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Review

Screening Methods for the Identification of Irradiated Foods

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Abstract

The exposure of food to ionizing radiation has been recognized as a safe and effective mode of food preservation in more than 55 countries. The benefits include eradication of insect pests, inactivation of food pathogens, extension of shelf-life, and improvement in food hygiene. Regulatory authorities around the world have emphasized the implementation of various national and international regulations to facilitate trade and development of consumers'confidence in purchasing irradiated foods. Therefore, the need for reliable irradiation detection methods has increased to enforce these regulations. At present, a number of promising analytical approaches have been developed and evaluated. Moreover, about 10 European Standards have been adopted as General CODEX Alimentarius methods for the detection of irradiated foodstuffs. However, most of these methods demand relatively expensive equipment and prolonged sample preparation. Therefore, simple and cost-effective approaches would be advantageous for rapid screening of foodstuffs. The suspected samples need to be analyzed further with more validated techniques to confirm the screening results. In this review, existing screening methods (i.e. physical, chemical, and biological) for the identification of irradiated foods have been outlined along with their principles, scopes and limitations.

Keywords : Irradiated food, Identification, Physical screening methods, 2-Alkylcyclobutanones, Biological screening methods

Introduction

Food irradiation is the process of exposing food to ionizing radiation such as gamma-rays or electrons and/or X-rays. Each application differs in the exposure time required to treat the food and penetration ability of the radiation, and for the safety of the workforce. The process of food irradiation is also called "cold pasteurization" because it kills harmful bacteria without the use of heat. Furthermore, irradiation treatment offers potential to control insect pests, reduce bacteria, deactivate molds, and prevent sprouting. It also serves as a quarantine treatment for many fresh fruits and vegetables to enhance their shelf-life and to facilitate international trade (Hallman 2001; Delincée 2002; Kwon et al. 2011; Crews et al. 2012).

In 1981, the FAO/IAEA/WHO Joint Expert Committees on the wholesomeness of irradiated food (JECFI) concluded, "the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard." The expert committee reviewed over 500 research studies and concluded that food irradiation creates no toxicological, microbiological, or nutritional problems to consumers (GAO 2000; Farkas 2006). Currently, more than 55 countries in the world approve national clearances of food irradiation processes. Food irradiation facilities (Table 1) in member countries are regulated jointly by the International Atomic Energy Agency (IAEA), the Food and Agriculture Organization (FAO), and the World Health organization (WHO). The database for clearance list of different irradiated foods and facilities is available (IAEA 2007; Farkas and Mohacsi-Farkas 2011).

Regulatory authorities have emphasized the implementation of various national and international regulations to enhance trade and to ensure consumers' free choice about irradiated food products. Labeling is mandatory, either "irradiated" or "treated with ionizing radiation" together with the radura symbol (Figure 1). Several methods have been developed so far to detect irradiated foods and many methods have been approved as standard methods in the Codex Alimentarius Commission in the Codex General Standard for Irradiated Foods in section 6.4 on 'Post-irradiation verification' (FAO/WHO 2003; Akram et al. 2012; Shin et al. 2012). The European Committee for Standardization (CEN) approved five methods (EN-1784 to EN-1788) as European standards for the detection of irradiated foods in 1996, while five more validated standard methods (EN-13783, EN-1384, EN-14596, EN-13708, and EN-13751) were authorized in 2004. The methods are based on minute

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Table 1. Some food irradiation facilities around the world.

