DOI:10.1021/jf103439c

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Identification of Irradiated Prawn (*Penaeus monodon*) Using Thermoluminescence and 2-Alkylcyclobutanone Analyses

Susu Chen,[†,‡] Yuka Morita,[†] Kimie Saito,[†] Hiromi Kameya,[†] Mitsutoshi Nakajima,[‡] and Setsuko Todoriki*,[†]

[†]Food Safety Division, National Food Research Institute, 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan, and [‡]Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tennodai, Tsukuba, Ibaraki 305-8572, Japan

Thermoluminescence (TL) and 2-alkylcyclobutanone (2-ACB) analyses were performed to identify irradiated prawns (*Penaeus monodon*). With the TL method, minerals were extracted from prawns using acid hydrolysis. The experimental results satisfied the evaluation criteria of European Norm (EN) 1788, even after low-dose irradiation (0.5 kGy) and a 60 day storage at −20 °C. With the 2-ACB method, 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) were successfully extracted from prawns by direct solvent extraction with purification using a conventional silica column and a sulfoxide column, which was used for 2-ACB for the first time. Both 2-ACB derivatives were absent from the non-irradiated samples but were identified in all irradiated samples by gas chromatography−mass spectrometry. Moreover, 2-DCB and 2-TCB production correlated with the applied dose (2.5−10 kGy), and the correlation did not diminish after 60 days of storage at −20 °C for any dose. Therefore, these two techniques provide rapid, simple, and promising methods for routine investigation of frozen prawns.

KEYWORDS: 2-Alkylcyclobutanone; thermoluminescence; solid-phase extraction; prawn; irradiation; detection

INTRODUCTION

In recent years, food irradiation technology has become successful in protecting food from contamination, with minimum interruption to the functional, nutritional, and sensory properties of food products (*1*). The Joint Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA) have indicated that health and safety authorities in over 60 countries worldwide have approved the irradiation of over 60 types of foodstuffs, including prawns (*2*). Prawn is quite popular in many regions of the world as a low-fat and high-protein health food. However, it was sometimes contaminated with pathogenic bacteria, such as *Vibrio* spp. (*3*). It was reported that frozen shrimp was irradiated to reduce mesophilic bacterial contamination at 2.5 kGy by Sinanoglou et al. (*4*). Also, to extend the shelf life, frozen prawn is irradiated in many countries, such as Belgium, China, Thailand, and Vietnam (*5*). Although properly irradiated food is safe and wholesome, consumers should be able to make their own choices between irradiated and non-irradiated foods. Thus, it is important to ensure adequate product labeling. Additionally, the development of simple, repeatable, and routine analytical methods for irradiated prawns is a priority.

Many studies on detection techniques have been performed with various irradiated food products, and some valid analytical methods are being adopted as global standards in Codex Alimentarius Commission (CAC) (*6*). Among them, the thermoluminescence (TL) method (*7*) has been extensively used for foodstuffs from which silicate minerals can be isolated, such as shellfish (*8*, *9*), herbs and spices (*10*, *11*), and fresh or dehydrated fruits and vegetables (*12*, *13*). At the same time, many prawns are processed to remove the shells or intestines before their importation. Because it would be almost impossible to obtain silicate minerals from prawn without its husks and intestines, the demand for another reliable detection method is growing in international trade.

2-Alkylcyclobutanones (2-ACBs), such as 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB), which are produced from triglycerides during irradiation (*14*), have been successfully used as unique radiolysis product markers to identify irradiated foodstuffs (*15*). The European Norm (EN) 1785 method (*16*), as a standard 2-ACB detection method, has proven applicable for a wide range of irradiated fat-containing foods, such as chicken (*17*, *18*), cured pork (*19*), liquid whole egg (*20*), Camembert cheese (*21*), fruits (*21*, *22*), and seafood (*23*, *24*). However, this method is a complex procedure involving a long Soxhlet extraction of 2-ACBs from the sample (6 h), followed by large-scale column chromatography (overall analysis time of 72 h). Alternatively, the introduction of supercritical fluid extraction (SFE) (*25*−*28*) or accelerated solvent extraction (ASE) (*29*) has been reported to improve the extraction and cleanup process for 2-ACB detection. However, these procedures require specialized equipment. Therefore, the development of other faster methods of extraction would be of value in routine detection, and Tewfik

*To whom correspondence should be addressed. Telephone: +81-29-838-8047. Fax: +81-29-839-1552. E-mail: setsuko@affrc.go.jp.

 Published on Web 12/07/2010

previously advanced a simple and rapid method, direct solvent extraction (DSE), for the detection of irradiated foods, such as chicken and liquid whole egg (*30*). However, this novel rapid extraction method has yet to be inspected for its applicability for low-fat food products, such as prawn.

Therefore, the purpose of this study was to investigate whether the TL detection method could be applied to identify imported frozen prawns that had been irradiated at a low dose (0.5 kGy) by the end of their shelf life (storage of 60 days at −20 °C). In addition, in the case of prawns that had their intestines removed before importation, 2-ACB detection using a novel small-scale solid-phase extraction (SPE) column was studied for its applicability for low-fat prawn.

MATERIALS AND METHODS

Reagents. Sodium polytungstate (SPT) was purchased from Sometu-Europe, Berlin, Germany). 2-Cyclohexylcyclohexanone (2-CHCH), as an internal standard (IS) for gas chromatography/mass spectrometry (GC/MS) determination, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), dissolved in *n*-hexane to create a 0.1 μg mL^{-1} stock solution, and preserved at −20 °C. The 2-ACB standards, including 2-DCB and 2-TCB, which were synthesized by Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan), were equally mixed and diluted with *n*-hexane in a range of 0.008−4 μg mL^{-1} for the running standards and spiking solution in recovery tests. Sodium sulfate (Na_2SO_4) was heated for 5 h at 600 °C before use.

Food Samples. Beheaded *Penaeus monodon*, commonly called black tiger prawn, was purchased from a local supermarket in Tsukuba, Ibaraki Prefecture, Japan, and preserved at −20 °C. For 2-ACB analysis, the husks and intestines were removed and edible parts were excised homogeneously with a food cutter.

