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Detection of Radiation-Induced Hydrocarbons and 2-Alkylcyclobutanones in Irradiated Perilla Seeds

HAE-JUNG LEE,[1] MYUNG-WOO BYUN,[2] AND KYONG-SU KIM[1]*

[1]*Department of Food and Nutrition, Chosun University, Kwangju 501-759, Korea; and* [2]*Department of Food Irradiation, Korea Atomic Energy Research Institute, Taejon 305-353, Korea*

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ABSTRACT

The method consists of the extraction of fat from perilla seeds, separation of hydrocarbons and 2-alkylcyclobutanones with florisil column chromatography, and identification of hydrocarbons and 2-alkylcyclobutanones by gas chromatography–mass spectroscopy (GC-MS). Concentrations of hydrocarbons and 2-alkylcyclobutanones increased with the irradiation dose. The major hydrocarbons in the irradiated perilla seeds were 8-heptadecene and 1,7-hexadecadiene from oleic acid and 6,9-heptadecadiene and 1,7,10-hexadecatriene from linoleic acid. One of the 2-alkylcyclobutanones, 2-(5′-tetradecenyl)cyclobutanone, was found in the highest concentration in the irradiated perilla seeds. Radiation-induced hydrocarbons in the perilla seeds were detected at doses of 0.5 kGy and higher, and radiation-induced 2-alkylcyclobutanones were detected at doses of 1 kGy and higher. These compounds were not detected in nonirradiated perilla seeds.

Perilla seeds have been used as perilla oil, one of the spices used in Korean foods. In Korea, a large amount of perilla seeds are imported, since domestic production has decreased. Insect infestation may result in high losses during shipment or after harvest. For insect disinfestation, ionizing irradiation is an alternative treatment to chemical fumigation. Food irradiation can also improve the safety and quality of foods by extending shelf life, decreasing microbial load, preventing sprouting, and delaying ripening *(3)*. Interest in the use of irradiation for the treatment and preservation of foods has increased throughout the world, and the need for the development of a detection method for irradiated food was recognized. Thus, a reliable method is needed to detect the irradiated food and the allowed absorbed dose of radiation.

In fat-containing foods, radiation-induced hydrocarbons or 2-alkylcyclobutanones can be detected by gas chromatography or gas chromatography–mass spectroscopy (GC-MS) *(5, 13)*. These methods are regarded as the most promising methods to detect whether fat-containing food has been irradiated.

Nawar and Balboni *(9)* reported that hydrocarbons were formed from fatty acids in irradiated fat-containing foods. LeTellier and Nawar *(4)* found 2-alkylcyclobutanones using simple triglycerides irradiated with 60 kGy. Hydrocarbons formed are one fewer carbon atom (C_{n-1}) and two fewer carbon atoms (C_{n-2}) than the parent fatty acid. 2-Alkylcyclobutanones are the same number of carbons as the parent fatty acid and substituted with an alkyl group located in the 2 position.

A number of studies on the use of these compounds as detection markers of irradiated foods have been performed *(1, 2, 7)*, but to date studies on irradiated perilla seeds have not yet been performed. This study was undertaken to identify and quantify hydrocarbons and 2-alkylcyclobutanones formed from irradiated perilla seeds and to detect whether perilla seeds were irradiated.

MATERIALS AND METHODS

Irradiation. Perilla seeds were purchased from a farmer in Naju, Korea. Samples were irradiated using a ^{60}Co γ-irradiator at the Korea Atomic Energy Research Institute at each of the following doses: 0.1, 0.5, 1, 3, 5, and 10 kGy. The dose rate was 2.5 kGy/h, and the dose rate error was ±0.02 kGy. Irradiation temperature was 10 ± 1°C. Absorbed doses were monitored with radical dosimeter and a ceric-cerous dosimeter. The irradiated samples and the nonirradiated controls were stored at −18°C before analysis.

Reagents. The hydrocarbon and 2-alkylcyclobutanone standards were purchased from TeLA (Berlin, Germany). High-performance liquid chromatography grade solvents (*n*-pentane, *n*-hexane, and isopropanol) were purchased from Fisher Scientific (Pittsburgh, Pa.) and distilled with a spiral-packed, double-distilling apparatus (Normschliff Geratebau, Wertheim, Germany) before use. Florisil (60 to 100 mesh) was obtained from Fisher Scientific and heated at 550°C overnight to remove the contaminants. Before use, florisil was heated for at least 5 h in a 130°C dry oven and cooled in a desiccator. After that, 3% water (wt/wt) was added to separate hydrocarbons, and 20% (wt/wt) water was added to separate 2-alkylcyclobutanones, individually shaken for at least 20 min. These mixtures were stored for 10 to 12 hours. Florisil deactivated in this way was used for 3 days. Otherwise, the florisil was reheated at 130°C and deactivated again.

