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Analysis of γ-Irradiated Melon, Pumpkin, and Sunflower Seeds by Electron Paramagnetic Resonance Spectroscopy and Gas Chromatography−Mass Spectrometry

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Seeds of melon (*Citrullus lanatus* var. sp.), pumpkin (*Cucurbita moschata*), and sunflower (*Heliantus annus*) were γ-irradiated at 1, 3, 5, and 10 kGy and analyzed by electron paramagnetic resonance (EPR) and gas chromatography−mass spectrometry (GC-MS) according to EN1787:2000 and EN1785:2003, respectively. Distinguishable triplet signals due to the presence of induced cellulose radicals were found at 2.0010−2.0047 g in the EPR spectra. The γ-irradiated radiolytic markers of 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) were identified in all irradiated seed samples. Both the free radicals and the alkylcyclobutanones were found to increase with irradiation dose. In general, linear relationships between the amount of radicals and irradiation dosage could be established. Studies at an ambient temperature (20−25 °C) in a humidity-controlled environment showed a complete disappearance of the cellulosic peaks for irradiated samples upon 60 days of storage. Such instability behavior was considered to render the usefulness of using EPR alone in the determination of irradiated seed samples. On the other hand, 2-DCB and 2-TCB were also found to decompose rapidly (>85% loss after 120 days of storage), but the radiolytic markers remained quantifiable after 120 days of postirradiation storage. These results suggest that GC-MS is a versatile and complimentary technique for the confirmation of irradiation treatment to seeds.

KEYWORDS: Alkylcyclobutanones; detection of irradiated seeds; EPR; GC-MS; storage influence

INTRODUCTION

Melon, pumpkin, and sunflower seeds are among the major agriculture exports in China and the most popular treats during the Lunar New Year among Chinese communities. Because of their amino acid- and fatty acid-rich contents, such seeds are also served as important nutritional diets in some African countries (*1*) and as traditional snacks after salting and roasting in Arabian countries (*2*). Like other agricultural products, these edible seeds frequently undergo microbial deterioration during storage and transport. They are particularly vulnerable to infections caused by fungi *Aspergillus flavus* and result in aflatoxin B_1 poisoning symptoms after consumption (*3*). To prevent public health threats and to meet international regulatory requirements, disinfection treatments are applied to control the spoilage process and to preserve the edible quality of the seeds. Fumigant-free high-energy irradiation such as γ-irradiation is recognized as a safe and a preferable technique in many countries worldwide (*4*). Evaluation on the effectiveness of γ-irradiation in various horticultural products showed that significant depletion of fungal populations on seeds commenced with an irradiation dose of 1−2 kGy and complete inhibition of the contaminating fungi at 4 kGy or above (*5*). At dose levels of 1.5 kGy, γ-irradiation could be an efficient quarantine means for eradicating most anthropoid pests in fruit and vegetable commodities (*6*). Furthermore, irradiation at higher doses (10−18 kGy) has the beneficial effects of reducing the phytic acid content and flatulence-causing oligosaccharides in broad beans, and it has been suggested that γ-irradiation is a good procedure to improve the edible quality of broad beans from a nutritional point of view (*7*). Because the use of γ-irradiation on crops is widespread, reliable detection methods for irradiated foodstuffs have recently been widely explored.

Electron paramagnetic resonance (EPR) has the merits of being simple and convenient and has been adopted in European official methods (*8*) for rapid qualitative analysis of irradiated bones (EN1786:1996), cellulose tissues (EN1787:2000), and sugar-containing foodstuffs (EN13708:2001). An unambiguous EPR spectrum for irradiated plant materials is evidenced with a triplet or a cellulosic signal (*9*) that originates from the imprisonment of free radicals within the matrices. The signal usually consists of a dominant central peak in the region of about $g = 2.0050 \pm 0.0050$ together with two small characteristic cellulosic peaks on both sides with a spacing of about 6 mT (*7*). Recent EPR studies on the behavior of these free radicals in various cellulose tissues (*10*−*13*) showed that the triplet signals declined readily in most cases, and their stability was mainly governed by the food types and storage environ-

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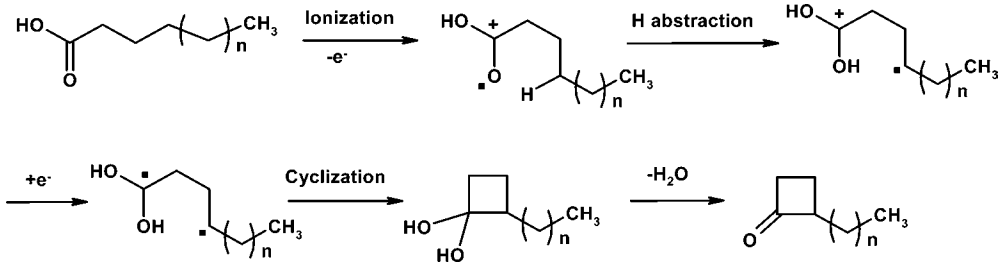

Figure 1. Radiolytic transformation of free fatty acids to cyclobutanones during γ-irradiation. Palmitic acid ($n = 11$) forms 2-DCB, and stearic acid ($n = 13$) forms 2-TCB.

ments after irradiation. In addition, the cellulosic peaks might not be detected in some irradiated herbs and spices (*14*). These unfavorable observations might limit the overall precision and effectiveness of using EPR for ascertaining irradiation doses in plant materials.

The use of 2-alkylcyclobutanones for the detection of irradiated foods was proposed in the early nineties (*15*) and became one of the European official methods (EN1785:2003) for irradiated lipid-containing foods. The detection mechanism is based on chromatographic determination of 2-alkylcyclobutanones that were uniquely formed from radiolysis of fatty acids via ionization and cyclic rearrangement processes (**Figure 1**). Among the existing identified 2-alkylcyclobutanone members, 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) are the most studied compounds. Because 2-alkylcyclobutanones have exclusively been confined to irradiated fat-containing foods, the method has been claimed to be highly specific to differentiate irradiated meat (*16*, *17*) and eggs (*18*). Its application in fruit seeds like mango, papaya (*19*), and perilla (*20*) was shown to be very successful as well.

