Food Sci. Biotechnol. Vol. 14, No. 1, pp. 1 ~ 5 (2005)

Food Science and Biotechnology

Physical and Microbiological Approach in Proving the Identity of Gamma-irradiated Different Teas

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Abstract Photostimulated luminescence (PSL), thermoluminescence (TL), electron spin resonance (ESR), and direct epiflourescent filter technique/aerobic plate count (DEFT/APC) were applied to detect dried green, black, and oolong teas irradiated between 0-10 kGy. Teas irradiated at 2.5 kGy and higher showed over 5000 photon counts/60 sec, while non-irradiated teas yielded 650-1000 photon counts/60 sec. TL glow curves for minerals separated from teas were detected at about 300°C with low intensity in non-irradiated samples, whereas around 150°C with high intensity in all irradiated samples. Ratio of TL_1/TL_2 based on re-irradiation step, showing lower than 0.1 and higher than 1.44 for non-irradiated and irradiated samples, respectively, enhanced reliability of TL results. ESR measurements for irradiated teas showed signals specific to irradiation. Log DEFT/APC ratio increased with irradiation dose; this result could be applied to identify irradiated tea samples.

Keywords: irradiated teas, identity, PSL, TL, DEFT/APC

Introduction

Tea is the most popular beverage worldwide due to its refreshing and mildly stimulating action (1). Many reports are available on the health benefits of tea including its protective role against cardiovascular disease and cancer resulting from the antioxidant activity of the leaf flavonoids (2, 3). Like other plant materials, tea leaves are also prone to microbial spoilage and pest damage from agricultural practices as well as problems that may occur during harvesting, handling, and processing of raw materials. Chemical fumigants including ethylene oxide, methyl bromide, and phosphine have been used for the control of pests, microorganisms, and overall quarantine importance. However, these are either prohibited or being increasingly restricted for use on foods or food ingredients in most countries due to their adverse effects on the health and environment. Therefore, alternative means of control, which also provide safety and food security, are in high demand.

Food irradiation has been widely accepted and is now becoming a reality in many countries. In particular, spices and dried vegetables including herbal teas are major items for irradiation practices in most countries, and consequently have been popular commodities in food trade among countries. In this regard, suitable analytical methods are needed for confirming the identity of irradiated products in terms of enhancing consumers confidence regarding the quality of irradiated commodities by differentiating them from non-irradiated ones.

Photostimulated luminescence (PSL), which uses whole samples, has been studied as a screening method for many irradiated foods including brown shrimp, herbs, spices, seasonings, and shellfish (4, 5), and white ginseng powder (6). Thermoluminescence (TL) technique has also shown potential for the identification of various irradiated foods, such as herbs and spice (7-9), fruits (10), vegetables (11), and sea foods (12, 13) and was adopted as a standard method for detecting food containing silicate minerals (14). Electron spin resonance (ESR) spectroscopy can be applied to detect irradiated foods by measuring ions or radicals produced from molecules dissociated by irradiation energy, with its results affected by the nature and water activity of the foodstuff (15-17). The characteristics of the microbial population in irradiated foods can also be used to detect irradiated food (15). In addition, microbiological method based on direct epiflourescent filter technique (DEFT) and aerobic plate count (APC) has been used for the detection of irradiated spices (18, 19). The objective of this study was to examine the suitability of the different detection methods for identifying irradiated green, black, and oolong teas over the non-irradiated ones.

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Received May 3, 2004; accepted September 1, 2004

Materials and Methods

Materials Green (Korean), black (Sri Lankan), and oolong (Chinese) teas were purchased from H Tea Co., Korea and packaged in a commercial PE film prior to irradiation. Gamma irradiation was carried in a ^{60}Co irradiator at the Korean Atomic Energy Research Institute (KAERI; Daejon, Korea) at doses ranging from 0 to 10 kGy for the prepackaged samples, and the target doses were assured by a ceric/cerous dosimeter (5.6%).