Fat extraction. A total of 30 g of ground perilla seeds was placed in beakers and mixed with 30 ml of solvent (*n*-pentane and isopropanol, 3:2, vol/vol). The mixture was homogenized for 2 min with a Ultra Turrax (IKA Labortechnik, Staufen, Germany) and centrifuged for 20 min at 1000 × *g* to obtain the fat. The

* Author for correspondence. Tel: 82.62.230.7724; Fax: 82.62.224.8880; E-mail: kskim@mail.chosun.ac.kr.

TABLE 1. *Radiation-induced hydrocarbons from fatty acids*

Fatty acid	C_{n-1}	C_{n-2}
Palmitic acid	Pentadecane ($C_{15:0}$)	1-Tetradecene ($C_{14:1}$)
Stearic acid	Heptadecane ($C_{17:0}$)	1-Hexadecene ($C_{16:1}$)
Oleic acid	8-Heptadecene ($C_{17:1}$)	1,7-Hexadecadiene ($C_{16:2}$)
Linoleic acid	6,9-Heptadecadiene ($C_{17:2}$)	1,7,10-Hexadecatriene ($C_{16:3}$)
Linolenic acid	6,9,12-Heptadecatriene ($C_{17:3}$)	1,7,10,13-Hexadecatetraene ($C_{16:4}$)

residue was re-extracted with one third of the amount of solvent and centrifuged again. The solvent phase was concentrated using a rotary vacuum evaporator (Büchi, Flawil, Switzerland) and nitrogen gas. Final volume of extracted fat was approximately 15 ml. The extracted fat was stored at −18°C.

Separation of hydrocarbons. A total of 25 g of deactivated florisil was packed into a 200 by 20-mm glass column. Anhydrous sodium sulfate was added on top of the florisil column in a 1-cm layer. A total of 1 g of extracted fat was mixed with an internal standard, 1 ml of *n*-eicosane (4 μg/ml of hexane), applied to the column of florisil, and eluted with 60 ml of hexane at a flow rate of 3 ml/min. The eluted hexane was concentrated to a volume of 2 ml using a rotary vacuum evaporator and further concentrated to a volume of 0.5 ml by means of nitrogen gas.

Separation of 2-alkylcyclobutanones. A total of 30 g of deactivated florisil was packed into a 200 by 20-mm glass column. Anhydrous sodium sulfate was added on top of the florisil column in a 1-cm layer. A total of 0.2 g of extracted fat was mixed with an internal standard, 1 ml of 2-cyclohexylcyclohexanone (1 μg/ml of hexane), applied to the column, and eluted with 150 ml of hexane followed by 120 ml of 2% diethyl ether in hexane (vol/vol) at a flow rate of 3 ml/min. This latter fraction was concentrated to a volume of 2 ml using a rotary vacuum evaporator and further concentrated to a volume of 0.2 ml by means of nitrogen gas.

GC-MS analysis of hydrocarbons. GC-MS analysis was carried out on a Shimadzu GC-MS QP-5050 spectrometer (Kyoto, Japan) in EI mode. The ionization voltage was 70 eV; injector and ion source temperatures were both kept at 250°C. The column was a DB-5 (30-m by 0.32-mm internal diameter, 0.25-μm film thickness, J & W Scientific, Folsom, Calif.). The oven temperature program was 60 to 170°C at 25°C/min, 205°C at 2°C/min, and then 270°C at 10°C/min. The carrier gas was helium at a flow rate of 1.0 ml/min. A total of 1 μl of sample was injected in splitless mode for 2 min and then in split mode (20:1). Hydrocarbons were identified by comparing retention time and mass spectrum of peaks in the total ion chromatogram with those of authentic hydrocarbon standards. The concentration of each hydrocarbon in the fat was determined by using an internal standard *n*-eicosane.

GC-MS analysis of 2-alkylcyclobutanones. GC-MS analysis was carried out on a Shimadzu GC-MS QP-5050 spectrometer (Kyoto, Japan) in EI mode. The injector and ion source temperatures were kept at 250 and 290°C. The column was a DB-5 (30-m by 0.32-mm internal diameter, 0.25-μm film thickness, J & W Scientific). The oven temperature program was 120°C (1 min) to 160°C at 15°C/min, 175°C at 0.5°C/min, and then 290°C at 30°C/min (10 min). A total of 2 μl of sample was injected in splitless mode for 1 min and then in split mode (20:1). The other conditions were the same as described for the hydrocarbons.

2-Alkylcyclobutanones were analyzed by GC-MS using the selected ion monitoring mode. For the quantitative analysis of 2-alkylcyclobutanones, authentic standards, 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, and 2-(5′-tetradecenyl)cyclobutanone, and an internal standard, 2-cyclohexylcyclohexanone, were prepared with 0.1 to 5 ppm (μg/ml). These solutions were experimented with the same procedure of separation of 2-alkylcyclobutanones and analyzed by selected ion monitoring method, and standard curves were calibrated. The concentration of each 2-alkylcyclobutanone in the fat was determined by using standard calibration curves with an internal standard 2-cyclohexylcyclohexanone. Selected ion monitoring of 2-alkylcyclobutanones was set for 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone using ions m/z 98 and m/z 112 and 2-(5′-tetradecenyl)cyclobutanone using ions m/z 67, m/z 81, m/z 98, and m/z 109 and produced the peaks with a retention time and ion ratio that corresponded to that of 2-alkylcyclobutanone standards. Mass spectra of 2-alkylcyclobutanones were confirmed by GC-MS with the full-scan mode.