Irradiation. The frozen prawn samples were irradiated with γ-rays from a cobalt 60 source (Gammacell 220, MDS Nordion International Co., Ltd., Ottawa, Ontario, Canada) at the National Food Research Institute (NFRI) of Japan. The dose rate was 6 kGy h^{-1}. For TL analysis, the frozen whole prawn samples were irradiated at doses of 0.5 and 2.5 kGy and minerals were separated from the samples after 1 and 60 days of storage at −20 °C. In the case of 2-ACB analysis, the prawn samples, for which about 15 g was wrapped in aluminum foil, were irradiated in the frozen state under refrigerant at doses of 2.5, 5, 7.5, and 10 kGy and stored at −20 °C until analysis. The temperature of the samples was measured before and after irradiation, and it was confirmed that there was no significant change in the sample temperature during the irradiation. To investigate the efficiency of 2-ACB extraction from the matrix, prawns irradiated at a dose of 64 kGy were prepared and used for method development. An alanine pellet dosimeter (Bruker Biospin, Ltd., Rheinstetten, Germany) was attached to the surface of each sample, and the absorbed dose was determined with an electron spin paramagnetic spectrophotometer (Bruker EMX, Bruker Biospin, Ltd., Rheinstetten, Germany). The non-irradiated prawns were used as the control and stored under the same conditions.

Isolation of Minerals for TL Analysis. The silicate minerals for TL analysis were separated from prawns by the acid hydrolysis procedure described in the EN 1788 method (*7*), with a slight modification. The intestinal tract (50−170 mg), which was removed from the prawns, was placed into a boiling tube containing 10 mL of 6 M HCl and refluxed at 50 °C for 15 min. The solution was sonicated at 5 min, after which about 40 mL of deionized water was added. After the solution sat for 5 min, it was discarded by decantation, and the mineral-rich fractions remaining at the bottom of the tube were obtained. The minerals were transferred into a centrifuge tube along with the SPT solution ($d = 2$ g mL^{-1}) and centrifuged for 2 min at 1000*g*. Then, the minerals were further purified by centrifugation twice with 3 mL of acetone under the same conditions given above, after rinsing twice with 10 mL of deionized water. Finally, about 1 mg or less of the minerals was weighed, transferred into a stainless-steel disk, and stored 16 h at 50 °C in a laboratory oven for the TL measurement.

TL Measurements. TL measurements for this study were performed using the TL reader (QS3500, Harshaw/Bicron, Solon, OH) with computer-controlled temperature ramping and automatic reheat facility. Before analysis, the silicate mineral samples were protected from light exposure. The TL glow curves (glow 1) were recorded for each sample disk from 70 to 400 °C at a heating rate of 6 °C s^{-1}, and the TL oven was purged of oxygen with nitrogen at a flow rate of 2 mL min^{-1}. For normalization of the glow 1 result, each sample was irradiated at a standard dose of 1 kGy and preheated for 16 h in an oven at 50 °C. The second TL glow curve (glow 2) was then recorded under the same conditions given above. The luminescent intensities, TL_1 and TL_2, were integrated for glows 1 and 2, respectively, in the temperature range of 150−250 °C, and the TL area ratio (TL_1/TL_2) was calculated for both irradiated and non-irradiated samples to verify the reliability of detection results from TL_1. All determinations were repeated a minimum of 6 times.

Extraction of 2-ACBs. The extraction of 2-ACBs from the prawn samples for GC/MS measurement was performed by following the DSE extraction method of Tewfik (*30*), with a slight modification. In a mortar and pestle, prawn meat (about 15 g) was homogenized with Na_2SO_4 (about 30 g) to form a fine filth, which was transferred to a separating funnel (300 mL). The mixture was extracted using an almighty shaker (AW-1, AS ONE, Co., Ltd., Tokyo, Japan) and was shaken with 70 mL of *n*-hexane for 10 min at 420 rpm. After the mixture sat for 5 min, it was shaking again for an additional 5 min under the same conditions described above. The extract solvent was transferred to a flask (200 mL) carefully, and the mixture was retained in the separating funnel. Then, another 70 mL of fresh *n*-hexane was added, and the shaking extraction was repeated as described above. Finally, the mixture was rinsed with 60 mL of *n*-hexane, and the extraction solutions were combined into the flask. The combined extracts were evaporated using a rotary evaporator with a water bath at 40 °C and carefully dried to 1 mL by nitrogen stream for column cleanup.

Cleanup of GC/MS Samples. About 1 mL of the prawn extract was added to a silica SPE cartridge column (1 g 8 mL^{-1}; Alltech Associates, Inc., Grace Co., Columbia, MD), in which the packing bed was rinsed with 10 mL of *n*-hexane for conditioning. A 10 mL aliquot of *n*-hexane was first eluted and discarded. A 14 mL aliquot of 2% diethyl ether/*n*-hexane (2:98, v/v) was eluted, and the 4−14 mL fraction was collected as the 2-ACB fraction. After concentration to 1 mL, the extract was further subjected to a Supelclean sulfoxide SPE column (3 g/6 mL; Supelco, Bellefonte, PA), which was conditioned with 10 mL of acetone to remove residual moisture and equilibrated with 20 mL of *n*-hexane, after which the 4−14 mL fraction was collected with *n*-hexane. Then, the eluted *n*-hexane was carefully concentrated into a volume of 1 mL in a rotary vacuum evaporator, further concentrated to mostly dryness under a stream of nitrogen at 40 °C, and then added to 0.2 mL of 2-CHCH (0.05 μg mL^{-1}) as an IS. Finally, the mixture was transferred into a glass vial insert for GC/MS analysis. A single sample was processed within 120−160 min.