Photostimulated luminescence (PSL) analysis PLS was measured using the SURRC Pulsed PSL irradiated food screening system (SURRC, Glasgow, UK) which was composed of a control unit, sample chamber, and detector head assembly. The control unit contained a stimulation source comprising of an array of infrared light (880-940) emitting diodes, which pulsed symmetrically on and off for equal periods. The PSL signal was detected by a bialkali cathode photomultiplier tube operating at a photon-counting

mode and automatically recorded in a computer connected with the system. Optical filtration was applied to define both the stimulation and detection wavebands. For screening, the signal levels were compared with two thresholds, a lower threshold T_1 of 700 counts/60 sec and an upper threshold T_2 of 5000 counts/60 sec (20). After checking the PSL apparatus by running an empty chamber test for possible contamination, followed by a test using irradiated and non-irradiated materials (in this case ginger powder supplied by SURRC, Glasgow, UK) the dispensed green, black, and oolong teas were introduced into the chamber of the SURRC Pulsed PSL system. PSL signals below the lower threshold were classified as those emitted from the non-irradiated samples, whereas those above the upper threshold were regarded to be from the irradiated ones. The signal levels between the two thresholds were classified as intermediates, which require further investigation.

Thermoluminescence (TL) analysis Separation of minerals was carried out through the method of DIN EN1788 (14). A sample was rinsed with water after ultrasonic agitation (5-10 min) using a nylon sieve (125 mm). Minerals were shifted to the test tube and separated from debris material by density separation using 5 mL sodium polytungstate (2.0 g/mL). For separation from organic constituents, minerals were treated with 1 N HCl, followed by NH_4OH. Subsequently, they were washed sequentially with water and acetone, fixed onto an aluminium disc using silicon, and placed in an oven at 50 °C overnight before analysis by the TLD system.

TL measurement was carried out using a TL reader (Harshaw TLD-4200, Hanger, Germany). Temperature was raised from 50 to 400°C at 5°C/sec and maintained at 400 °C for 5 sec. The light emission was recorded in a temperature-dependent mode as a glow curve in units of nano coulombs (nC). After the first glow curve was measured, the discs with the minerals were re-irradiated under a Co-60 irradiator at a normalizing dose of 1 kGy to eliminate the effect of different compositions and/or weights of minerals deposited on the discs. TL intensity was re-measured after the re-irradiation step (second glow curve). This glow curve was compared with the first glow curve for identification of irradiation treatment in terms of the ratio of the area of the first glow curve to that of the second glow curve (TL_1/TL_2).

Electron spin resonance (ESR) analysis ESR measurements were done using an ESR spectrometer (JES-TE300, Jeol Co., Tokyo, Japan). Tea samples were dried at 40°C for 42 hr and ground into powder. About 100 mg of each sample was placed in a quartz tube, which was placed in the resonator located between the opposite magnets, which could provide the intensity of magnetic field needed in the spectrometer (21). The irradiation conditions for the different teas with ESR spectroscopy were as follows: magnetic center field, 327.083±0.088 mT; microwave frequency, 9.193±0.005 GHz; microwave power, 400 μW; time constant of signal channel, 0.03 sec; sweep time, 0.5 min; and, modulation frequency, 100 kHz.

Direct epiflourescent filter technique/aerobic plate count (DEFT/APC) measurement Ten grams each non-irradiated and irradiated samples were added to peptone saline diluent (pH 7.2, 8.5 g sodium chloride and 1.0 g peptone/1000 mL), diluted between 10 and 20 times, and stirred vigorously. The solution was then filtered through a fast filter paper (Whatman No. 41), and the filtered solution was diluted with peptone saline diluents by a logarithmic dilution series (10^1-10^3 times). Each diluted solution was transferred to the filteration manifold tower for DEFT. The total viable count (APC) was determined after filteration through a fast filter paper (Whatman No. 4). Aliquots (0.2 mL) of suitable dilutants were spread on a plate count agar. The plates were incubated upside down at 30±1°C for 72 hr and then counted (19). All experiments were conducted in triplicates.