Sr. No.	Country	Facility name	Radiation type
1	Korea, Republic of	Korea Atomic Energy Research Institute (KAERI)	Gamma/E-beam
2	Hungary	Agroster Irradiation Co. Ltd.	Gamma
3	Viet Nam	AnPhu Binh Minh Irradiation Company	Gamma
4	Spain	ARAGOGAMMA S.A.	Gamma
5	China	Atomic Agriculture Institute of Hunan	Gamma
6	Syrian Arab Republic	Atomic Energy Commission of Syria	Gamma
7	Bangladesh	Bangladesh Atomic Energy Commission	Gamma
8	India	Bhabha Atomic Research Centre, BARC	Gamma
9	Bulgaria	Bulgamma, Sopharma Ltd.	Gamma
10	Brazil	CBE-Companhia Brasileira de Esterilizacao	Gamma
11	Indonesia	Center for the Application of Isotope and Radiation Technology	Gamma
12	Cuba	Centro de Irradiacion de Alimentos	Gamma
13	Chile	Chilean Nuclear Energy Commission	Gamma
14	Taiwan	China Biotech Corporation	Gamma
15	Argentina	ComisiÃ³n Nacional de EnergÃa AtÃ³mica	Gamma
16	Malaysia	Electron Beam Sdn Bhd	E-beam
17	Saudi Arabia	SureBeam Middle East	E-beam
18	Italy	Gammarad Italia SPA	Gamma
19	Ghana	Ghana Atomic Energy Commission	Gamma
20	Colombia	Instituto Colombiano de GeologÃa y MinerÃa - INGEOMINAS	Gamma
21	Mexico	Instituto Nacional de Investigaciones Nucleares	Gamma
22	Peru	Instituto Peruano de Energia Nuclear (IPEN)	Gamma
23	Venezuela	Instituto Venezolano de Investigaciones Científicas – IVIC.	Gamma
24	Turkey	Turkish Atomic Energy Authority	Gamma
25	Netherlands	Isotron	Gamma
26	Germany	Isotron Deutschland GmbH	Gamma
27	France	Isotron France	Gamma
28	United Kingdom	Isotron Ltd.	Gamma
29	South Africa	Isotron South Africa (Pty) Ltd.	Gamma
30	Portugal	ITN – Nuclear & Technological Institute	Gamma
31	Canada	MDS Nordion - CIC	Gamma
32	Egypt	National Center for Rad. Research & Technology	Gamma
33	Iran, Islamic Republic of	Nuclear Science and Technology Research Institute (NSTRI), Atomic Energy Organization of Iran (AEOI)	Gamma
34	Pakistan	Pakistan Radiation Services (PARAS Foods)	Gamma
35	Philippines	Philippine Nuclear Research Institute	Gamma
36	Croatia	Ruder Boskovic Institute	Gamma
37	Nigeria	Sheda Science and Technology Complex	Gamma
38	Japan	Shihoro Agriculture Cooperative Association	Gamma
39	Israel	Sor-Van Radiation Ltd.	Gamma/E-beam
40	Belgium	Sterigenics Belgium	Gamma
41	United States	Sterigenics Int'l. Inc.- Corona, Ca.	Gamma
42	Australia	Steritech, NSW	Gamma
43	Poland	Technical University of Lodz, Institute of Applied Radiation Chemistry	Gamma
44	Thailand	Thai Irradiation Center	Gamma

Source: http://nucleus.iaea.org Last accessed: February 11, 2013

physical, chemical, biological, and/or microbiological changes which occur in food products upon irradiation (Chauhan et al. 2009).

However, most of these methods are time-consuming and require expensive equipment for analysis. Therefore, cost-effective and time-efficient screening methods are valued to screen large food lots with minimal sample preparation (Delincée 1995). Although these screening techniques may not be able to provide clear judgment, they can contribute by indicating the possible irradiation treatment. Suspected samples can be analyzed further with validated techniques like thermoluminescence (TL) detection and/or electron spin resonance (ESR) spectroscopy (Cerda et al. 1997; EN1788 2001; Delincée 2002).

The aim of this review article is to describe the principles and proceeding protocols of screening techniques along with their importance and scope for rapid identification of different types of irradiated foods.

