J. Food Sci. Nutr.
Vol. 4, No. 4, p. 270~275 (1999)

Detection of Radiation-induced Hydrocarbons and 2-Alkylcyclobutanones from Peanuts

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Abstract

Radiation-induced hydrocarbons and 2-alkylcyclobutanones are formed from the fatty acids of irradiated fats. Peanuts were irradiated with a dose of 0.1~10 kGy. The method consists of the extraction of fat from peanuts, separation of hydrocarbons and 2-alkylcyclobutanones with florisil column chromatography and identification of hydrocarbons by the GC/MS method and 2-alkylcyclobutanones by GC/MS/selected ion monitoring (SIM). Concentrations of hydrocarbons and 2-alkylcyclobutanones were linearly increased with the dose levels of radiation. The major hydrocarbons in the irradiated peanut samples were 8-heptadecene and 1,7-hexadecadiene from oleic acid and 6,9-heptadecadiene and 1,7,10-hexadecatriene from linoleic acid. 2-(5'-Tetradecenyl)cyclobutanone, one of 2-alkylcyclobutanones, was the highest amount in the irradiated peanuts. Radiation-induced hydrocarbons in the peanuts were detected at doses of 0.5 kGy and over, and radiation-induced 2-alkylcyclobutanones were detected at doses of 1 kGy and over. These compounds were not confirmed in unirradiated peanuts.

Key words: peanut, hydrocarbons, 2-alkylcyclobutanones, GC/MS

INTRODUCTION

Ionizing irradiation of foods can improve the safety and quality of foods by extending shelf-life, decreasing microbial and insect infestations, preventing sprouting and delaying ripening (1). In 1981, a Joint Expert Committee stated that "The irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard, introduces no special nutritional or microbiological problems" (2). Thus 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. A number of methods have been investigated extensively to detect irradiated foods by international organizations such as IAEA, FAO and WHO. So far, there are three main methods for the detection of irradiated food. Physical methods use electron spin resonance (ESR) spectroscopy for the identification of stable free radicals trapped in bone and fiber (3) and thermoluminescence (TL) for the measurement of minerals adhering to the surface of spices and dried vegetables (4). Biological methods are DNA (5), limulus amoebocytes lysate (LAL) (6) and direct epifluorescent filter technique/aerobic plate count (DEFT/APC) (7) analyses. In addition, chemical methods can be used to detect hydrocarbons or 2-alkylcyclobutanones formed from fat-containing food irradiated by GC or GC/MS analyzer (8, 9). In fat-containing foods, chemical methods have been regarded as the most promising method to detect whether food has been irradiated or not.

The major volatile compounds formed from irradiated fats are hydrocarbons, aldehydes, methyl and ethyl esters and free fatty acids. Nawar (10) reported that hydrocarbons were formed from fatty acids in irradiated fat-containing foods. As well as major compounds, LeTellier and Nawar (11) found 2-alkylcyclobutanones using simple triglycerides irradiated with 60 kGy. A number of studies on the use of these compounds as detection markers of irradiated foods have proceeded (12-14), but to date, systematic data is insufficient to be applied to every sample.

Therefore, this study was performed to identify hydrocarbons and 2-alkylcyclobutanones formed from irradiated peanuts and to provide a quantitative identification basis for the detection of irradiated foods, and furthermore, to enhance consumer confidence by labelling detection results.

MATERIALS AND METHODS

Materials

Peanuts were irradiated at each of the following doses, 0.1 kGy, 0.5 kGy, 1 kGy, 3 kGy, 5 kGy and 10 kGy using a ^{60}Co γ-irradiator at the Korea Atomic Energy Research Institute. The irradiated peanuts and the control were stored at -18°C.

Reagents

The hydrocarbon and 2-alkylcyclobutanone standards were purchased from TeLA (Germany). HPLC grade solvents (n-pentane, n-hexane and isopropanol) were purchased from Fisher Scientific (USA) and distilled with spiral packed double distilling apparatus (Normschliff, Germany) prior to use. Florisil (60~100 mesh) was obtained from Fisher Scientific (USA) and heated at 550°C overnight to remove the contaminants. Before use, florisil was heated for at least 5 hours in a 130°C dry oven and cooled in a desiccator. After that,

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3% water (w/w) was added to separate hydrocarbons and 20% water (w/w) was added to separate 2-alkylcyclobutanones, and individually shaken for at least 20 min. These mixtures were stored for 10～12 hours. Florisil deactivated in this way was used for 3 days. Otherwise, the florisil was reheated at 130°C and deactivated again.

Extraction of fat from peanuts

Peanut fat was extracted by a method previously described by Schreiber et al. (15). 30 g of ground peanut samples was placed in beakers and mixed with 30 ml of solvent (n-pentane/isopropanol 3 : 2, v/v). The mixture was homogenized for 2 min with an Ultra Turrax (Janke & Kunkel, Germany) and centrifuged for 20 min at 900×g to obtain the fat. The residues were re-extracted with one third of the amount of solvent and centrifuged again. The solvent phase was concentrated using a rotary vacuum evaporator (Büchi, Switzerland) and nitrogen gas. The extracted fat was stored at -20°C.