Calculation:

The DEFT count (X) per gram was calculated as follows:

X= DEFT count/g = (N x MF x DF)/n

where N/n is the mean number of DEFT units per microscope filed, DF the dilution factor of the sample, and MF the microscope factor.

The DEFT count was then converted into a logarithmic value. The difference between the DEFT count and APC count was then obtained by subtracting the APC count (logarithmic value) from the DEFT count (logarithmic value).

Results and Discussion

PSL properties Triplicate samples of non-irradiated and irradiated green, black, and oolong teas were dispensed into clean petridishes to cover the bottom surface. The petridishes were covered with lids to reduce the possibility of contamination from outside minerals. After checking the PSL apparatus by running an empty chamber test for possible contamination, followed by a test with irradiated and non-irradiated standard materials (in this case ginger powder), the dispensed samples were introduced into the

Table 1. Pulsed photostimulated luminescence determinations of irradiated teas at different doses

Sample	Irradiation dose (kGy)				
	0	2.5	5	7.5	10
Green tea	1001±213[1](M)[3]	20773±150(+)[4]	21293±153(+)	31257±182(+)	36125±195(+)
Black tea	786±494(M)	5202±443(+)	6038±988(+)	7076±194(+)	7327±196(+)
Oolong tea	650±91(-)[2]	8353±101(+)	10894±113(+)	13714±125(+)	15117±130(+)

[1)]Means of triplicate±standard deviations.
[2)]Less than 700 counts/60 sec.
[3)]700-5000 counts/60 sec.
[4)]More than 5000 counts/60 sec.

chamber of the SURRC PPSL system.

Table 1 shows the PSL values for green, black, and oolong teas. The photon counts of non-irradiated samples were 1001, 786, and 650 for green, black, and oolong teas, respectively. In previous reports on PSL measurements for different foods such as dried spices, condiments, and shrimp, photon counts of 700-1000 were normally detected in non-irradiated foods, while irradiated foods showed much higher photon counts (4000-5000) (4, 5, 22). Threshold values indicated green and oolong teas were suitable for PSL measurements to detect irradiation. Non-irradiated green and black teas yielded intermediate signals, requiring the use of additional method such as TL or ESR. PSL method, however, is very quick and easy to perform, and may give valuable information.

TL properties TL glow curves of minerals isolated from the irradiated and non-irradiated green, black, and oolong teas were recorded within 1 month of irradiation. Figure 1-3 show the typical glow curves of non-irradiated and irradiated green, black, and oolong teas, respectively. The glow curve of irradiated samples peaked approximately at 150°C with high intensity. The intensity of the glow curves for both irradiated and non-irradiated samples showed a significant difference. European Committee for Standardization (CEN) has proposed thresholds not employing the whole integrated area of the glow curve, but just the interval (±10 up to ±40°C in the range of 150-250°C). When using this recommended temperature interval, TL ratios of irradiated samples are typically greater than 0.5, whereas those of non-irradiated samples are below 0.1. If the ratio is between 0.1 and 0.5, then the shape of the glow curve should be taken into account (14).

The TL results were normalized by carefully re-irradiating the stainless steel disc with the deposited minerals at 1 kGy to obtain a second glow curve. The ratio of the integrated areas of the first glow curve to that of the second glow curve (TL_1/TL_2) was calculated for irradiated and non-irradiated samples. The ratios (TL_1/TL_2) of all non-irradiated samples were less than 0.01 except for oolong tea, which showed a ratio of 0.06. For all irradiated samples, the ratio was 1.44 or higher. The results are summarized in Table 2. On the basis of the (TL_1/TL_2) ratio, it is possible to correctly identify all irradiated samples.

