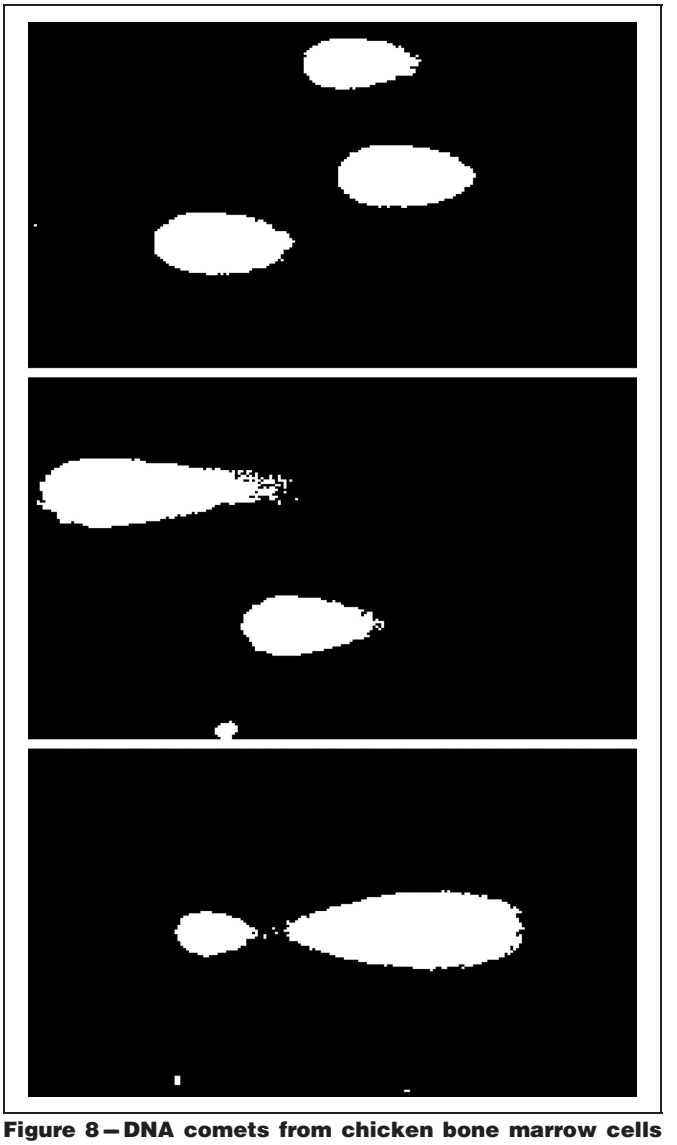

Figure 8—DNA comets from chicken bone marrow cells irradiated with 0 Gy (3 cells), 1 kGy (2 cells), and 5 kGy (1 cell), from top to bottom (adopted from Cerda 1998).

Marchioni and others (1996) concluded that the percentage of super-coiled DNA in animal foods was significantly reduced on irradiation (2 to 4 kGy), while the percentage of circular and linear mDNA increased. In nonirradiated food, super-coiled mDNA was shown to remain perfectly stable during storage of 25 d at 4 °C as well as during abrupt temperature changes (freezing at −20 °C and thawing at 20 °C). The mDNA test was also used to identify potatoes irradiated at 1 to 4 kGy (Bergaentzle and others 1994b). Compared to animal products, plant products are more complex and have high molecular weight DNA (200 to 250 K base pairs), so the analysis was carried out using pulsed electrophoresis. This technique has wide application in food,

particularly meat. The major disadvantage of this process is the extraction of DNA, which is time-consuming and complicated. Therefore, it warrants further research to simplify the process.

Immunological detection of modified DNA bases. DNA molecules contain various bases such as adenine, thymine, and so on. On irradiation, some proportion of thymine gets converted to dihydrothymidine (DiHT) due to its reaction with radiolytic products of water molecules under anoxic conditions (Deeble and others 1990). DiHT can be used as a potential internal marker for radiation-processing. Williams and others (1996) made a monoclonal antibody against DiHT for its isolation and ELISA-based assay was developed by Tyreman and others (1998). It was found that ELISA can detect prawn irradiated at 2 kGy and the same can be applied to the crude homogenate. Thus there is no need to extract the DNA prior to analysis. It can be utilized as a rapid and specific screening procedure.