GC/MS Analysis of 2-ACBs. 2-ACBs were analyzed using a QP2010 Plus model GC/MS system (Shimadzu, Kyoto, Japan). The data acquisition and control were performed using GCMS-Solution version 2.53 SU3 software. The gas chromatograph (GC) conditions were as follows: column, DB-5MS (Agilent Technologies J&W Scientific, Santa Clara, CA), 60 m × 0.25 mm inner diameter, and 0.25 μm film; column temperature program, 55 °C (2 min), 55−175 °C at 20 °C min^{-1}, 175−250 °C at 2 °C min^{-1}, 250−270 °C at 10 °C min^{-1}, 270 °C (5 min), 270−280 °C at 10 °C min^{-1}, and 280 °C (10 min); carrier gas, helium at 1.00 mL min^{-1}; injection temperature, 250 °C; injection single taper inlet linear (SGE Analytical Science, Brisbane, Australia); injection mode, splitless; and injection volume, 1 μL. The MS conditions were as follows: ionization mode, electron ionization; ion detection, selected ion monitoring (SIM); event time, 0.20 s; detector voltage, 0.81 kV; ion source temperature, 200 °C; and interface temperature, 280 °C. The monitored ions were m/z 98 and 112, and m/z 98 was selected for determination. All determinations were repeated at least 3 times.

Analysis of Fatty Acids. The fatty acid composition of prawn fat was performed by GC according to a previously reported method (*31*, *32*). In brief, total lipids were extracted according to the method by Folch et al. (*33*). Transesterification of fatty acids was performed by incubating the lipids in anhydrous methanol containing 5% HCl for 3 h at 100 °C. As an internal standard, tridecanoic acid (1 μmol) was added to the reaction mixture and used for quantification standards. The methyl esters were analyzed on a GC-2014 (Shimadzu, Kyoto, Japan) with a flame ionization detector (FID) and capillary column. The data acquisition and control were conducted with GC-Solution version 2.3 software. The GC conditions

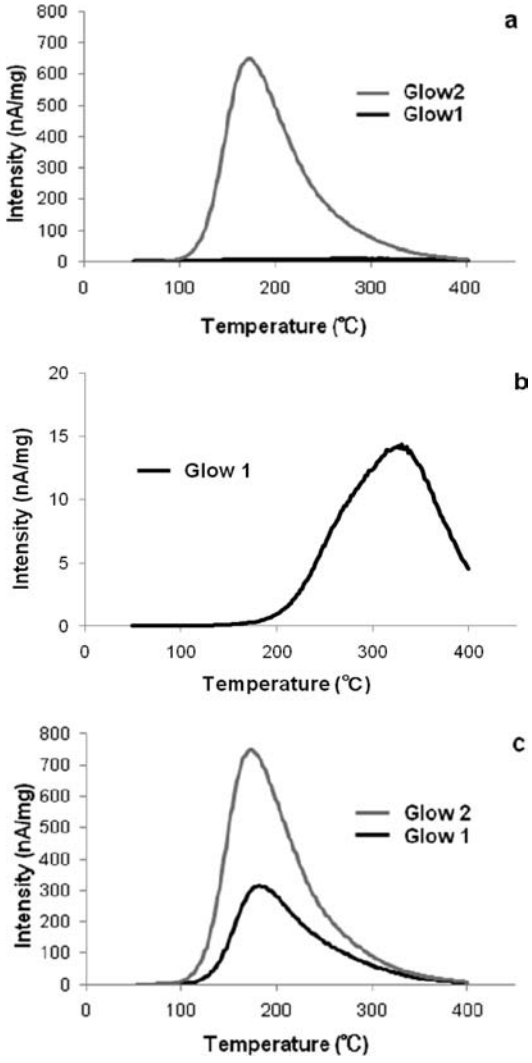

Figure 1. TL glow curves of minerals from frozen prawns that were irradiated and stored at −20 °C for 60 days: (a) 0 kGy, (b) glow 1 of 0 kGy, and (c) 0.5 kGy.

were as follows: column, DB-225 (Agilent Technologies J&W Scientific, Santa Clara, CA), 30 m × 0.25 mm inner diameter, 0.25 μm film; column temperature program, 150 °C (2 min), 150−200 °C at 2 °C min^{-1}, 200 °C (43 min); carrier gas, helium, 1.00 mL min^{-1}; injection temperature, 230 °C; injection volume, single taper inlet linear (SGE Analytical Science, Australia); injection mode, split at 30.0; injection volume, 1 μL. All determinations were performed at least 3 times.

Statistical Analysis. Statistical tests were performed using Microsoft Office Standard 2007 Excel software (Microsoft Corporation, Redmond, WA). Results were expressed as the mean ± standard deviation (SD) for each determination. Data were statistically analyzed by Welch's *t* test (SSRI Co., Ltd., Tokyo, Japan). A significance level of $p > 0.05$ between groups was accepted as being not significantly different.

RESULTS AND DISCUSSION

TL Characteristics of Minerals. It has been reported that the acid hydrolysis separation method required smaller quantities of food sample and reagents than the density gradient, as well as less time to be completed (*9*, *13*). Thus, acid hydrolysis extraction following the EN 1788 method (*7*) was employed to separate the silicate minerals from the prawn. Because the shelf life of the non-irradiated prawn is 33 days, while that for prawn irradiated at 1.8−3.6 kGy is 47 days by Lacroix et al. (*34*), in this study, the samples were irradiated at 0.5 and 2.5 kGy and subjected to TL analysis after storage for 1 or 60 days at −20 °C. **Figure 1** shows

Table 1. Peak Temperature of Glow 1 Curves during Storage at −20 °C[a]

	irradiation dose (kGy)		
storage period (day)	0	0.5	2.5
1	331.6 ± 5.9 a,x	169.5 ± 4.3 a,y	169.4 ± 6.0 a,y
60	329.3 ± 5.7 a,x	170.3 ± 6.8 a,y	168.7 ± 5.1 a,y

[a] Means in the same column with the same letter (a) are not significantly different according to Welch's *t* test results ($p > 0.05$). Means in the same row with the same letter (x and y) are not significantly different according to Welch's *t* test results ($p > 0.05$). Mean of six replications ± standard deviation.