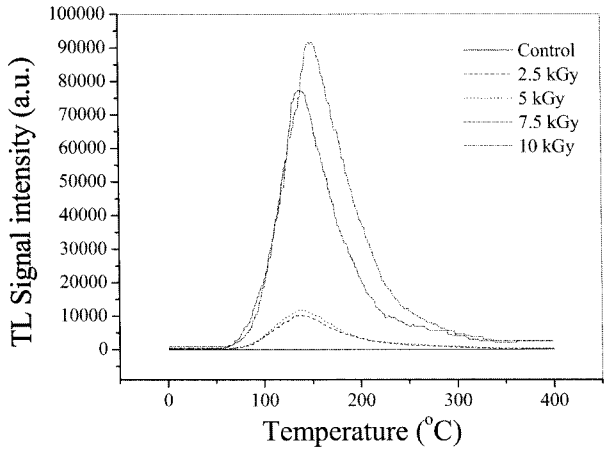

Fig. 1. Glow curves of minerals separated from irradiated green tea at different doses.

ESR properties For dried green, black, and oolong teas, although settings for the ESR spectrometer to detect the cellulose radical were applied, detection was not successful. Only non-specific central peaks were observed at g_1=2.008±0.0007, 2.007±0.0001, and 2.007±0.0001 with irradiated green, black, and oolong teas, respectively (Fig. 4). Similar tendencies were observed in different kinds of foods by Gorden *et al.* (23), Nam *et al.* (24), and Bayram and Delincée (25). The ESR signal intensities of irradiated green, and black oolong, teas, however, linearly increased with the increase in irradiation dose from 0 to 10 kGy, suggesting the supplementary use of ESR for the identification of irradiated teas (Fig. 5).

DEFT/APC properties Characteristics of the microbial population of irradiated foods have been used to develop detection methods for irradiated foods (26). A microbiological

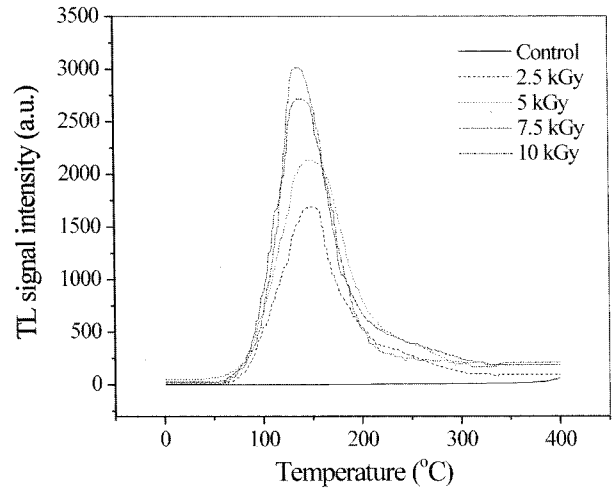

Fig. 2. Glow curves of minerals separated from irradiated black tea at different doses.

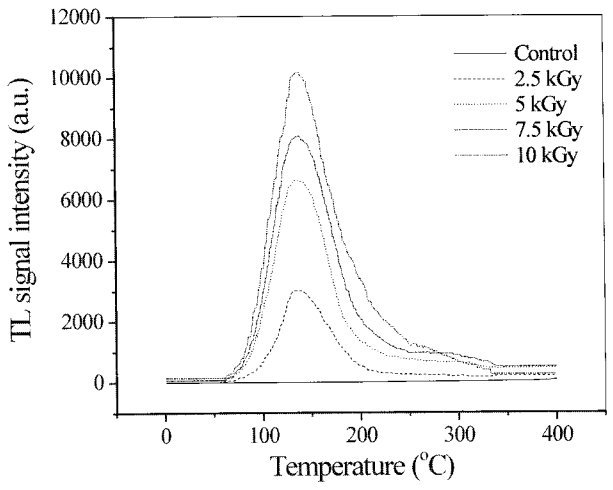

Fig. 3. Glow curves of minerals separated from irradiated oolong tea at different doses.

Table 2. TL ratio of minerals separated from dried teas after gamma irradiation

Sample	TL ratio[1]					Correlation equation and coefficient [2]	
	Control	2.5 kGy	5 kGy	7.5 kGy	10 kGy		
Green tea	0.01[3]	2.66	3.14	3.52	3.96	Y=0.8746x+0.03465	R^2=0.792
Black tea	0.01	1.44	2.04	4.10	4.78	Y=1.2179x-1.17900	R^2=0.973
Oolong tea	0.06	1.81	1.99	3.02	3.17	Y=0.7425x-0.21890	R^2=0.889

[1] Integrated TL_1/TL_2.
[2] x: irradiation dose (kGy), y: TL ratio.
[3] Means of duplicate.