Although there are a number of research studies on the identification of free radicals and radiolytic species that are generated after ionizing radiation treatment to some food species, to our best knowledge, this is the first study of its kind on irradiated melon, pumpkin, and sunflower seeds. The objectives of this study are to study the induced radicals and 2-alkylcyclobutanones in common edible seeds of melons (*Citrullus lanatus* var. sp.) (Two edible varieties of watermelon seeds, commonly known as red melon seeds and black melon seeds in China, were tested.), pumpkin (*Cucurbita moschata*), and sunflower (*Heliantus annus*) after exposure to γ-irradiation at different doses by EPR and gas chromatography−mass spectrometry (GC-MS); to investigate the stability of these chemical and radiolytic markers during storage at ambient temperature; and to compare the performance and applicability of the two detection methods.

MATERIALS AND METHODS

Reagents. Analytical grade *n*-hexane, octane, anhydrous sodium sulfate, and cyclohexylcyclohexanone were purchased from Sigma. Florisil (60−100 mesh) was conditioned to 340−350 °C overnight to remove moisture and organic contaminants and stored in desiccators before use. Standards of 2-DCB and 2-TCB were obtained from Laboratoire de Chimie Analytique et Sciences de l'Aliment, Université Louis Pasteur (Strasbourg, France), and the purity of both standards was >95%.

Setting of EPR Spectrometer. The X-band in EPR spectra was recorded with a Bruker EMS104 analyzer (Bruker Analytische Messtechnik, GmbH, Karlsruhe, Germany) at ambient temperature of (20−25 °C). Operation procedures detailed in EN1787:2000 for the detection of irradiated food containing cellulose were adopted, and instrumental settings were as follows: center of magnetic field, 38 mT; sweep width, 20 mT; microwave frequency, 9.76 GHz; modulation amplitude, 0.4 mT; sweep time, 42 s; time constant, 20.5 ms; and number of scans, three cycles. Because microwave distribution in the cavity might not be uniform, it was important to ensure a reproducible sample positioning. In this respect, a motorized height adjustment pedestal (EMS104 cavity template) was used. The accuracy of the *g* factor of the EPR spectrometer was regularly calibrated at the time of measurement using alanine standard (EMS 914−1005). The signal was computed as the peak-to-peak amplitude of the dominant peak in the EPR spectra and calculated in arbitrary units per sample weight (AU/mg).

Setting of GC-MS Instrument. A Shimadzu GC-MS (model QP-2010, Kyoto, Japan) equipped with an autosampler and connected with a HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film, J & W Scientific) was used. Helium carrier gas was set at a flow rate of 1.6 mL/min, and separation of 2-DCB and 2-TCB was carried out using a temperature program as follows: injector temperature, 250 °C; column temperature, 80 °C for 1 min, ramped to 220 °C at 15 °C/min, and held for 7 min, then to 300 °C at 25 °C/min, and held for 15 min. The transfer line and the ion source were set at 300 °C, and the ionization voltage for electron impact mode was 70 eV. Aliquots of 2 μL were injected into the GC-MS system under splitless mode, and 2-DCB and 2-TCB were analyzed at *m*/*z* 98 and 112 under selected ion monitoring mode.

Calibration of ^{137}Cs Irradiator. Irradiation was carried out using a ^{137}Cs irradiator (Gammacell-1000 Elite, MDS Nordion, Canada) at ambient temperature. Calibrations on (i) the absorbed dose distribution in blood equivalent in medium using a radiochromic film and (ii) central dose rate (CDR) with reference standard Fricke dosimeters (The Fricke dosimeter response was verified using a standard radiation field measured and certified by the National Institute of Standards and Technology, United States.) were regularly measured. The absorbed doses at the center were normalized to 30 Gy, and the maximum and minimum absorbed doses obtained were 32.0 ± 5.80 and 24.1 ± 0.63 Gy, respectively, and the uncertainty of the CDR was found to range from 2.7 to 6.6% at the 95% confidence level over the past 6 years.

Irradiation of Seeds and Sample Treatment. Four different lots of intact seed samples (black melon, red melon, pumpkin, and sunflower seeds), which were acquired from the local market, screened, and found to contain undetectable levels of irradiation-induced species, were used as the zero dose control samples. Four random portions (each about 500 g) of each type of these control seeds were separately placed in four polypropylene bags and subjected to irradiation at 1, 3, 5, and 10 kGy, respectively, at a dose rate of 3.52 Gy/min in the γ-irradiator at ambient temperature. The irradiation range studied covered the FDA's permitted doses for arthropod disinfections and pathogen controls in seeds (*21*) and mimicked the conditions that are used in commercial practice. The irradiated seeds were stored at 20 °C and kept in a humidity-controlled cabinet (relative humidity at 30%) prior to measurements. Storage influence on the stability of induced irradiated species, free radicals, and 2-alkylcyclobutanones in these seeds was determined. Triplicate measurements were performed, and data were analyzed using one-way analysis of variance followed by Tukey's multiple comparison test for significant differences (Sigmastat 2.0, Jandel Corp., United States) at the $p \leq 0.05$ level. Procedures for measuring nonirradiated and irradiated samples are described as follows.

EPR Measurements. Measurements were taken at days 0, 7, 14, 21, 28, 60, and 120 after irradiation, respectively. About 1 g of each seed sample was randomly taken from the irradiated lot, and seed pods