Table 2. Glow Ratio (TL_1/TL_2) Integrated between 150 and 250 °C during Storage at −20 °C[a]

	irradiated dose (kGy)		
storage period (day)	0	0.5	2.5
1	0.0013 ± 0.0002 a,x	0.583 ± 0.041 a,y	2.187 ± 0.216 a,z
60	0.0014 ± 0.0003 a,x	0.529 ± 0.081 b,y	1.864 ± 0.295 b,z

[a] Means in the same column with the same letter (a and b) are not significantly different according to Welch's *t* test results ($p > 0.05$). Means in the same row with the same letter (x−z) are not significantly different according to Welch's *t* test ($p > 0.05$). Mean of six replications ± standard deviation.

the typical TL glow curves of minerals from the (**a**) non-irradiated and (**c**) 0.5 kGy irradiated prawn intestines after storage for 60 days at −20 °C. The glow 1 of non-irradiated samples was observed at approximately 300 °C with little intensity (**b**), but that of irradiated prawns peaked at approximately 170 °C with a high intensity (**c**). A similar trend was also observed for samples irradiated at 2.5 kGy and stored for 1 or 60 days at −20 °C (data not shown). In addition, the peak temperature of glow 1 for samples irradiated at either 0.5 or 2.5 kGy was approximately 170 °C, while that for the non-irradiated sample was approximately 330 °C (**Table 1**). Few changes in the peak temperature were observed in irradiated samples stored for 1 versus 60 days.

Table 2 shows the TL ratios for each dose level. It was found that the TL ratio for non-irradiated samples was approximately 0.001 after storage for 1 or 60 days. On the contrary, the TL ratios for irradiated samples (0.5 and 2.5 kGy) were significantly larger than 0.1 and depended upon the radiation dose. Although some decreases in TL ratios were observed in irradiated samples after 60 days of storage at −20 °C, it was possible to discriminate between irradiated and non-irradiated samples. This result confirmed the numerous promising conclusions reported by Kwon et al. (*35*).

All of the experimental results discussed above satisfied the evaluation criteria of the EN 1788 method for both the glow 1 peak temperature and the TL ratios. Moreover, the storage of prawns at a freezing temperature (−20 °C) did not affect the glow 1 peak temperatures and TL ratios of irradiated samples even after 60 days.

However, it was reported that about 5% of seafood samples could not be successfully analyzed because no minerals or only non-thermoluminescent clays or carbonates were found (*8*). Therefore, another promising method, the 2-ACB method, was investigated for irradiation identification.

Fatty Acid Composition of Prawn. The fatty acid composition of non-irradiation samples was evaluated by GC analysis. The list of the fatty acids present in prawn is shown in **Table 3**. There were about 16 different fatty acids in the non-irradiation prawn, and the total of fatty acid content in per gram of sample was 15.58 ± 0.51 μmol g^{-1}. The palmitic acid content was 3.01 ± 0.08 μmol g^{-1}, accounting for 19.31 ± 0.26 of the fatty acid content, more than any other fatty acid. The stearic acid content was 2.32 ± 0.08 μmol g^{-1}, accounting for 14.92 ± 0.14 of all fatty acid.

Table 3. Fatty Acid Composition in Non-irradiated Prawn[a]

fatty acids	percent of total fatty acid (mol %)	content of fatty acid in per gram of sample (μmol/g of FW)
C14:0	2.05 ± 0.52	0.32 ± 0.09
C16:0	19.31 ± 0.26	3.01 ± 0.08
C16:1	7.68 ± 0.14	1.20 ± 0.04
C17:0	2.55 ± 0.05	0.40 ± 0.01
C18:0	14.92 ± 0.14	2.32 ± 0.08
C18:1 (*cis*)	9.37 ± 0.45	1.46 ± 0.08
C18:1 (*trans*)	3.57 ± 0.14	0.56 ± 0.02
C18:2	1.79 ± 0.07	0.28 ± 0.02
C20:0	0.77 ± 0.13	0.12 ± 0.02
C20:2	0.61 ± 0.04	0.09 ± 0.01
C20:4	8.59 ± 0.18	1.34 ± 0.04
C20:5	14.88 ± 0.18	2.32 ± 0.07
C22:0	0.87 ± 0.13	0.14 ± 0.02
C22:4	0.76 ± 0.18	0.12 ± 0.03
C22:5 (*n*-3)	1.82 ± 0.25	0.28 ± 0.04
C22:6	10.47 ± 0.26	1.63 ± 0.05
total	100.00 ± 3.10	15.58 ± 0.51

[a] Mean of three replications ± standard deviation.

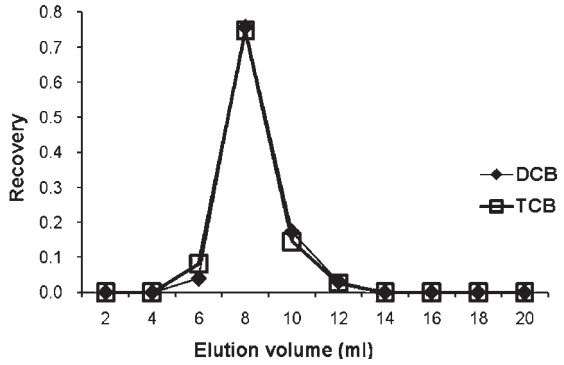

Figure 2. Elution patterns of 2-ACBs on the silica SPE column.

It was reported that γ irradiation causes a chemical reaction that produces 2-DCB from palmitic acid (16:0) and 2-TCB from stearic acid (18:0) (*16*, *17*). Therefore, 2-DCB and 2-TCB were chosen as the target 2-ACBs in this study. Incidentally, the ratio of palmitic acid/stearic acid in non-irradiation prawn was 1.31:1.

Method Development for 2-ACBs. The efficiency of direct solvent extraction was determined for prawn irradiated at a high dose (64 kGy). The prawn samples were shaken with 70 mL of *n*-hexane 5 times. The recovery of 2-ACBs from the first extract solvent was about 70% of the sum of that from five extractions, and the recoveries for the second and third extracts were about 18 and 7%, respectively. Collectively, repeating the shaking extract 3 times resulted in approximately 95% recovery; therefore, the shaking extraction was performed 3 times.