Figure 1. "Radura" symbol indicating irradiation-treated food. (Source: Codex 2005)

Physical Methods

Photostimulated luminescence (PSL)

PSL is a radiation-specific phenomenon based on energy being stored by trapped charge carriers following irradiation. Light is used rather than heat to stimulate the release of these trapped charge carriers (Figure 2). The method may in principle be applied to detect irradiated foods of any kind, which contain mineral debris, especially silicate mineral and bioinorganic materials such as calcite, which originate from shells or exoskeletons, or hydroxyapatite from bones or teeth (Sanderson et al. 1996; EN13751 2009). PSL was proposed by Sanderson (1991) to resolve the practical limitations of the TL method related to extended mineral separation/preparation procedures. Furthermore, this technique helps to perform multiple measurements in a few minutes without any sample preparation or the involvement of a re-irradiation step like in the case of TL. Therefore, it can be successfully applied as a rapid screening method for many irradiated foods (Sanderson et al. 1996).

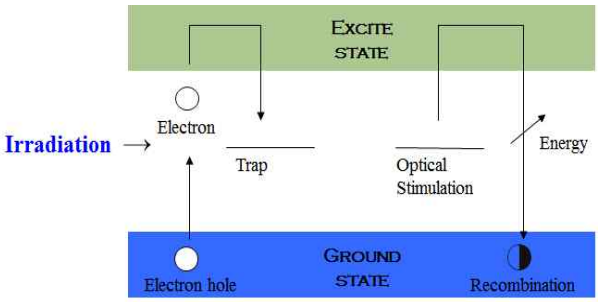

Figure 2. Principle of photostimulated luminescence technique. (Source: Tsoulfanidis 1995; Kwon et al. 2011)

A SURRC PPSL Irradiated Food Screening System (SURRC; Scottish Universities Research and Reactor Centre, Glasgow, U.K.) comprised of a control unit, sample chamber and detector head assembly is used for this analysis. The photon signals are divided into two threshold limits, i.e. an upper and lower one. For herbs and spices, the defined threshold limits after various interlaboratory trials are T_1= 700 counts/60 sec and T_2= 5000 counts/60 sec while for shellfish the threshold limits are T_1= 1000 counts/60 sec and T_2= 4000 counts/60 sec shown in Figure 3 (EN13751 2009). Irradiated food samples generate strong signals above the upper threshold limit and vice versa. The signal levels between these two limits are recognized as intermediate and require further investigation by TL (EN1788 2001) or another validated method such as ESR analysis (EN1787 2001).

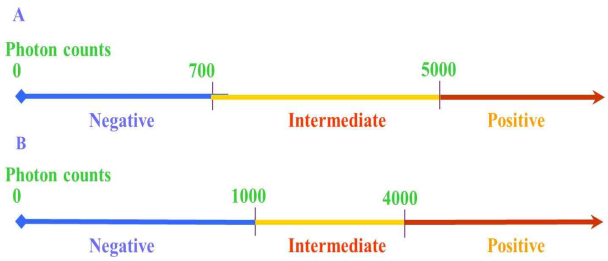

Figure 3. Threshold values of photostimulated luminescence photon counts. (A, spices and herbs; B, shellfish). (Source: Akram et al. 2012)

Sanderson et al. (1996) successfully segregated more than 90% of 45 un-irradiated and irradiated spices and seasoning samples

Table 2. Screening methods for detection of irradiated foods.

European standard No.	Detection method	Principle	Validated foods
EN 13751:2009	Photostimulated luminescence	Use of light to release trapped energy from mineral debris upon irradiation	Herbs and spices, shellfish, cereals, onion, fruits
EN 13783:2001	DEFT/APC microbiological screening	Comparison of APC with count obtained using DEFT following by irradiation	Herbs and spices
EN 14569:2004	LAL/GNB microbiological screening	Difference in number of viable Gram negative bacteria and concentration of bacterial endotoxin LPS	Poultry meat, all parts including breast, legs, wings of fresh, chilled or frozen carcasses
EN 1785:2003	GC/MS analysis of 2-alkylcyclobutanones	Measurement of 2-alkylcyclobutanones produced by irradiation of fat contain foods	Raw chicken, pork, salmon, liquid whole egg, camembert
EN 13784:2001	DNA comet assay screening	Micro-gel electrophoresis of single cells or nuclei to detect DNA fragmentation following irradiation treatment	Poultry meat, pork, fish, lamb, deer, beef, lentils, linseed, rose pepper, figs, sesame seeds, almonds, soybeans, strawberries, grapefruits, sunflower seeds
	Viscosimetry	Irradiation induced changes in physical characteristics of food polymers, particularly starch	Foods containing starch, cellulose, or pectin
	Germination and half embryo	Irradiated seeds germinate at significantly slower rates than control seeds	Vegetable seeds and some fruits