Separation of hydrocarbons

25 g of deactivated florisil was packed into a 200×20 mm glass column. Anhydrous sodium sulfate was added on top of the florisil column in a 1 cm layer. 1 g of extracted fat was mixed with an internal standard, 1 ml n-eicosane (4 μg/ml), applied to the column of florisil, and eluted with 60 ml 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

30 g of deactivated florisil was packed into a 200×20 mm glass column. Anhydrous sodium sulfate was added on top of the florisil column in a 1 cm layer. 0.2 g of extracted fat was mixed with an internal standard, 1 ml 2-cyclohexylcyclohexanone (1 μg/ml hexane) applied to the column, and eluted with 150 ml hexane followed by 120 ml of diethyl ether/hexane (2 : 98) 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-FID analysis

GC analysis was carried out with a Hewlett-Packard (HP) 5890 II Plus gas chromatograph equipped with a flame ionization detector (FID). The column used was DB-5 (30 m×0.32 mm i.d., 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA). The oven temperature programs used were : for the analysis of hydrocarbons, 60°C to 170°C at 25°C/min and to 205°C at 2°C/min then to 270°C at 10°C/min; and for the analysis of 2-alkylcyclobutanones, 120°C (1 min) to 160°C at 15°C/min and to 175°C at 0.5°C/min then to 290°C at 30 °C/min (10 min). The injector and detector temperatures were kept at 250°C and 300°C, respectively. The carrier gas was helium at a flow rate of 1.0 ml/min. To analyze hydrocarbons, 1 μl of sample was injected in splitless mode for 2 min and then in split mode (20 : 1). To analyze 2-alkylcyclobutanones, 2 μl of sample was injected in splitless mode for 1 min and then in split mode (20 : 1).

GC/MS analysis

GC/MS analysis was carried out on a Shimadzu GC/MS QP-5050 spectrometer in EI mode. The ionization voltage was 70 eV and ion source temperature was kept at 290°C. The column was a DB-5 (30 m×0.32 mm i.d., 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA). The other conditions were the same as described for the GC analysis. Hydrocarbons were identified by comparison of retention time and mass spectrum of peaks as shown in the total ion chromatogram with that of authentic hydrocarbon standards. The concentration of each hydrocarbon in the fat was determined by using an internal standard (n-eicosane 4 μg/ml). 2-Alkylcyclobutanones were analyzed by GC/MS with the selected ion monitoring (SIM) mode. For the quantitative analysis of 2-alkylcyclobutanones, standard materials, 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, and 2-(5′-tetradecenyl) cyclobutanone were prepared with 0.1～5 ppm (μg/ml) and standard curves were made. The SIM of the 2-alkylcyclobutanones formed from irradiated peanuts was set for 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone for ions m/z 98 and m/z 112, and 2-(5′-tetradecenyl)cyclobutanone for 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 which corresponded to that of standard 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone and 2-(5′-tetradecenyl)cyclobutanone. Mass spectra of 2-alkylcyclobutanones were confirmed by GC/MS with the full scan mode.

RESULTS AND DISCUSSION

Radiation-induced hydrocarbons from irradiated peanuts

Peanuts contain a large amount of palmitic, oleic and linoleic acids and a small amount of stearic acid. When these fatty acids are irradiated, mainly two types of hydrocarbons are formed. One hydrocarbon contains one less 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 less carbon atoms than its parent fatty acid. It also forms a double bond at the C_1 position (10).

Based on these, pentadecane ($C_{15\,0}$) and 1-tetradecene ($C_{14\,1}$) from palmitic acid, heptadecane ($C_{17,0}$) and 1-hexadecene ($C_{16\,1}$) from stearic acid, 8-heptadecene ($C_{17\,1}$) and 1,7-hexadecadiene ($C_{16\,2}$) from oleic acid and 6,9-heptadecadiene ($C_{17\,2}$) and 1,7,10-hexadecatriene ($C_{16\,3}$) from linoleic acid were formed and confirmed. For comparison of concentrations of hydrocarbons, peanuts were irradiated at doses of 0.1～10 kGy.