The extraction method of this study is based on the DSE method designed for foodstuffs with high lipid content (*30*). Considering the practical dose level for prawn irradiation, an efficient cleanup procedure would be needed to detect a very low amount of 2-ACBs by GC/MS. It was reported that silica SPE columns should be used for the purification step instead of existing large-scale Florisil columns because of its ability to retain 2-ACBs from the hexane extracts (*29*). Therefore, we first attempted silica SPE column purification; in this procedure, 0.05 μg each of 2-DCB and 2-TCB was added to the hexane extract from prawns and charged into a silica (1 g) SPE column with 14 mL of a mixture of diethyl ether/hexane (98:2, v/v). Only the 4−14 mL fraction was preserved. The recovery rates of 2-TCB and 2-DCB

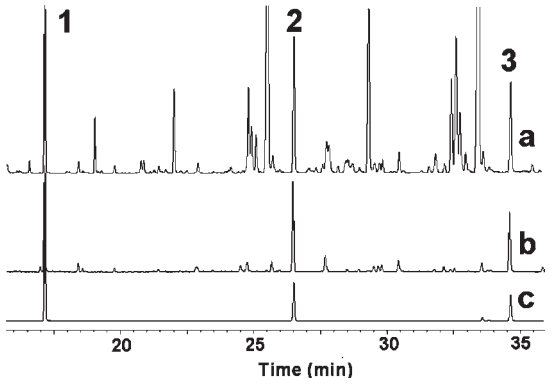

Figure 3. GC/MS chromatograms of 64 kGy irradiated frozen prawn samples at *m*/*z* 98. (a) Cleanup with silica SFE column. (b) Cleanup with silica and Supelclean sulfoxide SPE columns. (c) Standards for 2-DCB and 2-TCB at 1 μg/mL. Peak labels: 1, 2-cyclohexylcyclohexanone (IS); 2, 2-DCB; 3, 2-TCB.

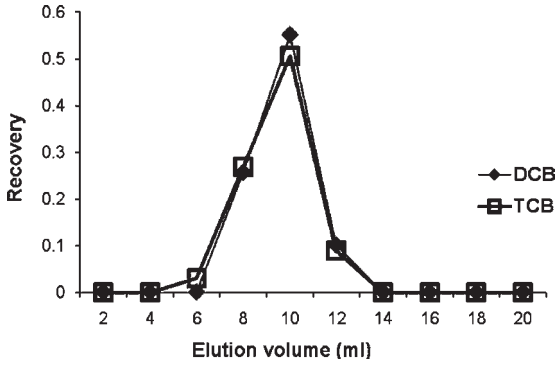

Figure 4. Elution patterns of 2-ACBs on the sulfoxide SPE column.

were 104.0 ± 7.7 and 101.3 ± 3.6%, respectively. **Figure 2** shows the elution patterns of 2-ACBs in the silica SPE column.

In the case of prawns, large amounts of impurities remain after silica SPE column purification. It was reported that 1 g of silica was sufficient to retain about 30 mg of the extract (*36*). In this study, fatty acid content analysis showed that 15 g of fresh prawn meat contained about 85 mg of fatty acids. The purification with silica SPE column was repeated twice, but the GC/MS chromatogram for the second purification was nearly identical to that for the first purification (data not shown). Therefore, a novel stationary phase Supelclean sulfoxide column, which was originally applied for the separation of polychlorinated biphenyls (*37*, *38*), was investigated. It was found that 2-ACBs were selectively eluted by the sulfoxide SPE column, resulting in better resolution between the impurities and the 2-ACBs. As a result, it was used for the second purification step for prawn samples, and it was observed that the impurity peaks that were not removed from the silica SPE column were completely defecated with the sulfoxide column, as shown in **Figure 3**. Moreover, 0.05 μg each of 2-DCB and 2-TCB was added to the hexane extract from the prawn and charged into a Supelclean sulfoxide SPE column with 14 mL of hexane, from which the 4−14 mL fraction was preserved (**Figure 4**). TCB and DCB were recovered at rates of 94.5 ± 3.8 and 97.9 ± 6.8%, respectively.

Detection and Identification of 2-ACBs. The analysis of 2-ACBs was performed on prawn irradiated at 2.5 kGy and stored frozen for 60 days, and the peaks corresponding to 2-DCB and 2-TCB in the obtained chromatogram were identified according to the conditions in the EN 1785 method (*16*).

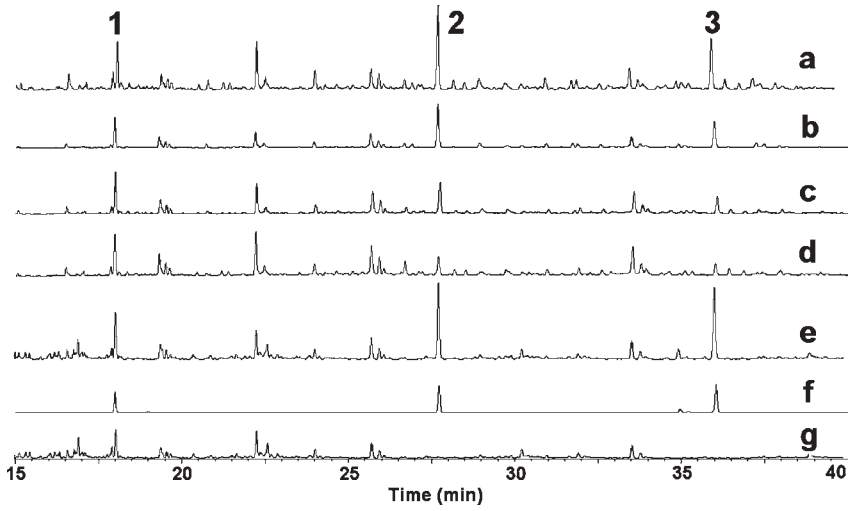

Figure 5. GC/MS chromatograms of irradiated frozen prawn samples confirming the presence of 2-DCB and 2-TCB at *m/z* 98. (a) Prawn irradiated at 10 kGy. (b) Prawn irradiated at 7.5 kGy. (c) Prawn irradiated at 5 kGy. (d) Prawn irradiated at 2.5 kGy. (e) Prawn spiked with 0.05 μg each of 2-DCB and 2-TCB. (f) Standards for 2-DCB and 2-TCB at 0.25 μg/mL. (g) Non-irradiated prawn (control). Peak labels: 1, 2-cyclohexylcyclohexanone (IS); 2, 2-DCB; 3, 2-TCB.