Biological Methods

Shift in microbial load. Generally, all types of food processing, including irradiation, cause destruction or changes in microbial load. On the basis of these changes a number of researchers have suggested the use of shift in microflora load as a simple test method to determine whether an irradiation treatment has been applied. Initial studies were carried out in fruits and vegetable products with the main focus on Gram-negative bacteria, as they are more sensitive to irradiation than other types of bacteria. For instance, the microflora on raw poultry meat showed a characteristic microbiological profile with significant numbers of Gram-negative bacteria, predominantly of the genus *Pseudomonas.* By contrast, the flora that develops on a raw chicken after irradiation at a dose of 2.5 kGy is mostly Gram-positive bacteria and yeasts. Tamminga and others (1975) studied the shifts in microbial load of strawberries and found that *Pseudomonas* of total population 10^5 to 10^6 colony forming units (CFU)/g present in the sample prior to irradiation was completely removed after irradiation at 2 kGy. However, this method has certain limitations as microbial load of product depends on a number of factors such as region of cultivation, postharvest condition, processing, and more. Thus, data obtained for a particular food under specific conditions cannot be valid for the same food obtained from different regions or conditions. These results were valid for strawberries grown outdoors but not for greenhouse strawberries that often contain lower numbers of microorganisms.

Other DNA methods. Fragmentation of DNA can also be analyzed by other methods such as filter elution, pulsed gel electrophoresis, and flow cytometry (Copin and Bourgeois 1991; Mayer and others 1993; Selven and Thomas 1997).

Limulus amebocyte lysate test combined with Gram-negative bacterial count (LAL/GNB). It is generally a screening method indicating the reduction of bacterial population of a food by irradiation. However, it cannot measure the reduction of bacterial toxins formed by the bacteria before their demise. The method determines the number of viable Gram-negative bacteria present in the test sample and the concentration of bacterial endotoxin present on the surfaces of Gram-negative bacteria as lipopolysaccharides (LPS). LPS is used to measure the amount of total Gram-negative bacteria, both viable and dead. If the difference between Gram-negative bacteria and endotoxin is high, it is assumed that the sample was treated by a method of preservation, possibly by treatment with ionizing radiation.

Scotter and others (1994) applied this test to chicken pieces and concluded that it can be used to identify a microbiological profile, although some difficulties can be encountered in differentiating between high-quality meat and irradiated samples. The test is presumptive and can be used as a screening method. This method can give only an indication of a possible treatment by ionizing radiation. A high amount of dead microorganisms in comparison to the viable fraction can be due to several other reasons. It is therefore necessary to confirm a possible treatment by ionizing radiation by standardized reference method for the detection of irradiated foods (EN 14569:2001).

Direct epifluorescent filter technique combined with aerobic plate count (DEFT/APC). Using both DEFT and APC, it is possible to devise a method that will give an indication of whether food is processed by irradiation. The method is based on a comparison of the APC with the count obtained using DEFT. The APC gives the number of viable microorganisms in the sample after a possible irradiation and the DEFT count indicates the total number of microorganisms, including nonviable cells, present in the sample (EN 13783:2001). The difference between the DEFT count and the APC in spices treated with doses of 5 to 10 kGy is generally about or above 3 to 4 log units. Similar differences between DEFT and APC counts can be induced by other treatments of the foods leading to death of microorganisms, such as heat, thus positive results must be confirmed.

A known volume of sample is passed through a membrane filter at reduced pressure to concentrate the microorganisms on the filter. The microorganisms are stained with a fluorochrome, acridine orange (AO), resulting in an orange and orange-yellow fluorescence under illumination with blue light at 450 to 490 nm. These microorganisms are counted using an epifluorescence microscope to give the DEFT count. However, microorganisms that were nonviable before irradiation show green fluorescence and are not counted. APC is determined from a 2nd portion of the same test sample. For nonirradiated samples DEFT counts are in close agreement with those obtained by APC. If APC count is found to be considerably less than obtained by DEFT, it indicates that the sample could have been irradiated (Figure 9) (Jones and others 1994). But this method has limitations when there are too few microbes in the sample (APC $< 10^3$ CFU/g). If fumigation or a heat treatment is used for decontamination, the DEFT/APC difference of counts can be similar to the difference of counts obtained after irradiation. However, the use of fumigation can be detected. Some spices such as cloves, cinnamon, garlic, and mustard seeds contain inhibitory components.