RESULTS AND DISCUSSION

Radiation-induced hydrocarbons from irradiated perilla seeds. When fatty acids are irradiated, mainly two types of hydrocarbons are formed. One hydrocarbon contains one fewer carbon atom than its parent fatty acid. This hydrocarbon is formed as a result of the loss of the carboxyl group. The other hydrocarbon contains two fewer carbon atoms than its parent fatty acid. It also forms a double bond at the C_1 position *(8)*.

Perilla seeds contain large amounts of oleic, linoleic, and linolenic acids and small amounts of palmitic and stearic acid. Oleic, linoleic, and linolenic acids have been found to be 9.5 to 21.4%, 9.1 to 20.4%, and 50.6 to 70.5% and palmitic and stearic acids were 4.1 to 10.0% and 0.6 to 4.1% of the total fatty acids in perilla seeds *(11)*. Table 1 shows the hydrocarbons formed from fatty acids. Figure 1 shows the gas chromatograms of hydrocarbons from non-irradiated and 10-kGy irradiated perilla seeds. Except 6,9,12-heptadecatriene and 1,7,10,13-hexadecatetraene formed from linolenic acid, hydrocarbons formed from palmitic, stearic, oleic, and linoleic acids were detected and determined quantitatively (Table 2). 6,9,12-Heptadecatriene and 1,7,10,13-hexadecatetraene were detected but not measured, because standard solutions are not available. Because of the composition of fatty acids in perilla seeds, 8-heptadecene and 1,7-hexadecadiene from oleic acid and 6,9-heptadecadiene and 1,7,10-hexadecatriene from linoleic acid are predicted to be the major radiation-induced hydrocarbons in perilla seeds. Table 2 shows that this is what is observed. Figure 2 shows mass spectra of hydrocarbons originated from oleic and linoleic acids.

Figure 3 demonstrates that hydrocarbons were detected

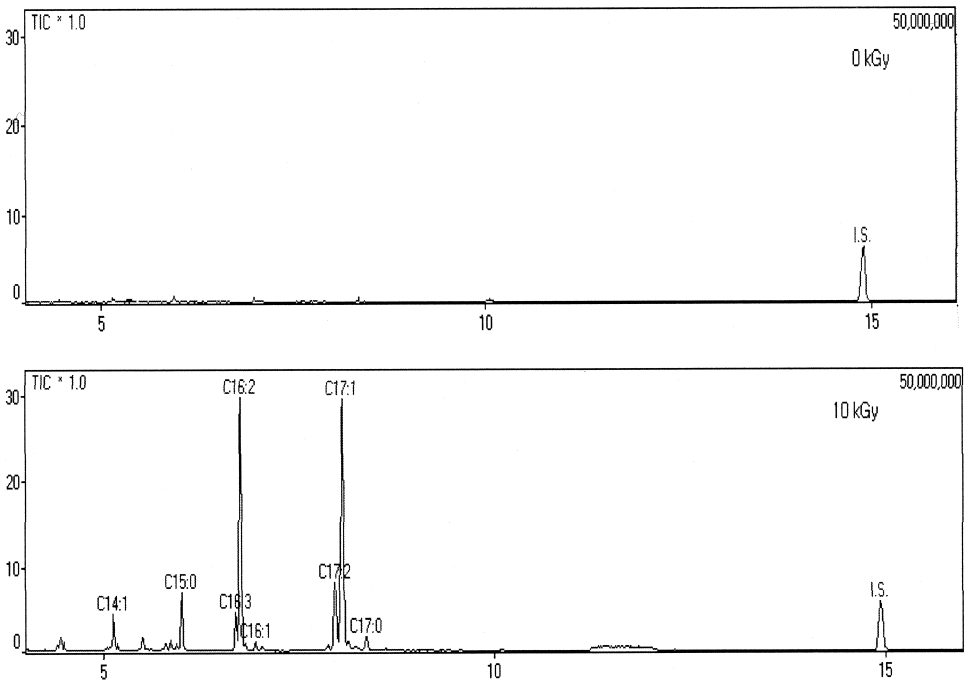

FIGURE 1. *Chromatograms of hydrocarbons from nonirradiated and 10-kGy irradiated perilla seeds.*

with different concentrations at the same dose, depending on composition of fatty acids in perilla seeds. This has been previously noted for beef, pork, and chicken *(12)*, shrimps and chicken *(6)*, and various other foods *(10)*. Also, Figure 3 indicates that C_{n-1} hydrocarbons are formed in higher concentrations than C_{n-2} hydrocarbons. This is different from the results of hydrocarbons in irradiated Brazilian beans *(15)*.