In the SIM mode, the *m/z* 98 ion was used for quantization of 2-ABCs, with the *m/z* 112 ion used as a relative ion. 2-DCB extracted from prawn samples produced peaks for ion *m/z* 98 and 112 at a ratio of 3.87−4.04:1, while for 2-TCB, the corresponding ratio was 3.71−3.94:1, both of which were similar to those obtained for the standards. Moreover, **Figure 5** shows that the retention times of 2-DCB and 2-TCB in irradiated prawns (**a**−**d**) were 27.7 ± 0.1 and 36.2 ± 0.1 min, respectively, which were the same as the pure standards (**f**), and neither was detected in the control sample (**g**). In addition, the signal-to-noise (S/N) ratio of each of these ions was greater than 3:1.

Figure 6 shows the mass spectra of (**a**) 2-DCB and (**b**) 2-TCB under the scan mode. The relative intensities of ions *m/z* 98 and 112 were compared to 2-DCB and 2-TCB standards with MS spectra that were practically the same in the detected *m/z* range of 50−270. It was observed that the molecular ions of 2-DCB and 2-TCB were (**a**) *m/z* 238 and (**b**) *m/z* 266, respectively.

Accordingly, the qualitative and quantitative presence of 2-DCB and 2-TCB were confirmed in prawn irradiated at 2.5 kGy using GC/MS detection. For the same reason, 2-DCB and 2-TCB were considered present in other samples irradiated at higher doses, such as 5, 7.5, and 10 kGy.

Evaluation of the Method. Finally, the full sample preparation procedure was evaluated with the recovery tests, in which prawn samples were spiked with 3.33 ng g^{-1} 2-DCB and 2-TCB (100 μL of 0.5 μg mL^{-1} in 15 g samples). Recovery studies were conducted for six experiments, and 2-DCB and 2-TCB were recovered at rates of 82.9 ± 15.8 and 83.3 ± 9.2%, respectively.

Furthermore, the reproducibility of the quantitative results was examined for samples irradiated at 2.5 kGy. Average concentrations of 2-TCB and 2-DCB of 0.42 ± 0.06 and 0.64 ± 0.03 ng g^{-1}, respectively, were obtained for six repetitions. The results demonstrate that this method is rapid, simple, and robust and, thus, suitable for examining prawn samples.

Dose Dependence and Changes during Storage. Table 4 provides a summary of the results of 2-DCB and 2-TCB content. Both 2-DCB and 2-TCB were present in all irradiated prawn samples at all irradiation doses (2.5, 5, 7.5, and 10 kGy). On the other hand, neither 2-DCB nor 2-TCB was detected in the non-irradiated samples.

Moreover, at 2.5 kGy, the mean yield of 2-DCB was 0.66 ng g^{-1} of sample compared to 0.64 ng g^{-1} of sample after frozen storage for 1 and 60 days, and the same trend was observed for the other

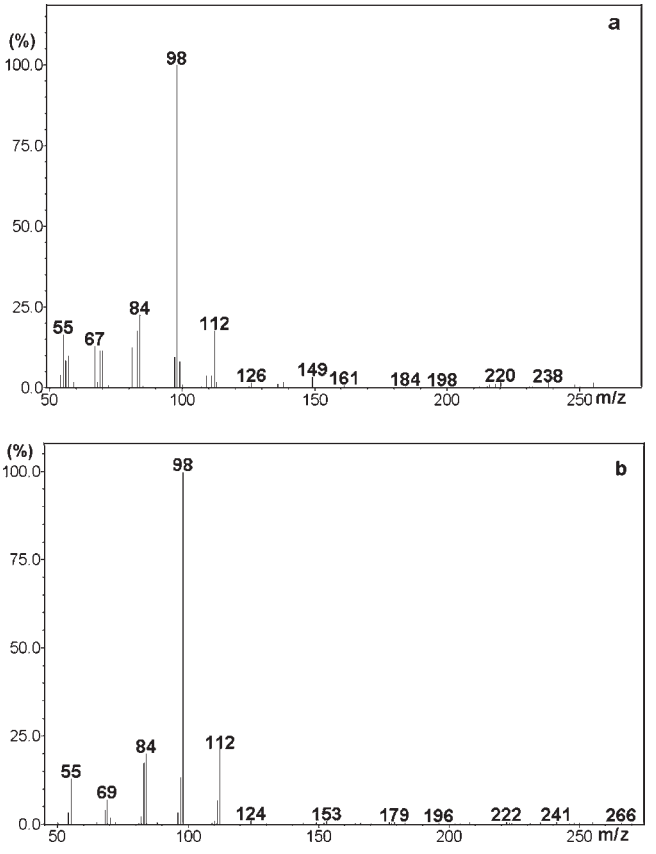

Figure 6. Mass spectra of (a) 2-DCB and (b) 2-TCB from the 2.5 kGy irradiated prawns.

irradiation doses (**Table 4**). Therefore, regardless of the dose, the concentration of 2-DCB did not show any clear reduction after frozen storage for 60 days at −20 °C. The same trend was observed for 2-TCB. The persistence of 2-ACBs during the shelf life of foods was confirmed by the earlier report (*18*) and provided further evidence of their usefulness as markers for the detection of irradiated prawns after frozen storage. Moreover, the results of this study showed that the 2-ACB content did not diminish noticeably during the 60 day frozen storage, as was reported previously (*39*, *40*).

Table 4. Concentrations of Radiation-Induced 2-DCB and 2-TCB after Frozen Storage (ng/g of Sample)[a]

2-ACBs	storage period (day)	irradiation dose (kGy) 0	2.5	5	7.5	10	regression equation and coefficient[b]
2-DCB	1	ND[c]	0.66 ± 0.06 a	1.00 ± 0.07 a	1.68 ± 0.06 a	2.23 ± 0.29 a	$y = 0.216x \pm 0.028$; $r^2 = 0.988$
	60	ND	0.64 ± 0.02 a	1.02 ± 0.06 a	1.63 ± 0.14 a	2.23 ± 0.18 a	$y = 0.215x \pm 0.035$; $r^2 = 0.990$
2-TCB	1	ND	0.42 ± 0.02 a	0.64 ± 0.04 a	0.97 ± 0.04 a	1.36 ± 0.18 a	$y = 0.127x \pm 0.055$; $r^2 = 0.987$
	60	ND	0.41 ± 0.02 a	0.59 ± 0.05 a	1.02 ± 0.07 a	1.33 ± 0.08 a	$y = 0.128x \pm 0.037$; $r^2 = 0.981$

[a] For each compound, the concentrations of 2-DCB and 2-TCB were compared by Welch's *t* test ($p > 0.05$). Means in the same column of the same compound with the same letter (a) are not significantly different ($p > 0.05$). Mean of three replications ± standard deviation. [b] *x*, irradiation dose (kGy); *y*, 2-ACB concentration (in ng/g of sample). [c] ND = not detected.