with this technique. Other scientists also have reported the efficiency of the PSL method for correctly identifying irradiated samples including pepper powder, dried herbs, potatoes, soybeans, dried figs, sesame seeds, chestnuts (Hwang et al. 1998; Chung et al. 2000; Lee et al. 2008; Pal et al. 2010), and white ginseng powder (Chung et al. 2000). However, storage conditions can slightly affect the measurements of the PSL photon counts. Ahn et al. (2012) considered the effect of different light conditions such as sunlight, artificial light, and a dark room on the luminescence characteristics of contaminating minerals separated from irradiated onions. They observed a slight change in PSL PCs under different applied conditions, but all the irradiated samples were effectively identified even after 2 years of storage.

Marchesani et al. (2012) compared the application of standardized methods based on luminescence techniques (PSL and TL) for identifying X-ray irradiated oysters at different dose levels. The applicability and feasibility of the proposed methods was successfully analyzed. Kim et al. (2012) reported on the limitation of the PSL screening method for the identification of sample mixtures containing a small proportion of irradiated component. Different spice blends with small quantities of different irradiated spice powders such as red pepper, garlic or ginger, were studied using the PSL and TL techniques. Recently, Yunoki et al. (2012) evaluated the possibility of irradiated packaging material as alternative specimens for screening detection of irradiated foods. The core of irradiated corrugated fiberboards (CFs) exhibited PSL signals sufficient for differentiating irradiated from non-irradiated samples even after CFs were exposed to light and environmental stresses raised to 50℃ for over 6 months post-irradiation. However, a difficulty was observed to establish a well-defined "positive" threshold limit due to the marked variations of the PSL signals among CFs.

Viscosimetry

The method is principally based on the change induced by irradiation in the physical state of food polymers particularly starch explained in Figure 4. Radiolysis will alter the rheological properties of foodstuffs containing starch, cellulose, pectin, etc. as a major component. Various scientists have reported that gamma-irradiation of certain foodstuffs resulted in solutions of reduced viscosity (Glidewell et al. 1993; Stevenson and Stewart 1995; Rahman et al. 1995). The viability of apparent viscosity as a method for identifying electron beam and gamma-irradiation treated black and white peppers was also investigated (de Alwis

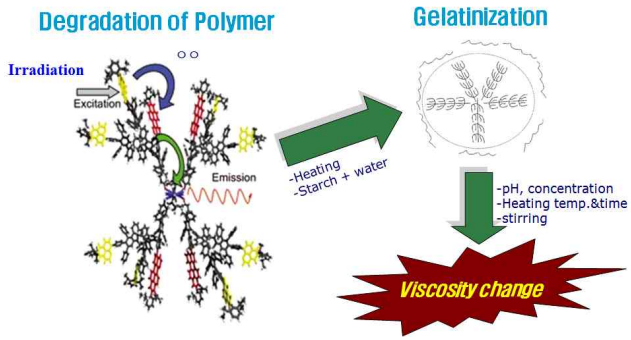

Figure 4. Mechanism of change in viscosity of starch-based foods upon irradiation. (Source: Yew et al. 2005; Wittaya 2012)

and Grandison 1992; Esteves et al. 1995; Hayashi and Todoriki 1996).