Pentadecane, 1-tetradecene, heptadecane, and 1,7-hexadecadiene could be detected in perilla seeds irradiated with the dose of 0.1 kGy; 1-tetradecene, 1,7,10-hexadecatriene, 8-heptadecene, and 6,9-heptadecadiene could be detected at a dose of 0.5 kGy; and 1-hexadecene was detected at a dose of 1 kGy. Radiation-induced hydrocarbons were not detected in nonirradiated perilla seeds.

Pentadecane was detected in a relatively large amount compared with 1-tetradecene at a dose of 10 kGy and at an almost similar level at as low as 5 kGy. The amount of these hydrocarbons was low because of the small amount of stearic acid in perilla seeds but increased with the irradiation dose. 8-Heptadecene and 1,7-hexadecadiene were present at high concentrations compared with other hydrocarbons. Similar results have been previously noted for Corioca bean, a kind of Brazilian bean *(15)*. 6,9-Heptadecadiene and 1,7,10-hexadecatriene were also in high concentrations because of the large amount of linoleic acid in perilla seeds. 6,9-Heptadecadiene was much more concentrated than 1,7,10-hexadecatriene. The major hydrocarbons formed based on the composition of fatty acids and degradation mechanism in the perilla seeds were 8-heptadecene, 1,7-hexadecadiene, 6,9-heptadecadiene, and 1,7,10-hexadecatriene. Ratios of C_{n-2} (1,7-hexadecadiene and 1,7,10-hexadecatriene) to C_{n-1} (8-heptadecene and 6,9-heptadecadiene) in 10-kGy irradiated perilla seeds were 1.03 and 2.26, respectively. Concentrations of radiation-induced hydrocarbons in perilla seeds increased linearly with irradiation dose. These major hydrocarbons could be used to detect gamma-irradiated perilla seeds.

Radiation-induced 2-alkylcyclobutanones from irradiated perilla seeds. 2-Alkylcyclobutanones are formed

TABLE 2. *Concentrations of radiation-induced hydrocarbons in perilla seeds*[a]

Irradiation dose (kGy)	$C_{15:0}$	$C_{14:1}$	$C_{17:0}$	$C_{16:1}$	$C_{17:1}$	$C_{16:2}$	$C_{17:2}$	$C_{16:3}$
0	—	—	—	—	—	—	—	—
0.1	0.065 ± 0.01	—	0.092 ± 0.02	—	—	0.075 ± 0.03	—	—
0.5	0.254 ± 0.02	0.066 ± 0.03	0.112 ± 0.07	—	0.256 ± 0.12	0.193 ± 0.08	0.053 ± 0.03	0.054 ± 0.04
1	0.327 ± 0.08	0.087 ± 0.03	0.130 ± 0.07	0.048 ± 0.03	0.545 ± 0.18	0.425 ± 0.21	0.232 ± 0.05	0.073 ± 0.08
3	0.442 ± 0.12	0.16 ± 0.06	0.173 ± 0.09	0.064 ± 0.05	1.682 ± 0.35	1.991 ± 0.62	0.502 ± 0.24	0.420 ± 0.12
5	0.771 ± 0.18	0.415 ± 0.08	0.298 ± 0.11	0.128 ± 0.05	4.277 ± 0.68	4.267 ± 0.58	1.34 ± 0.11	0.509 ± 0.15
10	1.894 ± 0.15	1.05 ± 0.09	0.683 ± 0.12	0.314 ± 0.04	10.634 ± 0.89	10.254 ± 0.85	3.006 ± 0.32	1.33 ± 0.18

[a] Values are expressed in μg per g of fat as mean ± standard deviation ($n = 3$).