Furthermore, the concentrations of 2-DCB and 2-TCB were found to increase with an increasing radiation dose in all samples, demonstrating a linear relationship from 2.5 to 10 kGy. The coefficients of correlation (*r*) for 2-DCB were 0.988 and 0.991 after 1 and 60 days of frozen storage, while those for 2-TCB were 0.987 and 0.981, respectively. Because 2-DCB and 2-TCB were almost unchanged during the shelf life of frozen prawns, the actual dose assessment could be predicted according to this standard curve.

In conclusion, two different techniques were applied for analyzing frozen prawns that were exposed to γ radiation, and both techniques were sufficiently rapid and simple to be performed routinely. With regard to the TL method, the minerals separated from prawns irradiated at a low dose (0.5 kGy) showed typical TL glow curves relative to those of non-irradiated samples, facilitating discrimination between irradiated and non-irradiated samples even after 60 days of storage at −20 °C. For the 2-ACB method, the DSE extraction of 2-ACBs can be used to identify irradiated prawn qualitatively as well as quantitatively without the use expensive extraction equipment, such as SFE or ASE. In addition, with the novel cleanup procedure employing the combination of silica and sulfoxide SPE columns, impurities can be effectively removed from the sample matrix, and 2-DCB and 2-TCB could be isolated and detected using GC/MS analysis at very low concentration levels after irradiation at 2.5 kGy, which is the expected practical dose. Moreover, reductions in the extraction time from 6 h to 45 min and purification time from 5 h to 60 min make this method safe and economical for routine analysis.

ABBREVIATIONS USED

2-ACB, 2-alkylcyclobutanone; ASE, accelerated solvent extraction; CAC, Codex Alimentarius Commission; CEN, European Committee for Standardization; 2-DCB, 2-dodecylcyclobutanone; DSE, direct solvent extraction; EN, European Norm; 2-TCB, 2-tetradecylcyclobutanone; FAO/IAEA, Food and Agriculture Organization of the United Nations and International Atomic Energy Agency; GC, gas chromatograph; GC/MS, gas chromatography/mass spectrometry; NFRI, National Food Research Institute; SD, standard deviation; SFE, supercritical fluid extraction; SIM, selected ion monitoring; SPE, solid-phase extraction; SPT, sodium polytungstate; TL, thermoluminescence.

LITERATURE CITED

(1) Chauhan, S. K.; Kumar, R.; Nadanasabapathy, S.; Bawa, A. S. Detection methods for irradiated foods. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 4−16.

(2) Joint Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (Joint FAO/IAEA). *Food Irradiation: A Powerful Nuclear Tool for Food Safety*; FAO/IAEA: Vienna, Austria, 2006; http://www-naweb.iaea.org/nafa/news/food-irrad-tool0807.pdf (accessed on Oct 30, 2010).

(3) Ito, H.; Rashid, H. O.; Sangthong, N.; Adulyatham, P.; Rattagool, P.; Ishigaki, I. Effect of γ-irradiation on frozen shrimps for decontamination of pathogenic bacteria. *Radiat. Phys. Chem.* **1993**, *42*, 279−282.

(4) Sinanoglou, V. J.; Batrinou, A.; Konteles, S.; Sflomos, K. Microbial population, physicochemical quality, and allergenicity of mollusks and shrimp treated with cobalt-60 γ radiation. *J. Food Prot.* **2007**, *70*, 958−966.

(5) Kume, T.; Furuta, M.; Todoriki, S.; Uenoyama, N.; Kobayashi, Y. Status of food irradiation in the world. *Radiat. Phys. Chem.* **2009**, *78*, 222−226.

(6) Codex Alimentarius Commission (CAC). *General Codox Methods for the Detection of Irradiated Foods*; CAC: Rome, Italy, 2001 (revised 2003), http://www.codexalimentarius.net/web/index_en.jsp# (accessed on Aug 10, 2010).

(7) European Committee for Standardization (CEN). *EN 1788, Foodstuffs—Detection of Irradiated Foods from Which Silicate Minerals Can Be Isolated—Method by Thermoluminescence*; CEN: Brussels, Belgium, **1996** (revised **2001**).

(8) Pinnioja, S.; Lindberg, A. Effect of mineral composition on thermoluminescence detection of irradiated seafood. *Radiat. Meas.* **1998**, *6*, 651−661.

(9) Carmichael, L. A.; Sanderson, D. C. W. The use of acid hydrolysis for extracting minerals from shellfish for thermoluminescence detection of irradiation. *Food Chem.* **2000**, *68*, 233−238.

(10) Calderon, T.; Correcher, V.; Millan, A.; Beneitez, P.; Rendell, H.; Larsson, M.; Townsend, P. D.; Wood, R. A. New data on thermoluminescence of inorganic dust from herbs and spices. *J. Phys. D: Appl. Phys.* **1995**, *28*, 415−423.

(11) D'Oca, M. C.; Bartolotta, A.; Cammilleri, M. C.; Brai, M.; Marrale, M.; Triolo, A.; Parlato, A. Qualitative and quantitative thermoluminescence analysis on irradiated oregano. *Food Control* **2007**, *18*, 996−1001.

(12) Khan, H. M.; Bhatti, I. A.; Delincee, H. Thermoluminescence of contaminating minerals for the detection of radiation treatment of dried fruits. *Radiat. Phys. Chem.* **2002**, *63*, 403−406.

(13) D'Oca, M. C.; Bartolotta, A. The identification of irradiated crustaceans and evaluation of the dose by thermoluminescence: Intercomparison between two methods for extracting minerals. *Food Res. Int.* **2010**, *43*, 1255−1259.