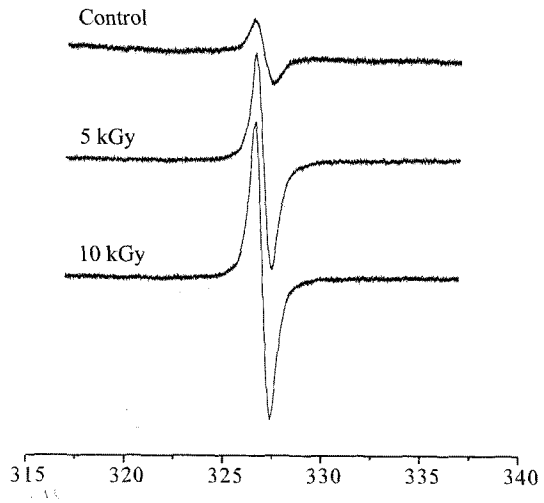

Fig. 4. Typical ESR spectra of irradiated green tea.

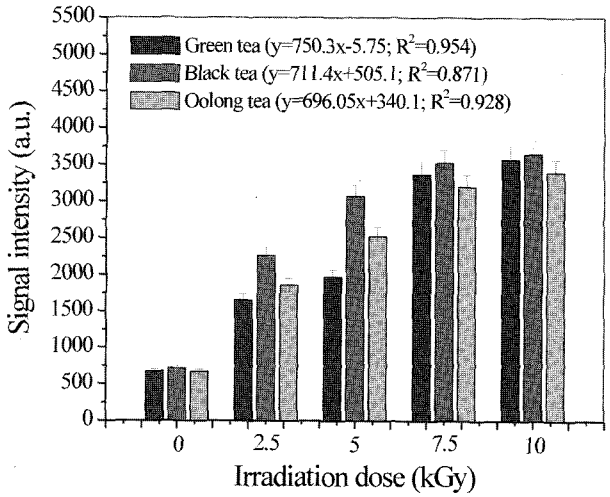

Fig. 5. Dose-dependent ESR signal intensities in irradiated teas.

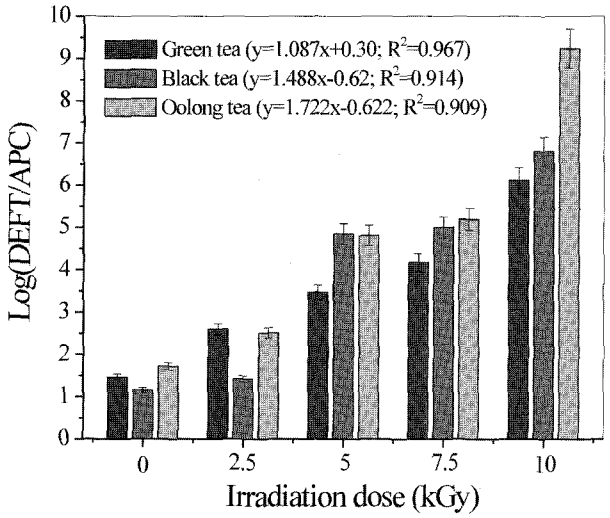

Fig. 6. Histogram of log DEFT/APC ratio of irradiated teas.