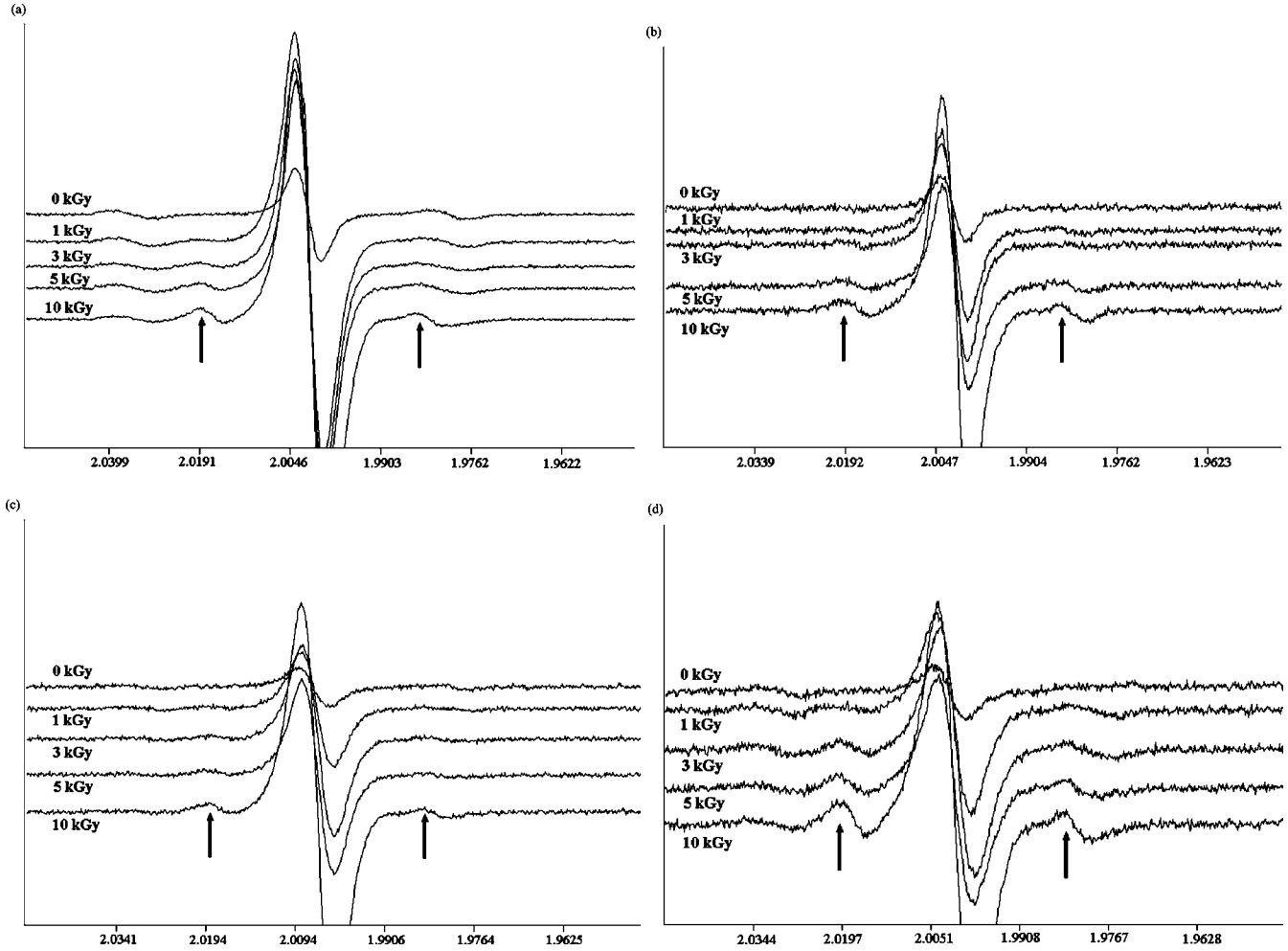

Figure 2. Typical EPR spectra (*y*-axis, the signal intensities in arbitrary units/weight; *x*-axis, *g* values) of samples of (**a**) black melon seeds, (**b**) red melon seeds, (**c**) pumpkin seeds, and (**d**) sunflower seeds at day 21 after γ-irradiation at various irradiation doses. The cellulosic peaks (shown by arrows) in low-dosed samples started to fade away.

were removed from the flesh and blended. A quantity of 100−200 mg of homogenized seed pod was accurately weighed and packed into an EPR quartz tube with tapping to reduce the void volume. The quartz tube was put into the sample chamber of the EPR spectrometer, and the EPR signals intensity was measured.

GC-MS Measurements. Prior to GC-MS analysis, about 50 g of seeds was randomly sampled and an amount of 10−20 g of edible pulp (freshly removed from seed pods) was blended, accurately weighed, and mixed with about 20 g of anhydrous sodium sulfate. The mixture was put into an extraction thimble, and 100 mL of *n*-hexane was added into a 250 mL round-bottomed flask and fitted into a Soxhlet extractor. The extraction thimble was positioned in the extractor, and 40 mL of *n*-hexane was added. The whole mixture was refluxed for 6 h at about three cycles per hour. The final *n*-hexane extract was dried in a rotary evaporator and then reconstituted with approximately 10 mL of *n*-hexane. The concentrate was transferred into a cylinder, then adjusted to 100 mL with *n*-hexane, and dried with 10 g of anhydrous sodium sulfate. About 30 g of deactivated Florisil powder was packed into a glass column (200 mm × 20 mm i.d.) and topped with 1 cm of anhydrous sodium sulfate. An aliquot equivalent to 200 mg of lipid (The amount of lipid present in a sample had been determined according to EN1785:2003 method) was applied to the column and eluted at a flow rate of 2−5 mL/min. The column was washed with 150 mL of *n*-hexane and then eluted with 150 mL of 1% diethyl ether in *n*-hexane. The eluant containing 2-DCB and 2-TCB was collected in a 250 mL round-bottomed flask. The ether fraction was concentrated to about 5−10 mL using a rotary evaporator, then decanted into a 10 mL glass tube, and dried under a gentle stream of nitrogen at 25 °C. The concentrate was reconstituted with 0.2 mL of *n*-hexane containing the internal standard of 2-cyclohexylcyclohexanone and finally transferred to a vial for GC-MS analysis. Measurements were taken at days 0, 7, 14, 28, 60, and 120 for black melon seeds and at days 0 and 120 for red melon, pumpkin, and sunflower seeds, respectively. The concentrations of 2-DCB and 2-TCB were expressed in μg/g on a lipid basis.

Quality Control for GC-MS. Identification of 2-DCB and 2-TCB in irradiated samples was confirmed by the presence of mass fragments at *m*/*z* 98 and 112 at the defined retention time windows and matching of the relative ion abundance ratios (*m*/*z* 98−112) to those obtained from the calibration standards. Estimation of the concentrations of 2-DCB and 2-TCB was performed using five-point calibration curves with $r^2 > 0.995$. Minimum quantification limits (MQL) were calculated as 10 times the standard deviation of seven replicate analyses of a standard mixture solution of cyclobutanones each at the concentration of 0.05 μg/mL. The detection limits were further verified with the GC-MS measurement of 2-DCB and 2-TCB from seed samples that received a 1 kGy-irradiation dose.