Germination and half embryo test. It is very well known that ionizing radiation affects the viability of the germ or embryo, delaying or inhibiting germination. Various researchers have shown that this test could be utilized to differentiate irradiated commodities from nonirradiated ones. It relies on the fact that irradiated seeds germinate at significantly slower rates than control seeds. Sprout inhibition of potatoes by irradiation is irreversible and may

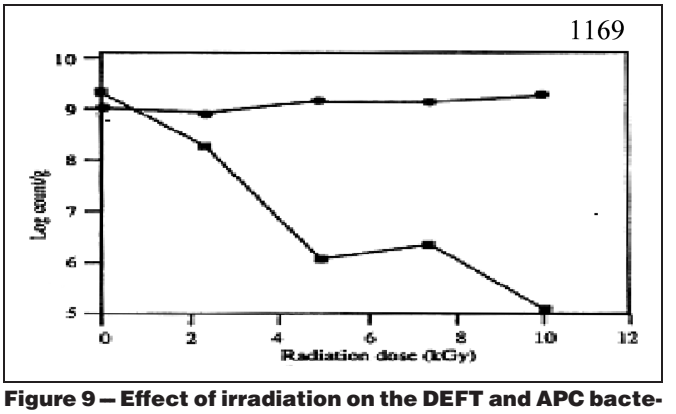

Figure 9—Effect of irradiation on the DEFT and APC bacterial counts in ground beef (adopted from Jones and others 1996).

serve as proof of irradiation, but the method is too slow for routine analysis (even if growth hormones are used to accelerate sprouting). This assay is limited to vegetable seeds. It is very simple and inexpensive, but slow since it takes several days to get results. The germination of irradiated grapefruits with doses over 0.15 kGy showed markedly reduced root growth and shoot elongation (Kawamura and others 1989). Enzymatic changes in irradiated potatoes could be histochemically visualized for a few weeks with a tetrazolium stain (Jona and Fronda 1990). Kawamura and others (1989) developed an improved germination test known as "half embryo test" for detection of irradiated grapefruit and other fruits. In this test, the embryo was used for germination instead of seeds thereby accelerating the germination process. At a dose of 0.15 kGy radiation treatment could be detected within 2 to 4 d, whereas germination takes place in 6 to 14 d for grapefruit seeds. Cutrubinis and others (2004) have tested whether the germination test can be used to detect irradiated garlic. The results showed that the germination test could be used as a detection method in the dormancy period. It is even reliable for samples treated with 25 kGy. But for garlic irradiated after the dormancy period this test does not function properly. However, in this case the sprout-inhibiting effect of irradiation is also inadequate. So for such samples some other reliable methods should be adopted.

Other biological methods. Other biological and histochemical measurements have been suggested for the specific identification of irradiated potatoes (Thomas 1983) and onions (Thomas 1984). In some biological methods, the effect of irradiation itself is used as internal radiation marker. Some researchers have indicated that the bacterial spoilage profile could potentially be used as a tool of identifying irradiated seafoods. The ability of bacterial spoilage can be measured with the generation of total volatile acids (TVAs) and total volatile basic nitrogen (TVBN). It was shown that bacterial growth was found in both irradiated and nonirradiated foods, whereas formation of TVA and TVBN was comparatively low in irradiated fish (Alur and others 1991). Detection of radiation-induced changes in insects has been suggested as a marker for identifying radiation processes in fruits, vegetables, and cereals. A simple biological method is a test for a specific enzyme present in food products, such as polyphenol oxidase in fruits.

Conclusions

Detection methods for irradiated foods are being developed continuously. Ideally such methods should be simple, accurate, easy to perform, rapid, and inexpensive. It is recognized that availability of such detection methods would augment standard regulatory procedures, which would help to strengthen national regulations on the irradiation of specific foods and would be of assistance in establishing a system of legislative control and enhance consumer confidence in such regulations and acceptance of irradiated foods. Unfortunately, no single method can be applied to all food systems. Different foods vary in their chemical composition, physical, and quality attributes. Selection of a suitable detection method generally depends on type of food, dose used for irradiation, degree of precision required, and cost. 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, preferably 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 demands specialized skills and facilities. Modern methods of multi-component analysis, combined with multivariate statistical evaluation, might be a solution to this complex problem. DNA methods and chemical methods require further research for simplification of test procedures. Development of ELISA kits for the detection of various chemical indicators such as 2-DCB, TCB, and others would be a boon to irradiation processing. Development of cost-effective ESR and luminescence equipment for irradiation testing has vast scope.

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