Table 3. The logarithmic microbial accounts of the irradiated dried teas

Sample		Irradiation dose (kGy)				
		0	2.5	5	7.5	10
Green tea	Log DEFT	6.43[1]	6.49	6.43	6.43	6.36
	Log APC	4.39	2.49	1.85	1.54	1.04
Black tea	Log DEFT	6.45	6.43	6.41	6.41	6.46
	Log APC	5.58	4.51	1.32	1.28	0.95
Oolong tea	Log DEFT	6.38	6.39	6.36	6.39	6.38
	Log APC	3.69	2.54	1.32	1.23	0.69

[1] Means of triplicate.

method based on DEFT and the conventional APC has been used for the detection of irradiated spices (18). DEFT count enumerates the total number of contaminated microorganisms, irrespective of the viabilities of untreated and treated samples. APC indicates the number of viable microorganisms capable of forming colonies on an agar plate and is expressed as a colony forming unit (CFU). If samples are irradiated, most viable microorganisms are killed and the ratio of DEFT/APC increases. Thus, the quotient of the two counts is used in assessing whether the sample is irradiated.

The results of both non-irradiated and irradiated teas are presented as logarithmic microbial counts in Table 3. The DEFT counts of all samples were nearly equal independent of irradiation, whereas the APC counts decreased gradually with irradiation dose increments. Therefore, the log DEFT/APC ratio increased with increasing dose level. In green tea, the log DEFT/APC ratio of non-irradiated sample was 1.46 and increased to higher than 2.5 as the irradiation dose increased. In the case of black tea, the ratios were 1.15 and 1.42 for samples non-irradiated and irradiated with 2.5 kGy, respectively, and samples irradiated at 5 kGy or higher showed a log DEFT/APC ratio of more than 4.85. In oolong tea, the non-irradiated and irradiated samples showed 1.72 and greater than 2.5, respectively (Fig. 6). In a

previous research carried out by Oh *et al.* (27, 28), cereal grains and beans showed log DEFT/APC ratios between 2.0 and 3.0 at 0.5 kGy or higher.

These results thus suggest irradiation treatment of dried teas can be identified through a combination of physical and microbiological methods. PSL and DEFT/APC have been proven useful as screening methods, when involving a large number of samples, to identify in a limited period whether or not the samples have been irradiated. However, to obtain a definite proof of irradiation for the unknown samples through both screening, TL is recommended as a means of verification due to its success on teas.

Acknowledgments

This work was supported by Korea Institute of Science & Technology Evaluation and Planning (KISTEP), and Ministry of Science & Technology (MOST), Korea, through the National Nuclear Technology Program.