RESULTS AND DISCUSSION

EPR Signals of Irradiated Seeds. Typical EPR spectra of irradiated seed pods are composed of a dominant central peak and two small cellulosic peaks, whereas only a single and less intense peak was observed for unirradiated samples as shown in **Figure 2**. The dominant central peaks were detected with a *g* value of 2.0010 for black melon seeds, 2.0014 for red melon seeds, 2.0047 for pumpkin seeds, and 2.0026 for sunflower seeds, respectively. The signals produced due to cellulose

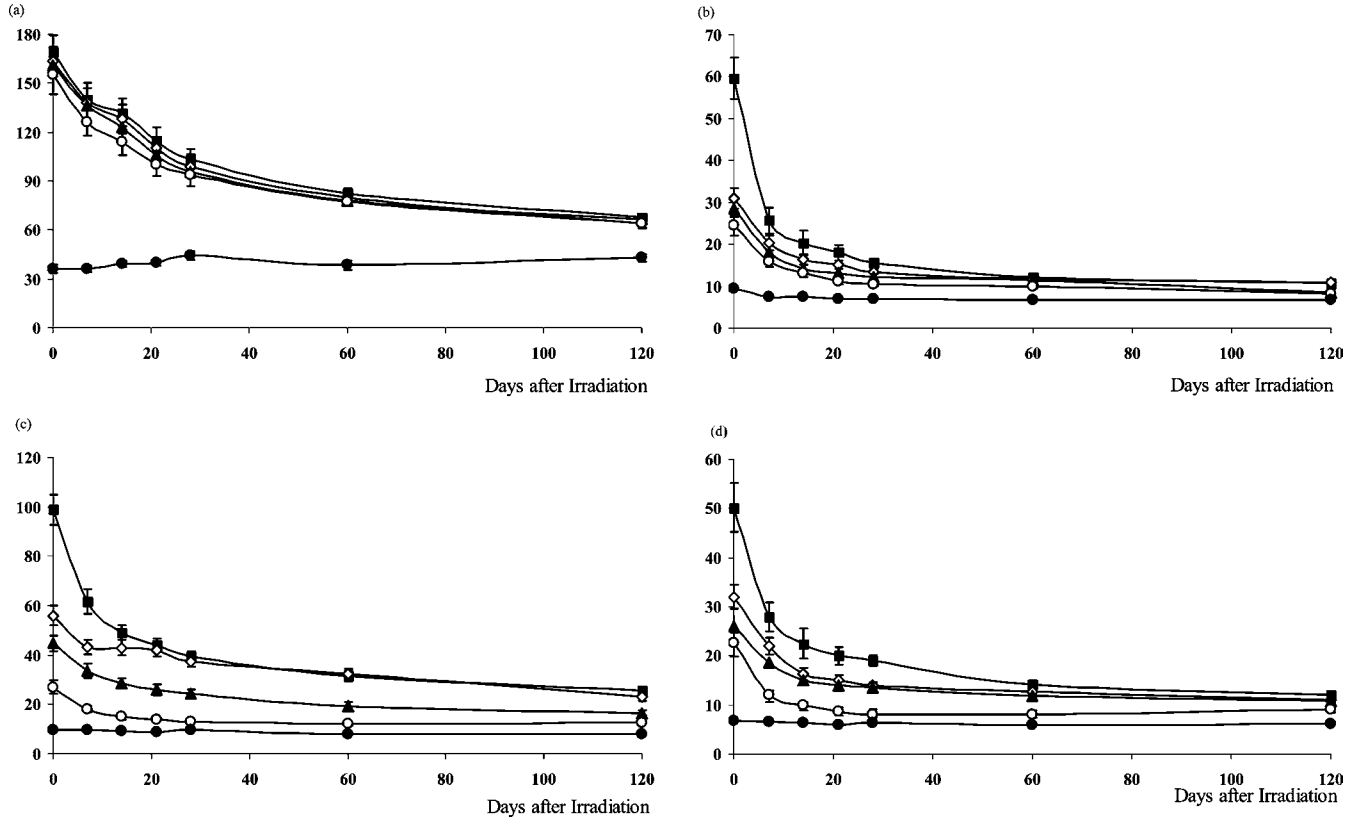

Figure 3. Decay of mean ± SD EPR signal intensity from triplicate measurements in samples of (**a**) black melon seeds, (**b**) red melon seeds, (**c**) pumpkin seeds, and (**d**) sunflower seeds after γ-irradiation at 0 (●), 1 (○), 3 (▲), 5 (◊), and 10 kGy (■).

radicals were found to increase with the irradiation doses from 1 to 10 kGy with good correlation coefficients of 0.934 in red melon seeds, 0.986 in pumpkin seeds, and 0.923 in sunflower seeds. These findings agreed well with the previous studies (*22*, *23*) that linear dose−response relationships were found in some food species, and this enabled a fairly good estimation of irradiation doses by measuring the intensity of the EPR signals. Such correlations were, however, not observed in black melon seeds ($r^2 = 0.387$) where the signal intensities only slightly increased with an increase in irradiation dose from 1 to 10 kGy. When compared to other seed types under study, a comparatively large difference in EPR signal intensities was noted between the unirradiated and the irradiated black melon seeds (**Figure 3**).

All irradiated seed samples under study produced distinguishable triplets in EPR spectra, although the characteristic cellulosic peaks (*9*) were found to fade away upon storage. After storage for 21 days, the two small peaks in low dose samples (1−3 kGy dose) diminished significantly (**Figure 2**) and the peaks faded away completely at day 60 in all samples under study (1−10 kGy). Instability of the cellulosic signals has also been recently reported in other fruit species. After storage for 3 weeks at 1 °C, the cellulosic signals in strawberry samples (irradiated at 1.5−3 kGy) decreased significantly and were almost not detected in "low dose" (0.5 kGy) samples (*13*), and approximately 40−58% of the cellulosic signals in kiwi fruits, which had previously irradiated at 1−2 kGy decreased upon storage for 6 weeks at 4 °C (*24*). Unlike bone samples where free radicals were stabilized in the rigid and hard matrices (*25*), the central EPR signals in seed samples were also shown to decline over the 120 day study period (**Figure 3**). The loss of free radicals in black melon seeds (13−18%) during the first week of storage was less severe then that of red melon (35−57%), pumpkin (22−43%), and sunflower seeds (31−50%). The signals, however, were found to be significantly higher than the unirradiated samples and could still be detected at the end of the study period or approximately the normal shelf life of seed samples. The results indicated that EPR could be applicable to the detection of irradiation treatment to the above seed types under study.

Table 1. Composition of Total Fat and Major Fatty Acids in Edible Pulps of Melon, Pumpkin, and Sunflower Seeds[a]

	mean composition ± SD (%)			
	black melon seed	red melon seed	pumpkin seed	sunflower seed
total fat	24.4 ± 1.2	38.3 ± 1.6	42.5 ± 1.6	32.9 ± 1.1
palmitic acid (16:0)	10.5 ± 0.06	9.48 ± 0.05	13.8 ± 0.06	5.71 ± 0.04
stearic acid (18:0)	7.45 ± 0.02	7.17 ± 0.02	7.01 ± 0.02	5.43 ± 0.02
oleic acid (18:1)	10.6 ± 0.04	13.2 ± 0.05	20.7 ± 0.08	29.2 ± 0.08
linoleic acid (18:2)	70.1 ± 0.10	69.7 ± 0.12	56.0 ± 0.11	58.5 ± 0.12

[a] The concentrations determined were the means of 10 replicates for total fat and three replicates for fatty acids, respectively. Myristic acid (14:0) and linolenic acid (18:3) were not detected.