The process is revealed to be quick, relatively easy to perform and comparatively inexpensive. In addition, viscosity changes occurred in food materials that are quite stable in storage; therefore; irradiation detection is possible even after years of storage. However, storage conditions are significant for the correct measurement of viscosity over prolonged storage times. The conditions for the measurement of viscosity of spice suspensions have been defined by a number of studies. Farkas et al. (1990) observed a change in viscometric properties of spices stored for a long time under humid conditions. The other contributing factors are the concentration of the suspension, particle size and distribution, the pH, soaking time and temperature (Heide et al. 1990 Anon 1990; Sharif and Farkas 1993; Hayashi and Todoriki 1996).

Chemical Methods

GC/MS analysis of 2-Alkylcyclobutanones (2-ACBs)

The European Standard protocol EN1785 (2003) specifies a method based on the measurements of 2-ACBs produced by the irradiation of foods which contain fat including meat, fish, shrimp, cheese, liquid egg products, etc. (Morehouse and Ku 1990; EN1785 2003).

The principle relies on the fact that during irradiation, the acyl-oxygen bond in triglycerides is cleaved and this reaction results in the formation of 2-ACBs containing the same number of carbon atoms as the parent fatty acid, and the alkyl group is located in ring position 2 as indicated in Figure 5. Thus, if the fatty acid composition is known, the 2-ACBs formed can be predicted (Stewart et al. 2001; EN1785 2003; Crews et al. 2012).

Horvatovich et al. (2000) suggested a time-saving (4-5 h) and cost-effective modified method for the detection of fat-containing irradiated foods compared to the reference methods EN1784 (1996) (volatile hydrocarbons) and EN1785 (2003) (2-ACBs). Barba et al. (2012) reported the capability of solid phase microextraction (SPME), coupled with either gas chromatography-ionization flame detector (CG-FID) or multidimensional gas chromatography-mass spectrometry (MDGC-MS) for the detection of radiolytic markers or volatile hydrocarbons produced during the irradiation of cooked ham. The proposed screening method provides potential for the rapid detection of most n-alkanes and n-alkenes produced during the irradiation of most fatty acids (oleic acid, stearic acid, and palmitic acid) in cooked ham.

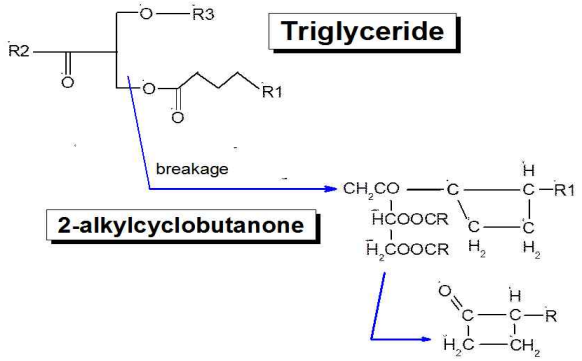

Figure 5. Radiation-induced cleavage of triglyceride molecule resulting in the formation of 2-alkylcyclobutanones. (Source: Akram et al. 2012)

Improvements and optimization is necessary for the detection of hydrocarbons in foods containing low fat content (<1%) and irradiated at low doses (0.5 kGy) (Ndiaye et al. 1999). Food processing technologies other than irradiation can also lead to the formation of ACBs. Therefore; to eliminate the possibility of false positive results, improvements are required in the EN1785 (2003) standard to verify whether 2-ACBs can be detected in non-irradiated foods or foods treated/processed by any means other than irradiation. Improvements are possible to widen the applicability spectrum of the 2-ACB technique. It may be possible by increasing the sample size to increase the extracted fat, optimizing the chromatographic separation of the 2-ACBs from the extracted fat, improving the sensitivity of the detector, optimizing the injection parameters, and permitting a variety of extraction techniques such as acetonitrile or supercritical fluid extraction (Crews et al. 2012).