(14) Lettelier, P. R.; Nawar, W. W. 2-Alkylcyclobutanones from the radiolysis of triglycerides. *Lipids* **1972**, *7*, 75−76.

(15) Stevenson, M. H.; Crone, A. V. J.; Hamilton, J. T. G. Irradiation detection. *Nature* **1990**, *344*, 202−203.

(16) European Committee for Standardization (CEN). *EN 1785, Foodstuffs—Detection of Irradiated Foods Containing Fat—Gas Chromatographic/Mass Spectrometric Analysis of 2-Alkylcyclobutanone*; CEN: Brussels, Belgium, **2003**.

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

(18) Crone, A. V. J.; Hamilton, J. T. G.; Stevenson, M. H. Detection of 2-dodecylcyclobutanone in radiation-sterilized chicken stored for several years. *Int. J. Food Sci. Technol.* **1992**, *27*, 691−696.

(19) Zanardi, E.; Battaglia, A.; Ghidini, S.; Conter, M.; Badiani, A.; Ianieri, A. Evaluation of 2-alkylcyclobutanones in irradiated cured pork products during vacuum-packed storage. *J. Agric. Food Chem.* **2007**, *55*, 4264−4270.

(20) Crone, A. V. J.; Hand, M. V.; Hamilton, J. T. G.; Sharma, N. D.; Stevenson, M. H. Synthesis, characterization and use of 2-tetradecylcyclobutanone together with other cyclobutanones as markers for irradiated liquid whole egg. *J. Sci. Food Agric*. **1993**, *62*, 361−367.

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

(22) Stewart, E. M.; Moore, S.; McRoberts, W. C.; Graham, W. D.; Hamilton, J. T. G. 2-Alkylcyclobutanones as markers for exotic fruits. *Food Sci. Technol. Today* **1998**, *12*, 103−106.

(23) 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.

(24) Kim, J. H.; Seo, H. Y.; Kim, K. S. Analysis of radiolytic products of lipid in irradiated dried squids (*Todarodes pacificus*). *J. Food Prot*. **2004**, *67*, 1731−1735.

(25) 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*, 1890−1983.

(26) Stewart, E. M.; McRoberts, W. C.; Hamilton, J. T.; Graham, W. D. Isolation of lipid and 2-alkylcyclobutanones from irradiated foods by supercritical fluid extraction. *J. AOAC Int*. **2001**, *84*, 976−986.

(27) Gadgil, P.; Hachmeister, K. A.; Smith, J. S.; Kropf, D. H. 2-Alkylcyclobutanones as irradiation dose indicators in irradiated ground beef patties. *J. Agric. Food Chem*. **2002**, *50*, 5746−5750.

(28) Horvatovich, P.; Miesch, M.; Hasselmann, C.; Marchioni, E. Supercritical fluid extraction for the detection of 2-dodecylcyclobutanone in low dose irradiated plant foods. *J. Chromatogr., A* **2002**, *968*, 251−255.

(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) Tewfik, I. A rapid direct solvent extraction method for the extraction of cyclobutanones from irradiated chicken and liquid whole egg. *Int. J. Food Sci. Technol*. **2008**, *43*, 108−113.

(31) Todoriki, S.; Hayashi, T.; Kouchi, H. Effects of γ-irradiation on lipid metabolic changes of potato tubers in response to mechanical injury. *Biosci., Biotechnol., Biochem*. **1992**, *56*, 1991−1994.

(32) Todoriki, S.; Hayashi, T.; Nakamura, Y. Effects of γ-irradiation on the activity of tonoplast H^+ATPase from potato tubers (*Solanum tuberosum* L.). *Plant Cell Physiol*. **1994**, *35*, 829−836.

(33) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem*. **1957**, *226*, 497−509.

(34) Lacroix, M. L.; Charbonneau, R.; Jobin, M.; Thibault, C.; Nouchpramool, K.; Charoen, S.; Gagnon, M. A feasibility study of γ irradiation on Thailand frozen shrimps (*Penaeus monodon*). *Radiat. Phys. Chem*. **1995**, *46*, 739−744.

(35) Kwon, J. H.; Kausar, T.; Noh, J.; Kim, D. H.; Byun, M. W.; Kim, K. S.; Kim, K. S. The identification of irradiated seasoned filefish (*Thamnamconus modestus*) by different analytical methods. *Radiat. Phys. Chem*. **2007**, *76*, 1833−1836.

(36) Horvatovich, P.; Werner, D.; Jung, S.; Miesch, M.; Delincee, H.; Hasselmann, C.; Marchioni, E. Determination of 2-alkylcyclobutanones with electronic impact and chemical ionization gas chromatography/mass spectrometry in irradiated foods. *J. Agric. Food Chem*. **2006**, *54*, 1990−1996.

(37) Numata, M.; Kaneko, T.; Mi, Q.; Ye, M.; Kawamata, S.; Matsuo, M.; Yarita, T. Preparation of a sulfoxide group and ammonium-salt bonded silica stationary phase for separation of polychlorinated biphenyls from mineral oils. *J. Chromatogr., A* **2008**, *7*, 68−75.

(38) Numata, M.; Aoyagi, Y.; Tsuda, Y.; Yarita, T.; Takatsu, A. Preparation of sulfoxide residue bonded silica stationary phase for separation of polychlorinated biphenyls from mineral oils. *Anal. Chem*. **2007**, *79*, 9211−9217.

(39) Obana, H.; Furuta, M.; Tanaka, Y. Detection of irradiated meat, fish and their products by measuring 2-alkylcyclobutanones levels after frozen storage. *Shokuhin Eiseigaku Zasshi* **2007**, *48*, 203−206.

(40) Tewfik, I.; Tewfik, S. Would it still be possible to identify irradiated lipid-containing foods towards the end of their shelf-life. *Food Sci. Technol. Int*. **2008**, *14*, 519−524.

Received for review September 4, 2010. Revised manuscript received November 13, 2010. Accepted November 17, 2010. This work was supported by a Grant-in-Aid "Development of Evaluation and Management Methods for Supply of Safe, Reliable, and Functional Food and Farm Produce" from the Ministry of Agriculture, Forestry, and Fisheries of Japan.