Determination of Fatty Acids. The major fatty acid composition of seed samples was determined by GC-flame ionization detection after conversion of the triglycerides into methyl esters by boron trifluoride based on an official AOAC method (*26*). The total fat content of all seed samples under study ranged from 24.4 to 42.5%, and the fatty acid profiles were relatively similar (**Table 1**). Palmitic (5.71−13.8%), stearic (5.43−7.45%), oleic (10.6−29.2%), and linoleic (56.0−70.1%) acids were the major fatty acid precursors in the four seed types that, respectively, gave radiolytic products of 2-DCB, 2-TCB, 2-(5′-

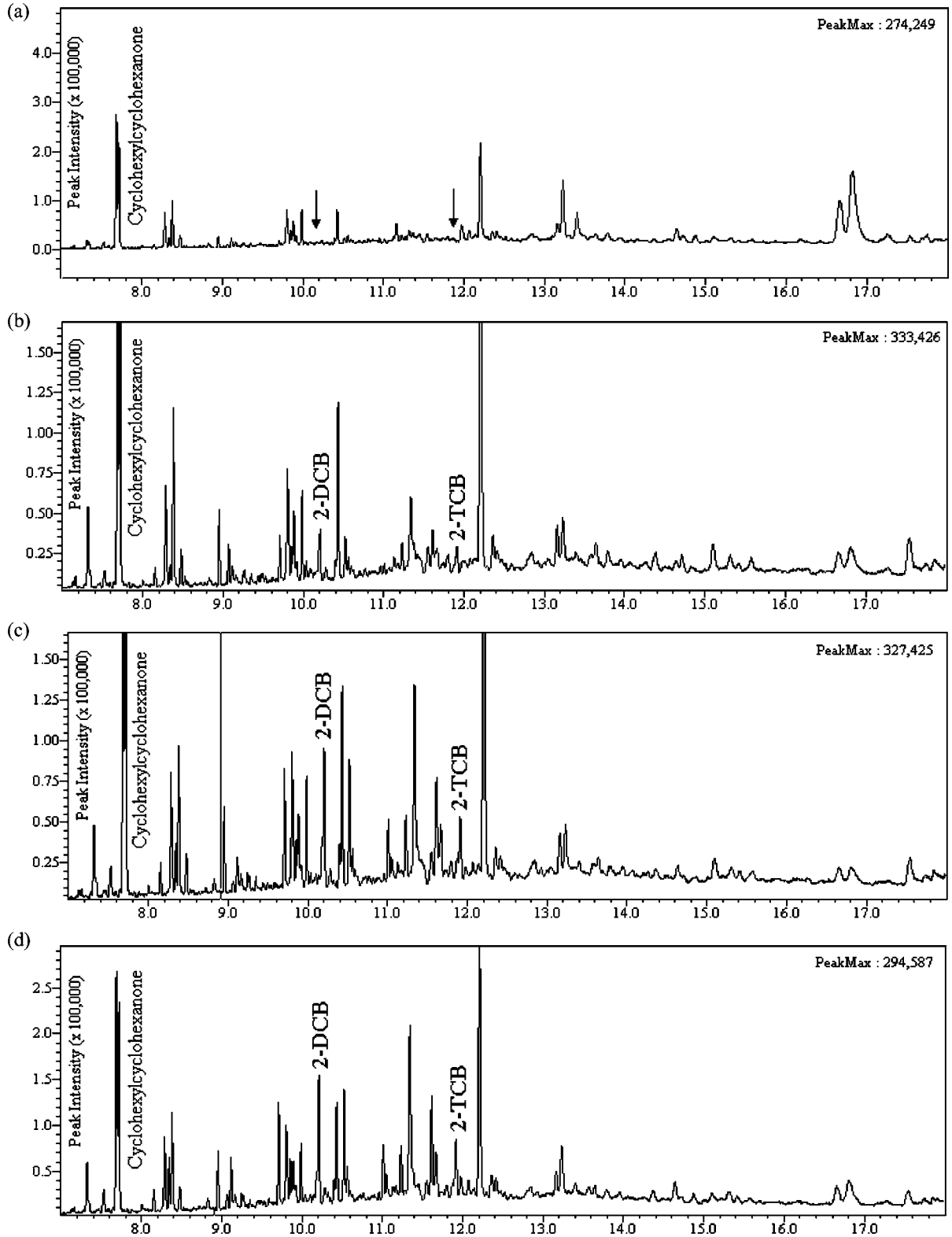

Figure 4. Total ion chromatograms showing cyclohexylcyclohexanone (internal standard), 2-DCB, and 2-TCB in black melon seed samples after irradiation at (**a**) 0, (**b**) 1, (**c**) 3, and (**d**) 5 kGy. Arrows indicate the retention times of 2-DCB and 2-TCB.

tetradecenyl)cyclobutanone, and 2-(5′,8′-tetradecadicenyl)cyclobutanone during irradiation (*16*). Because the latter two unsaturated alkylcyclobutanones were not commercially available and required special technique for synthesis (*27*), the present study was therefore limited to the determination of 2-DCB and 2-TCB in seed samples.

Performance of GC-MS Method. Typical chromatograms of unirradiated and irradiated (1−5 kGy) black melon seeds are shown in **Figure 4**. Alkylcyclobutanones were not detected in all unirradiated seed samples, and the chromatograms for other seed types were very similar to those of black melon seeds. Under the described chromatographic conditions, 2-DCB and 2-TCB were consistently eluted at 10.2 ± 0.1 and 11.9 ± 0.1 min, respectively, and interference to the analytes and internal