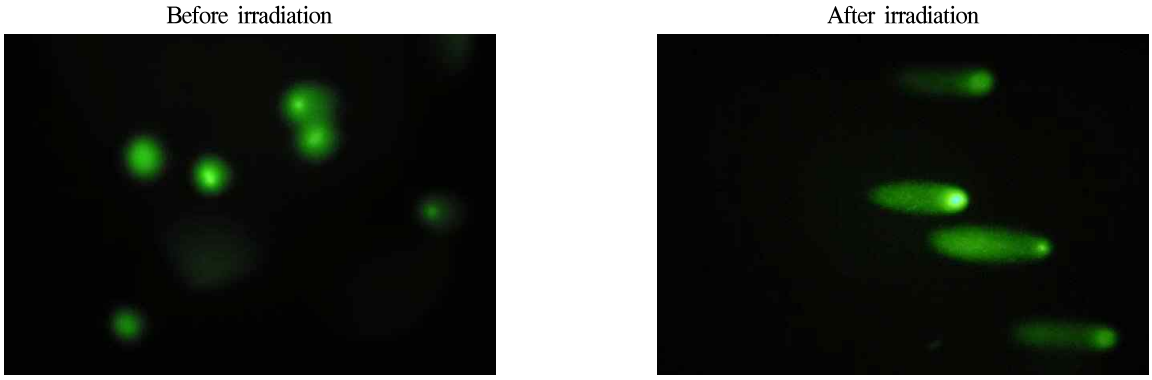

Figure 6. DNA comet assay results from non-irradiated (Left: round and intact) and irradiated poultry meat (Right: with clear tails). (Source: Khawar et al. 2011)

Biological Methods

DNA comet assay

DNA is the major cellular target for ionizing radiation. This radiation-induced DNA damage is responsible for inactivation of microorganisms, inhibition of growth, and other lethal effects. High energy ionizing radiation causes damage to DNA molecule such as denaturation, fragmentation, strand breaks, etc. Therefore, it is logical to investigate whether radiation damage to DNA in food can be utilized as a means of detecting ionization treatment (Delincee 1996; Chauhan et al. 2009). Considerably increased extension of the DNA from the nucleus towards the anode is exhibited by irradiated cells compared to non-irradiated ones shown in Figure 6 (Haine et al. 1995). Microgel electrophoresis is used to study DNA fragmentation of single cells or nuclei. Leakage of DNA subunits from the nuclei of lysed cells during electrophoresis yields 'comet' characteristics. Non‐irradiated samples do not exhibit this feature (Ostling and Johanson 1984; Stevenson 1992). The comet assay has wide application in different research areas and has been modified for the detection of irradiated foods by Cerda et al. (1993). Both alkaline and neutral protocols are available to measure DNA damage (EN13784 2001). This method is cheap and simple to perform in a short time and has been successfully tested for plant foods as well as animal foods with some limitations (Cerda et al. 1993, 1997; Delincee 1998; Khan et al. 2002, 2005; Verbeek et al. 2008).

Direct epifluorescent filter technique and aerobic plate count (DEFT/APC)

This method is based on a comparison of the APC with the count obtained using DEFT. The APC helps to calculate the number of viable microorganisms in a sample after a possible irradiation treatment while DEFT gives the total number of microorganisms, including nonviable cells, present in the sample shown in the microscopic fluorescent image of Figure 7 (Betts et al. 1988; EN13783 2001).

Oh et al. (2003) successfully evaluated the applicability of the DEFT/APC method for the screening of irradiated spices produced in Korea. Arau´jo et al. (2009) reported that the microbiological method DEFT/APC could be satisfactorily applied for the screening and identification of the hygienic status of minimally processed irradiated vegetables. The DEFT microorganism count is generally unaffected by irradiation. Particularly, microorganisms may be inactivated by irradiation and thus undetectable by the APC technique. However, it is important that sample storage may affect the DEFT/APC results (Jones et al. 1995).