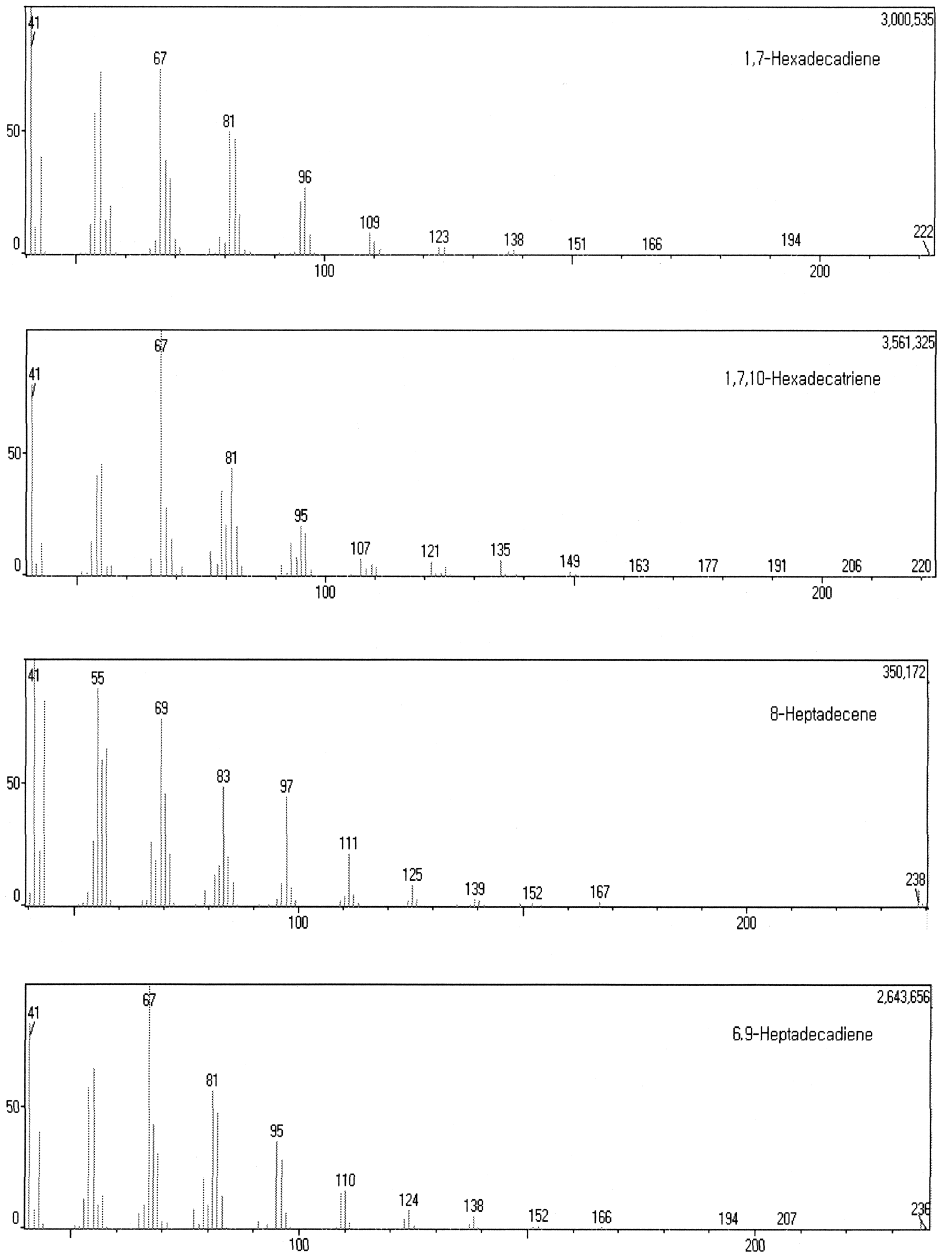

FIGURE 2. *Mass spectra of the major radiation-induced hydrocarbons from perilla seeds.*

in irradiated fat by chemical degradation and have the same number of carbon atoms as the parent fatty acids from which they are formed, with an alkyl group located in ring position 2. These compounds are cyclic compounds formed by the loss of an electron from the oxygen on the carbonyl of a fatty acid or triglyceride, followed by a rearrangement process to produce 2-alkylcyclobutanones specific to their parent fatty acids *(4).*

On the basis of this process, the major fatty acids in irradiated perilla seeds can be degraded to the corresponding 2-alkylcyclobutanones (Table 3). 2-(5′,8′-Tetradecadienyl)cyclobutanone from linoleic acid and 2-(5′,8′,11′-tetradecatrienyl)cyclobutanone from linolenic acid could not be confirmed, since standard solutions are not available. Except 2-alkylcyclobutanones from linoleic and linolenic acids, quantitative data of 2-alkylcyclobutanones by the selected ion monitoring method are shown in Table 4. Figure 4 shows the chromatograms of the 2-alkylcyclobutanones of nonirradiated and 10-kGy irradiated perilla seeds. Figure 5 shows mass spectra of 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, and 2-(5′-tetradecenyl)cyclobutanone by GC-MS with full-scan mode.

2-Dodecylcyclobutanone was found at higher concentration than 2-tetradecylcyclobutanone, since palmitic acid had a greater presence compared with stearic acid in perilla seeds. This result was similar to a report on the use of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone as an irradiation marker in liquid whole egg *(14).* Among 2-alkylcyclobutanones, 2-(5′-tetradecenyl)cyclobutanone formed from oleic acid had the highest concentration.