Table 2. Concentrations ± SD of 2-DCB and 2-TCB in Irradiated (1–10 kGy) Seed Samples at Day 0 and Mean Ratios of Palmitic Acid to Stearic Acid (PA/SA) and 2-DCB/2-TCB ($n = 3$)[a]

	irradiation dose (kGy)									
	2-DCB level (μg/g)				2-TCB level (μg/g)					
	1	3	5	10	1	3	5	10	PA/SA	2-DCB/2-TCB
black melon	0.20 ± 0.02 (0.03 ± 0.01)	0.61 ± 0.06 (0.04 ± 0.01)	0.90 ± 0.10 (0.07 ± 0.01)	1.93 ± 0.21 (0.14 ± 0.01)	0.10 ± 0.01 (0.02 ± 0.01)	0.31 ± 0.03 (0.02 ± 0.01)	0.46 ± 0.05 (0.03 ± 0.01)	0.93 ± 0.12 (0.05 ± 0.01)	1.4	2.0
red melon	0.38 ± 0.04 (0.03 ± 0.01)	1.36 ± 0.16 (0.15 ± 0.03)	2.12 ± 0.24 (0.18 ± 0.05)	3.65 ± 0.32 (0.31 ± 0.09)	0.25 ± 0.29 (0.02 ± 0.01)	0.95 ± 0.11 (0.06 ± 0.03)	1.51 ± 0.20 (0.11 ± 0.03)	2.55 ± 0.31 (0.15 ± 0.04)	1.3	1.5
pumpkin	0.15 ± 0.02 (0.02 ± 0.01)	0.33 ± 0.04 (0.05 ± 0.02)	0.68 ± 0.09 (0.08 ± 0.03)	1.07 ± 0.12 (0.12 ± 0.03)	0.05 ± 0.01 (0.02 ± 0.01)	0.09 ± 0.02 (0.02 ± 0.01)	0.21 ± 0.04 (0.03 ± 0.01)	0.36 ± 0.05 (0.04 ± 0.01)	2.0	3.4
sunflower	0.21 ± 0.02 (0.02 ± 0.01)	0.58 ± 0.08 (0.07 ± 0.02)	0.81 ± 0.11 (0.12 ± 0.03)	1.77 ± 0.26 (0.17 ± 0.04)	0.16 ± 0.02 (0.02 ± 0.01)	0.43 ± 0.06 (0.04 ± 0.01)	0.62 ± 0.07 (0.06 ± 0.02)	1.46 ± 0.22 (0.16 ± 0.04)	1.1	1.3

[a] Concentrations of 2-DCB and 2-TCB after 120 days of storage are shown in parentheses.

standard was not observed. Within the retention time windows, the ion abundance ratios of m/z 98−112 were 4.0−4.4 with RSD < 2% for 2-DCB and 3.8−4.2 with RSD < 5% for 2-TCB in all irradiated seed samples. Throughout the study, the ion abundance ratios for both analytes in all samples matched with those of the calibration standard (at 0.5 μg/mL) and were within the ranges as specified in EN1785:2003. Recovery of the method was evaluated by calculating the peak area ratios of alkylcyclobutanones to internal standard in fortified samples with reference to those of the calibration standards of equivalent concentrations. Fortified samples were prepared by spiking 10 g homogenized portions of nonirradiated sample ($n = 7$) of each seed type with 200 μL of 10 μg/mL of 2-DCB and 2-TCB standards, holding for about an hour for equilibration, and then subjecting the samples to extraction and analytical procedures as described above. Both alkylcyclobutanones were satisfactorily recovered (2-DCB, 89.9 ± 8.6% in black melon; 83.9 ± 10.0% in red melon; 78.0 ± 8.2% in pumpkin; and 85.6 ± 7.9% in sunflower seeds respectively; 2-TCB, 89.4 ± 9.6% in black melon; 83.3 ± 8.5% in red melon; 75.1 ± 6.2% in pumpkin; and 84.3 ± 7.3% in sunflower seeds, respectively) from fortified samples, and the results showed no statistical difference between analytes and sample types. Recoveries of 2-DCB and 2-TCB from the seed types under study were close to those from mango seeds but were better than those from meat such as chicken and pork (*28*). This would probably be accounted for by the smaller complexity in plant matrices. Although Soxhlet extraction for alkylcyclobutanones as detailed in EN1785:2003 was said to be less efficient than supercritical fluid extraction (*17*, *18*) and accelerated solvent extraction (*29*) techniques, the traditional extraction method still shows the distinct advantages of requiring simple instrumentation and expertise while producing reproducible results (RSD of ≤12% in this study). MQLs were found to be 0.015 μg/mL (15 ppb) for both 2-DCB and 2-TCB at 10 times the standard deviation of replicate analysis. Verification using irradiated seeds at 1 kGy, MQLs of the cyclobutanones ranged from 0.01 to 0.02 μg/g (10−20 ppb) on lipid base at signal-to-noise ratios of not less than 10; hence, the MQLs were set at 0.02 μg/g. The calibration range from 0.02 to 2 μg/mL was all linear with $r^2 > 0.995$.

Characteristics and Stability of 2-DCB and 2-TCB. Previous studies reported that the concentrations of induced alkylcyclobutanones increased proportionally with irradiation doses (0.5 to >10 kGy) in dried shrimps (*16*), beef (*17*), poultry meat (*18*), mango, papaya, cheese, salmon meat (*19*), perilla seed (*20*), pork (*28*), and fish meat (*29*), and the correlations were applicable to estimation of irradiation doses of various food matrices (*30*). As shown in **Table 2**, a linear relationship was also obtained for the induced 2-DCB ($r^2 = 0.979-0.998$) and 2-TCB ($r^2 = 0.987-0.998$) in all seed samples. The relative radiolytic yields of 2-DCB from palmitic acid and 2-TCB from stearic acid were found to be consistent in all seed types under study at different irradiation doses. Ratios of 2-DCB to 2-TCB (2-DCB/2-TCB) from 1 to 10 kGy were estimated to be 2.0−2.1 for black melon seeds, 1.4−1.5 for red melon seeds, 3.0−3.8 for pumpkin seeds, and 1.2−1.4 for sunflower seeds, respectively, but the ratios of the respective fatty acid precursors varied with the seed types. For instance, the mean ratios of palmitic acid/stearic acid (PA/SA) and (2-DCB/2-TCB) were 1.4 and 2.0, respectively, in black melon seeds and 2.0 and 3.4 in pumpkin seeds. Closer ratios of 1.3−1.5 and 1.1−1.3 were noted for red melon and sunflower seeds. These results indicated that the yield of 2-DCB from palmitic acid in all concerned seed types was higher than that of 2-TCB but the degree varied. The stability of 2-DCB and 2-TCB in irradiated black melon seeds (1−10 kGy) kept at ambient temperature was monitored from day 0 to day 120 after irradiation. Irrespective of the irradiation dose applied, concentrations of both alkylcyclobutanones decreased rapidly in all irradiated samples upon storage (**Figure 5**). The degradation was primarily due to the oxidation of the cyclobutanone part to form the respective 4-alkyl-γ-butyrolactone and claimed to be independent of the saturated hydrocarbon chain (*18*). Excluding the data on 2-TCB at 1 kGy dose as the respective concentrations were close to the MQL of the method, an average of 69 ± 9.3% loss for 2-DCB and 75 ± 4.7% for 2-TCB upon storage for 28 days was estimated. The losses were slightly higher than those in fish samples (at 53% for both 2-DCB and 2-TCB when stored at 25 °C for 21 days) (*18*) but were found to be lower than those in papayas (*17*). After storage for a period of 120 days, more than 85% of the alkylcyclobutanones (85−93% for 2-DCB and 90−97% for 2-TCB) in the black melon seed samples already decomposed. Similar findings were noted for irradiated red melon, pumpkin, and sunflower seeds upon prolonged (120 days) storage (**Table 2**). Nonetheless, a positive identification and detection of both irradiation markers in seed samples after a postirradiation storage period of 120 days confirmed that GC-MS was an accurate and reliable chemical technique for determining the irradiation status of seed samples.