The method has limitations regarding the use of fumigation or heat as decontamination treatments. In such a case, the DEFT/APC difference in counts can be similar to the difference in counts obtained after irradiation (EN13783 2001; Chauhan et al. 2009).

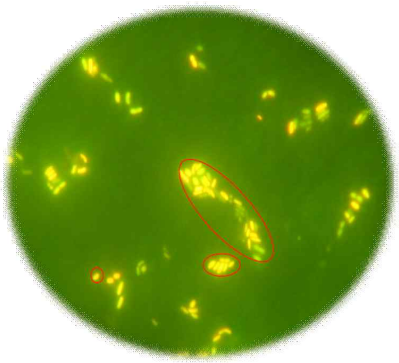

Figure 7. Microscopic fluorescent image of a DEFT/APC test for spices. (Source: Betts et al. 1988)

Limulus amebocyte lysate/ Gram-negative bacteria (LAL/GNB)

This microbiological assay is analogous to the DEFT/APC test and not totally specific to irradiated foods just as the DNA comet assay. The EN14569 (2004) protocol specifies its application on whole or different parts of poultry meat.

The technique evaluates the number of viable Gram-negative bacteria present in the sample and the concentration of bacterial endotoxins existing on the surfaces of the Gram-negative bacteria as lipopolysaccharides (LPS). The total amount of Gram-negative bacteria, both viable and dead, is calculated with the LPS. If the difference between the Gram-negative bacteria and the endotoxin is high, it is presumed that the sample was treated most probably with ionizing radiation (Haire et al. 1997; EN14569 2004).

The test is presumptive and economical. It has been validated as screening method for a variety of foodstuffs (Glidewell et al. 1993; Scatter et al. 1994; EN14569 2004). However, a significant amount of dead microbes in comparison to the viable fraction may be due to other reasons which can limit its useful application.

Germination and half embryo test

The basic principle of the germination test depends on the fact that irradiated seeds germinate at a considerably slower rate than control seeds (Stevenson and Stewart 1995). This seedling assay is mainly confined to vegetable seeds. It is easy and cheap but too slow for routine analysis. Kawamura et al. (1989; 1996) worked to reduce the analysis time and to accelerate the germination process. They established an improved germination test called the “half embryo test” for some fruits, particularly for irradiated grapefruit and citrus fruit, in which the embryo was used for germination in place of seeds. This test is based on the inhibition of shoot growth by γ-irradiation.

Kawamura et al. (1992) reported that the germination test can discriminated between γ-irradiated and non-irradiated rice even after long storage. Simlarly, Chaudhuri (2002) found that the germination method was efficient and reliable to detect γ-irradiated lentil seeds even after 12 months storage. Cutrubinis et al. (2004) successfully evaluated the applicability of the germination test as a detection method for garlic treated with up to 25 kGy in the dormancy period.

Conclusion

The screening methods for foods treated with ionizing radiations are based upon the changes in physical, chemical and biological aspects in food itself (Delincée 2002; Kwon et al. 2011). As physical methods, first PSL relies on the use of light to release energy stored by trapped charge carriers upon irradiation, while the viscosimetry technique depends on changes in the rheological properties of starch, cellulose, or pectin based foodstuffs after irradiation. Likewise, chemical method analyzing 2-ACB is based on the production and detection of hydrocarbons and 2-ACBs formed by the irradiation of fat based foods. Among biological tests, the DNA comet assay depends on the detection of radiation-induced DNA fragmentation, and DEFT/APC method identifies irradiation by making a comparison of the number of viable microorganisms (APC) present in the sample after irradiation with the total number of microorganisms. Similar to the DEFT/APC assay, the total amount of Gram-negative bacteria, both viable and dead, are calculated via LAL/GNB test. The difference between the number of Gram-negative bacteria and endotoxin level determines whether or not the food has been irradiated. Finally, the germination test simply verifies that irradiated seeds germinate at a significantly slower rate than control seeds.

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