References

1. Hamilton MJMT. Antimicrobial properties of tea (*Camellia sinenses* L.). Antimicro. Agents Chemiother. 39: 2375-2377 (1995)
2. Blot WJ, Chow WH, McLaughlin K. Tea and Cancer: a review of the epidemiologic evidence. Eur. J. Cancer Prevent. 5: 425-436 (1996)
3. Dreosti IE, Wargovish MJ, Yang CS. Inhibition of carcinogenesis by tea: the evidence from experimental studies. Crit. Rev. Food Sci. Nutr. 37: 761-770 (1997)
4. Sanderson DCW, Carmichael LA, Naylor JD. Recent advances in thermoluminescence and photostimulated luminescene detection methods for irradiated food. pp. 124-138. In: Detection Methods for Irradiated Foods. McMurray CH, Stewart EM, Gray R, Pearce J (eds). The Royal Society of Chemistry, Cambridge, UK (1996)
5. Sanderson DCW, Carmichael LA, Spencer JQ, Naylor JD. Luminescence detection of shellfish. pp. 139-148. In: Detection Methods for Irradiated Foods. McMurray CH, Stewart EM, Gray R, Pearce J (eds). The Royal Society of Chemistry, Cambridge, UK (1996)
6. Chung HW, Delincée H, Kwon JH. Photostimulated luminescence-thermoluminescence application to detection of irradiated white ginseng powder. Korean J. Food Sci. Technol. 32: 265-270 (2000)
7. Khan HM, Delincée H. Detection of radiation treatment of spices and herbs of Asian origin using thermoluminescence of mineral contaminants. Appl. Radiat. Isot. 46: 1071-1075 (1995)
8. Lesgards G, Fakirian A, Raffi J. Thermoluminescence identification of irradiated foodstuffs: LARQUA research. pp. 158-167. In: Detection Methods for Irradiated Foods. McMurray CH, Stewart EM, Gray R, Pearce J (eds). The Royal Society of Chemistry, Cambridge, UK (1996)
9. Mamoon A, Abdul-Fattah AA, Abulfaraj WH. Thermoluminescence of irradiated herbs and spices. Radiat. Phys. Chem. 44: 203-206 (1996)
10. Pinnioja S, Autio T, Niemi E, Pensala O. Import control of irradiated foods by the thermoluminescence method. Z. Lebensm. Unters. Forsch. 196: 111-115 (1990)
11. Kiyak N. Application of thermoluminescence technique to identify radiation processed foods. Radiat. Phys. Chem. 46: 721-723 (1995)
12. Schreiber GA, Hoffmann A, Helle N. Bögle KW. Method for routine control of irradiated food: determination of the irradiation status of shellfish by thermoluminescence analysis. Radiat. Phys. Chem. 43: 533-544 (1994)
13. Pinnioja S. Suitability of the thermoluminescence method for detection of irradiated food. Radiat. Phys. Chem. 42: 397-400 (1993)
14. EN 1788. Foodstuffs-Detection of irradiated food from which silicate minerals can be isolated, method by thermoluminescence. European Committee for Standardization, Brussels, Belgium (1996)
15. Delincée H. Analytical methods to identify irradiated food-a review. Radiat. Phys. Chem. 63: 455-458 (2002)
16. Helle N, Linke B, Bögle KW, Schreiber GA. Elektronen-spin-resonanz-spektroskopie an Gewürzproben. Nachweis winer Behandlung mit ionisierenden Straheln. Z. Lebensm. Unters. Forsch.195: 129-132 (1992)
17. Stewart EM. Detection methods for irradiated foods. In: Food Irradiation: Principles and applications. pp. 347-386. Molins RA (ed). John Wiley and Sons, New York, NY, USA (2001)
18. Boisen F, Skovgaard N, Ewalds S, Olsson G, Wirtane G. Quantitation of microorganisms on raw minced meat using the direct epiflourescenct filter technique: NMKL Collaborative Study. J. AOAC Int. 75: 465-473 (1992)
19. Wirtanen G, Sjöberg AM. A microbiological method (DEFT/APC) for the identification of irradiation of spices and seafood. pp. 25-34. In: Recent advances on detection of irradiated food. Leonardi M, Raffi JJ, Belliardo JJ (eds). BCR Information. 1993, Luxembourg: Commission of the European Communities (Report EUR/14315/en) (1993)
20. prEN 13751. Foodstuffs-Detection of irradiated food using photo-stimulated luminescence, European Committee for Standardization, Brussels, Belgium (1997)
21. EN 1787. Foodstuffs-Detection of irradiated food containing cellulose, method by ESR spectroscopy. European Committee for Standardization, Brussels, Belgium (2000)
22. Sanderson DCW, Carmichael LA, Fisk S. An international collaborative blind trial of photostimulated luminescence detection of irradiated herbs, spices and seasonings. Final Report, MAFFIB073 (1997)
23. Gorden TT, John RP, Don RH, Charles RH. and Graham, L.W. EPR detection of free radical in coffee and gamma-ray irradiated foodstuffs. Appl. Radiat. Isot. 40: 1223-1226 (1989)
24. Nam HS, Suh CS, Yang JS. Identification of gamma-irradiated teas by ESR spectroscopy. Food Sci. Biotechnol. 10: 602-605 (2001)
25. Bayram G, Delincée H. Identification of irradiated Turkish foodstuffs combining various physical detection methods. Radiat. Phys. Chem. 15: 81-91 (2003)
26. Delincée H. Detection of food treated with ionizing radiation. Trends Food Sci. Technol. 9: 73-82 (1998)
27. Oh KN, Lee SY, Yang JS. Microbial identification of beans exposed to gamma irradiation (DEFT/APC method). Food Sci. Biotechnol. 11: 257-259 (2002)
28. Oh KN, Lee SY, Yang JS. Detection of gamma-irradiated grains by DEFT/APC method. Korean Food Sci. Technol. 34: 380-3847 (2002)