EPR vs GC-MS. The obvious advantages of using EPR over GC-MS for the detection of irradiated samples were relatively simple instrumentation, easy sample preparation, and fast

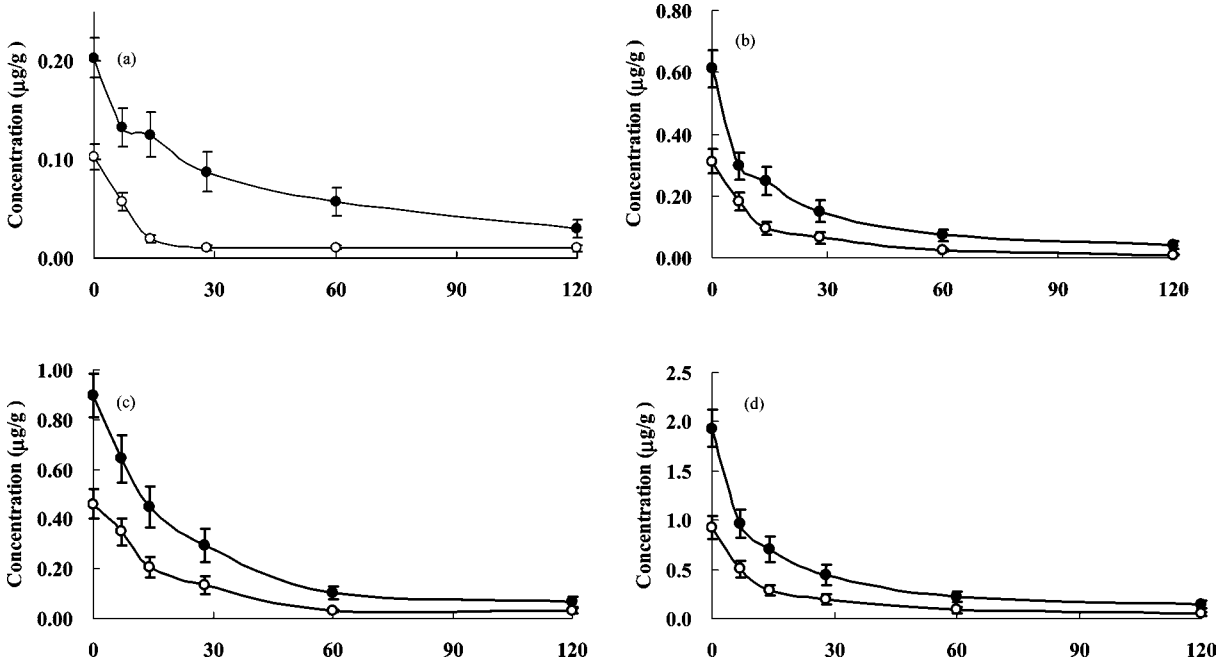

Figure 5. Evolution of mean concentrations ± SD (μg/g) of triplicate measurements of induced 2-DCB (●) and 2-TCB (○) in black melon seed samples during storage after γ-irradiation at (**a**) 1, (**b**) 3, (**c**) 5, and (**d**) 10 kGy.

operation time (requiring about 3−5 min for scanning of a sample in the spectrometer). The entire analytical procedure could be completed within the same working day when applying EPR methods, while it would be hardly possible when using methods based on GC-MS and the Soxhlet extraction technique. The 6 h Soxhlet extraction (although a shorter time if using supercritical fluid extraction) followed by sample concentration and Florisil cleanup prior to GC-MS analysis normally requires about two working days for a batch of six samples. Besides, the running costs for consumables like solvents, Soxhlet extraction apparatus, Florisil powder, internal standards, and expensive alkylcyclobutanone standards are the other contributing factors that need to be considered. Apart from the maintenance and calibration expenses for instruments, we estimated that an extra running cost of 100 HKD (~12 USD) per sample was required for performing GC-MS when compared to EPR analysis. It is worth reiterating that the concerned European protocols were established to prove a tangible means for ascertaining whether a foodstuff has been irradiated. The unstable cellulosic signals upon storage in seed samples under study verified the statement "a positive identification of cellulosic signals in samples is evidence of irradiation, but the absence of these signals does not constitute evidence that the sample is unirradiated" that pinpointed the limitation of EN1787:2000. To this end, it is opined that EPR could well be employed as a screening method because of its rapid turnaround time, but using it as a definitive method for confirmation of irradiation treatment to sample matrices should be cautious. Confirmation of the irradiation status by a more specific technique such as GC-MS was proven in this study to be more rational. Given different levels of expertise, instrumentation, budgetary constraints, sensitivity, and selectivity required for best fitting the data objectives and intended purposes of analysis, testing laboratories should work out their own analytical strategy and protocol for detection of irradiation treatment to concerned food types.

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LITERATURE CITED

(1) Nwokolo, E.; Sim, J. S. Nutritional assessment of defatted seed meal of melon (*Colocynthis citrullus L.*) and flutted pumpkin (*Telfaria occidentals Hook*) by chick assay. *J. Sci. Food Agric.* **1987**, *38*, 237−246.

(2) Al-Khalifa, A. S. Physiochemical characteristics, fatty acid composition and lipoxygenase activity of crude pumpkin and melon seeds oils. *J. Agric. Food Chem.* **1996**, *44*, 964−966.

(3) Bankole, S. A.; Ogunsanwo, B. M.; Mabekoje, O. O. Natural occurrence of moulds and aflatoxin B_1 in melon seeds from markets in Nigeria. *Food Chem. Toxicol.* **2004**, *42*, 1309−1314.