When the concentrations of radiation-induced 2-alkylcyclobutanones were plotted versus the amounts of fat, the linear response with irradiation dose is demonstrated (Fig. 6). The concentrations of these compounds increased with

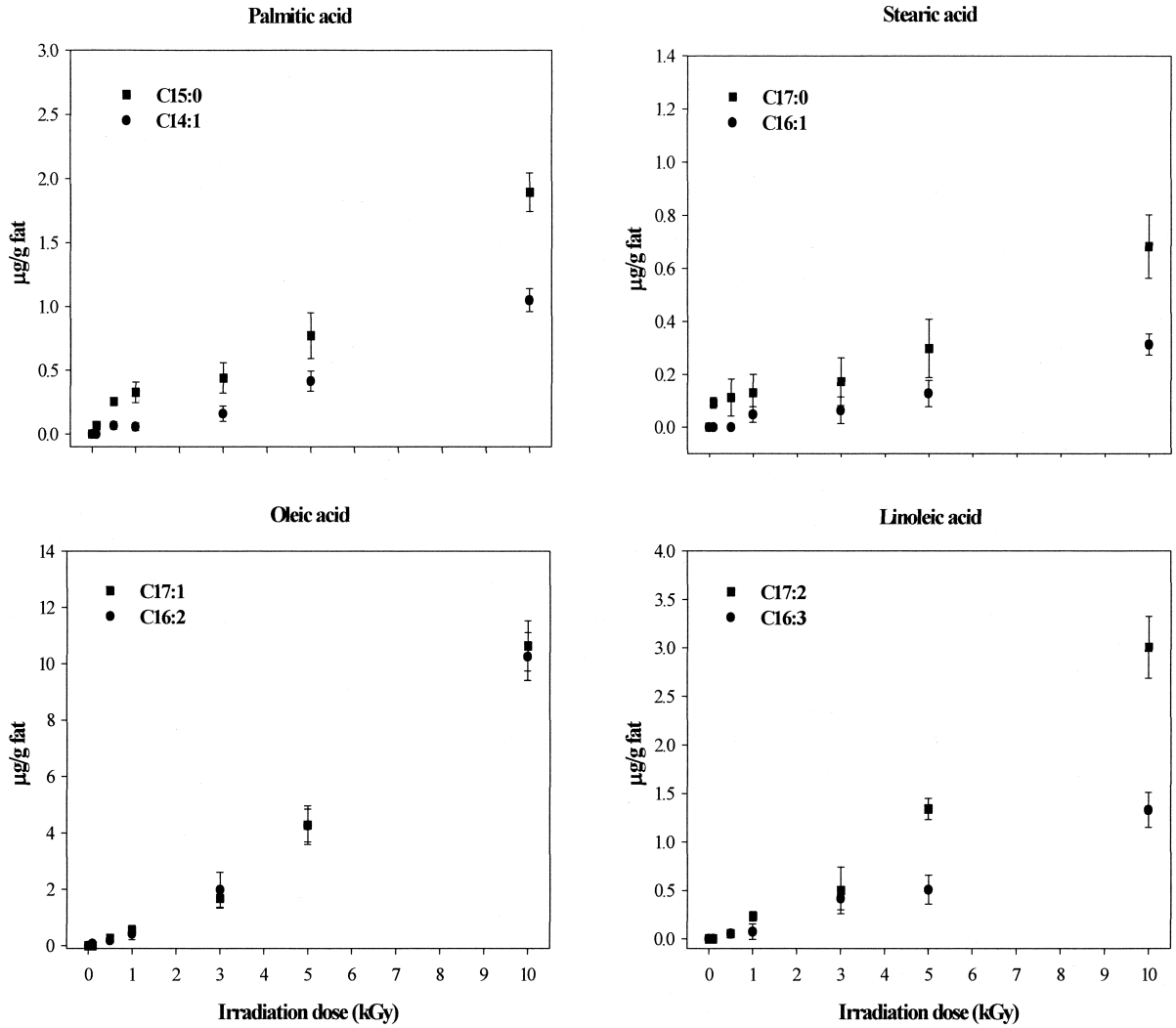

FIGURE 3. *Irradiation dose dependence of radiation-induced hydrocarbons from perilla seeds.*

the irradiation dose. 2-Dodecylcyclobutanone and 2-(5′-tetradecenyl)cyclobutanone could be detected at doses of 0.5 kGy and higher, whereas 2-tetradecylcyclobutanone was present in trace amounts and could be identified at doses of 1 kGy and higher. 2-Dodecylcyclobutanone, 2-tetradecylcyclobutanone, and 2-(5′-tetradecenyl)cyclobutanone were not detected in nonirradiated perilla seeds.

Finally, hydrocarbons and 2-alkylcyclobutanones formed during the irradiation of perilla seeds increased with the irradiation dose. Hydrocarbons could be detected at doses of 0.5 kGy and higher, and 2-alkylcyclobutanones could be confirmed at 1 kGy and higher. The major hydrocarbons were 8-heptadecene, 1,7-hexadecadiene, 6,9-heptadecadiene, and 1,7,10-hexadecatriene. One of the 2-alkylcyclobutanones, 2-(5′-tetradecenyl)cyclobutanone, was found at the highest concentration in the perilla seeds. These major hydrocarbons and 2-alkylcyclobutanone would be used for the detection of irradiated perilla seeds. Further research is needed to establish the influence of processing variables such as storage temperature and period. This process should