(4) Delincée, H. Detection of food treated with ionizing radiation. *Trends Food Sci. Technol.* **1998**, *9*, 73−82.

(5) Maity, J. P.; Chakraborty, A.; Saha, A.; Santra, S. C.; Chanda, S. Radiation-induced effects on some common storage edible seeds in India infested with surface microflora. *Radiat. Phys. Chem.* **2004**, *71*, 1065−1072.

(6) Follett, P. A. Irradiation to control insects in fruits and vegetables for export from Hawaii. *Radiat. Phys. Chem.* **2004**, *71*, 163−166.

(7) Al-Kaisey, M. T.; Alwan, A. H.; Mohammad, M. H.; Saeed, A. H. Effect of gamma irradiation on antinutritional factors in broad bean. *Radiat. Phys. Chem.* **2003**, *67*, 493−496.

(8) http://ec.europa.eu/food/food/biosafety/irradiation/anal_methods_en.htm.

(9) Raffi, J; Agnel, J. P. Electron spin resonance identification of irradiated fruits. *Radiat. Phys. Chem.* **1989**, *34*, 891−894.

(10) Calucci, L.; Pinzino, C.; Zandomeneghi, M.; Capocchi, A.; Ghiringhelli, S.; Saviozzi, F.; Tozzi, S.; Galleschi, L. Effect of γ-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J. Agric. Food Chem.* **2003**, *51*, 927−934.

(11) Polat, M.; Korkmaz, M. Use of electron spin resonance technique for the detection of irradiated rice seeds (*Oryza sativa* L.). *Food Res. Int.* **2003**, *36*, 653−659.

(12) Korkmaz, M.; Polat, M. The effect of temperature and storage time on the resonance signals of irradiated pea (*Pisum sativum* L.). *Food Res. Int.* **2003**, *36*, 857−862.

(13) Franco, R. W. A.; Martin-Neto, L.; Kato, M. S. A.; Furlan, G. R.; Walder, J. M. M.; Colnago, L. A. Identification of irradiation treatment in black pepper by electron paramagnetic resonance. *Int. J. Food Sci. Technol.* **2004**, *39*, 395−401.

(14) Delincée, H; Soika, C. Improvement of the ESR detection of irradiated food containing cellulose employing a simple extraction method. *Radiat. Phys. Chem.* **2002**, *63*, 437−441.

(15) Boyd, R. D.; Crone, A. V. J.; Hamilton, J. T. G.; Hand, M. V.; Stevenson, M. H.; Stevenson, P. J. Synthesis, characterization, and potential use of 2-dodecylcyclobutanones as a marker for irradiated chicken. *J. Agric. Food Chem.* **1991**, *39*, 789−792.

(16) Kim, K. S.; Seo, H. Y.; Lee, J. M.; Park, E. R.; Kim, J. H.; Hong, C. H.; Byun, M. W. Analysis of radiation-induced hydrocarbons and 2-alkylcyclobutanones from dried shrimps (*Penaeus aztecus*). *J. Food Prot.* **2004**, *67*, 142−147.

(17) Gadgil, P.; Smith, J. S.; Hachmeister, K. A.; Kropf, D. H. Evaluation of 2-dodecylcyclobutanone as an irradiation dose indicator in fresh irradiated ground beef. *J. Agric. Food Chem.* **2005**, *53*, 1890−1893.

(18) Horvatovich, P.; Miesch, M.; Hasselmann, C.; Delincée, H.; Marchioni, E. Determination of monounsaturated alkyl side chain 2-alkylcyclobutanones in irradiated foods. *J. Agric. Food Chem.* **2005**, *53*, 5836−5841.

(19) Stewart, E. M.; Moore, S.; Graham, W. D.; McRoberts, W. C.; Hamilton, J. T. G. 2-Alkylcyclobutanone as markers for the detection of irradiated mango, papaya, camembert cheese and salon meat. *J. Sci. Food Agric.* **2000**, *80*, 121−130.

(20) Lee, H. J.; Byun, M. W.; Kim, K. S. Detection of irradiation-induced hydrocarbons and 2-alkyl-cyclobutanones in irradiated perilla seeds. *J. Food Prot.* **2000**, *63*, 1563−1569.

(21) Morehouse, K. M. Food irradiation—U.S. regulatory considerations. *Radiat. Phys. Chem.* **2002**, *63*, 281−284.

(22) Desrosiers, M. F. Current status of the EPR method to detect irradiated food. *Appl. Radiat. Isot.* **1996**, *44*, 1621−1628.

(23) Kwon, J. H.; Chung, H. W.; Byun, M. W. ESR spectroscopy for detecting gamma-irradiated dried vegetables and estimating absorbed doses. *Radiat. Phys. Chem.* **2000**, *57*, 319−324.

(24) Jo, D.; Kwon, J. H. Detection of radiation-induced markers from parts of irradiated kiwifruits. *Food Control*. **2006**, *17*, 617−621.

(25) Sin, D. W. M.; Wong, Y. C.; Yao, M. W. Y.; Marchioni, E. Identification and stability study of irradiated chicken, pork, beef, lamb, fish and mollusk shells by electron paramagnetic resonance (EPR) spectroscopy. *Eur. Food Res. Technol.* **2005**, *221*, 684−691.

(26) AOAC. Official Method 969.33. *AOAC Official Methods of Analysis*; AOAC: Arlington, VA, 2000; Chapter 41, pp 17−18.

(27) Miesch, M.; Ndiaye, B.; Hasselmann, C.; Marchioni, E. 2-Alkylcyclobutanones as markers for irradiated foodstuffs. I. Synthesis of saturated and unsaturated standards. *Radiat. Phys. Chem.* **1999**, *55*, 337−344.

(28) Sin, D. W. M.; Wong, Y. C.; Yao, M. W. Y. Application of pentafluorophenyl hydrazine on detecting 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in irradiated chicken, pork and mangoes by gas chromatography-mass spectrometry. *Eur. Food Res. Technol.* **2006**, *222*, 674−680.

(29) Obana, H.; Furuta, M.; Tanaka, Y. Analysis of 2-alkylcyclobutanones with accelerated solvent extraction to detect irradiated meat and fish. *J. Agric. Food Chem.* **2005**, *53*, 6603−6608.

(30) Ndiaye, B.; Jamet, G.; Miesch, M.; Hasselmann, C.; Marchioni, E. 2-alkylcuclbutanones as markers for irradiated foodstuffs. II. The CEN (European Committee for Standardization) method: field of application and limit of utilization. *Radiat. Phys. Chem.* **1999**, *55*, 437−445.

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