TABLE 3. *Radiation-induced 2-alkylcyclobutanones from fatty acids*

Fatty acid	Radiation-induced 2-alkylcyclobutanones
Palmitic acid	2-Dodecylcyclobutanone
Stearic acid	2-Tetradecylcyclobutanone
Oleic acid	2-(5′-Tetradecenyl)cyclobutanone
Linoleic acid	2-(5′,8′-Tetradecadienyl)cyclobutanone
Linolenic acid	2-(5′,8′,11′-Tetradecatrienyl)cyclobutanone

TABLE 4. *Concentrations of radiation-induced 2-alkylcyclobutanones in perilla seeds*[a]

Irradiation dose (kGy)	2-Dodecylcyclobutanone	2-Tetradecylcyclobutanone	2-(5′-Tetradecenyl)cyclobutanone
0	—	—	—
0.5	0.022 ± 0.005	0.005 ± 0.004	0.053 ± 0.02
1	0.033 ± 0.01	0.011 ± 0.003	0.143 ± 0.05
3	0.06 ± 0.02	0.033 ± 0.009	0.503 ± 0.08
5	0.085 ± 0.03	0.048 ± 0.011	0.858 ± 0.11
10	0.195 ± 0.05	0.117 ± 0.03	2.887 ± 0.22

[a] Values are expressed in µg per g of fat as mean ± standard deviation ($n = 3$).

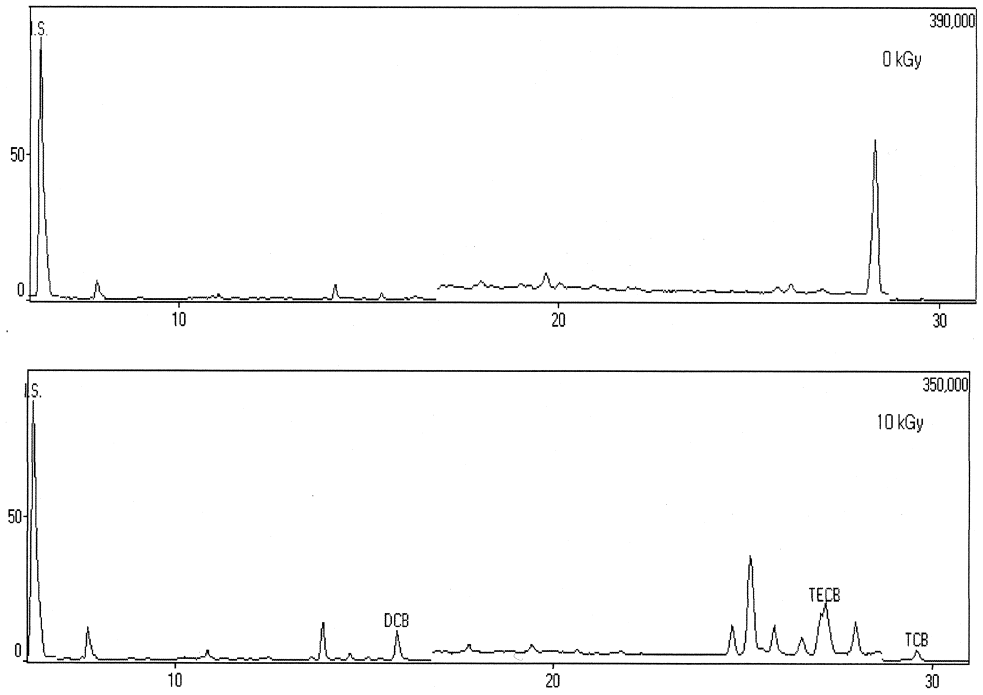

FIGURE 4. *Chromatograms of 2-alkylcyclobutanones from nonirradiated and 10-kGy irradiated perilla seeds. DCB, 2-dodecylcyclobutanone; TECB, 2-(5′-tetradecenyl)cyclobutanone; TCB, 2-tetradecylcyclobutanone.*

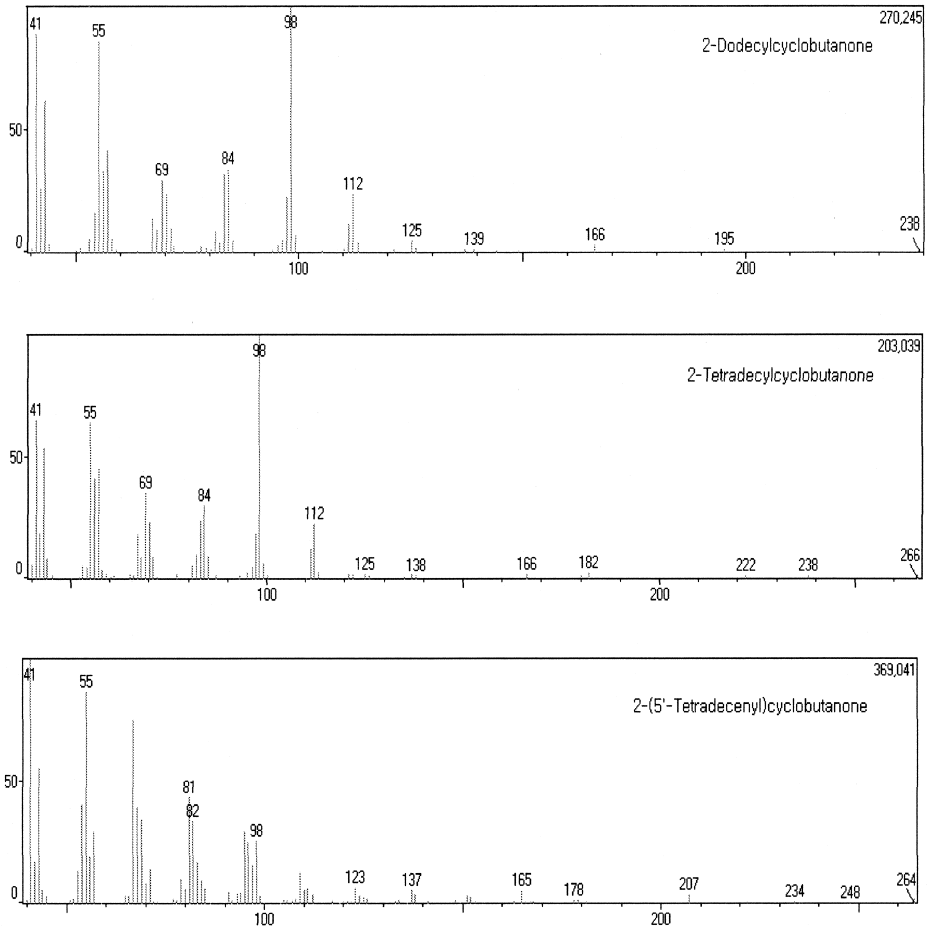

FIGURE 5. *Mass spectra of radiation-induced 2-alkylcyclobutanones from perilla seeds.*

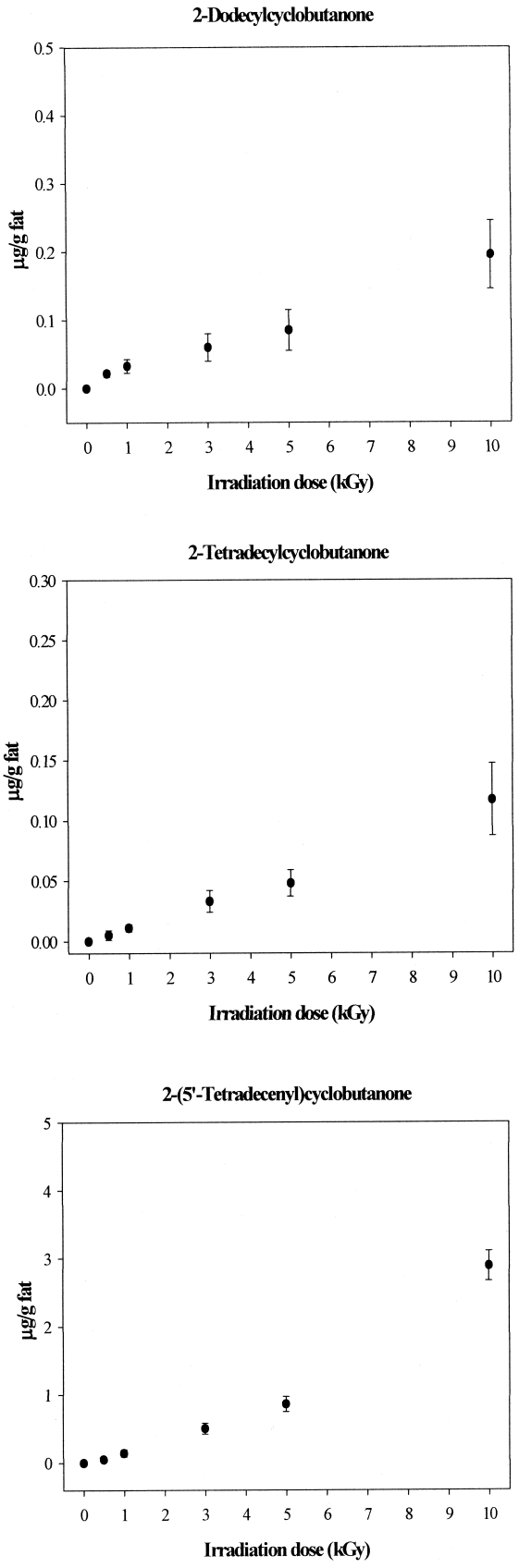

FIGURE 6. *Irradiation dose dependence of radiation-induced 2-alkylcyclobutanones from perilla seeds.*

be applied to a variety of foods, since concentrations of radiation-induced hydrocarbons and 2-alkylcyclobutanones in various foods are different.

ACKNOWLEDGMENT

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