Fig. 1. shows the gas chromatograms of hydrocarbons of unirradiated and 10 kGy irradiated peanuts. In agreement with the lipid degradation patterns during irradiation as proposed by Nawar (10), a number of radiolytic hydrocarbons can be detected. Concentrations of hydrocarbons increased with the irradiation dose (Table 1). Also as Fig. 2. indicates, hydrocarbons were detected with different concentrations at

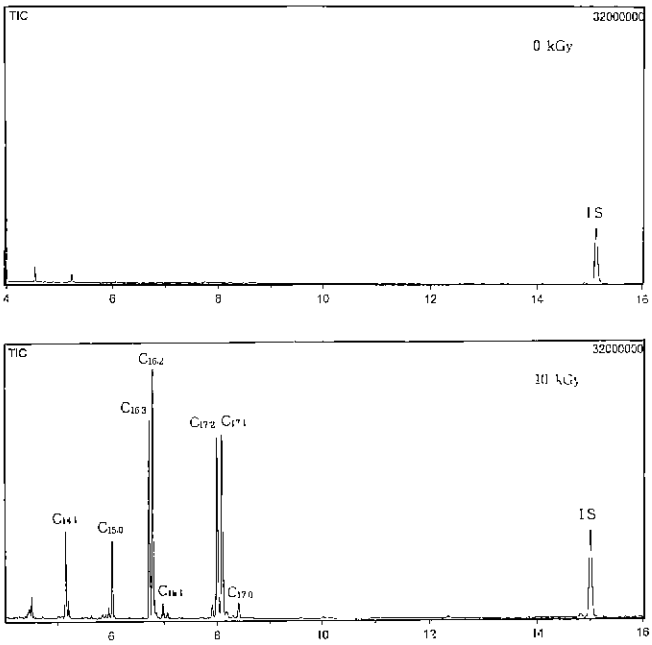

Fig. 1. Gas chromatograms of radiation-induced hydrocarbons of unirradiated and 10 kGy irradiated peanuts.

the same dose. It depends on the composition of fatty acids in peanuts. This has been previously noted for beef, pork and chicken (15), shrimps and chicken (16) and various other foods (17).

Pentadecane and 1-tetradecene were hydrocarbons formed from palmitic acid. Pentadecane was detected in a relatively large amount as compared to 1-tetradecene at a dose of 10 kGy and at an almost similar level at as low as 5 kGy. Heptadecane and 1-hexadecene were formed from stearic acid. These hydrocarbons were increased with the irradiation dose but had low concentrations because of the small amount of stearic acid in peanuts. Therefore, these hydrocarbons cannot be available for detection whether irradiated or not. 8-Heptadecene and 1,7-hexadecadiene were the major hydrocarbons in irradiated peanuts. Because of the large amount of oleic acid, these hydrocarbons were present as a high concentration compared with other hydrocarbons. Similarly, it has been previously noted for the Corioca bean, a kind of Brazilian bean (18). 6,9-Heptadecadiene and 1,7,10-hexadecatriene were also high concentrations because of the large amount of linoleic acid in peanuts. 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 peanuts were 8-heptadecene, 1,7,-hexadecadiene, 6,9-heptadecadiene and 1,7,10-hexadecatriene. These hydrocarbons would be used to detect gamma-irradiated foods.

Pentadecane, 1-tetradecene, heptadecane and 1,7-hexadecadiene could be detected in peanuts irradiated with the dose of 0.1 kGy, and other hydrocarbons could be detected at a dose of 0.5 kGy. Radiation-induced hydrocarbons were remarkably detected at doses of 0.5 kGy and higher and were not detected in unirradiated peanuts.

Radiation-induced 2-alkylcyclobutanones from irradiated peanuts

2-Alkylcyclobutanones are formed 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 were 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 (11). During irradiation of the peanuts, 2-dodecylcyclobutanone from palmitic acid, 2-tetradecylcyclobutanone from stearic acid, 2-(5′-tetradecenyl)cyclobutanone from oleic acid and 2-(5′,8′-tetradecadienyl)cyclobutanone from linoleic acid are formed. But 2-(5′,8′-tetradecadienyl)cyclobutanone could not be confirmed since a standard solution is not available. To identify 2-alkylcyclobutanones formed based on this process, peanuts

Table 1. Concentrations of radiation-induced hydrocarbons in peanuts (μg/g fat)

Radiation dose (kGy)	Palmitic acid		Stearic acid		Oleic acid		Linoleic acid	
	$C_{15:0}$	$C_{14:1}$	$C_{17:0}$	$C_{16:1}$	$C_{17:1}$	$C_{16:2}$	$C_{17:2}$	$C_{16:3}$
0	-	-	-	-	-	-	-	-
0.1	0.039 (±0.02)[1)]	0.029 (±0.01)	0.021 (±0.01)	-	-	0.030 (±0.01)	-	-
0.5	0.068 (±0.03)	0.104 (±.0.06)	0.035 (±0.02)	0.010 (±0.01)	0.025 (±0.01)	0.076 (±0.06)	0.065 (±0.02)	0.053 (±0.02)
1	0.104 (±0.08)	0.151 (±0.05)	0.041 (±0.04)	0.017 (±0.01)	0.114 (±0.05)	0.322 (±0.11)	0.114 (±0.09)	0.196 (±0.09)
3	0.274 (±0.10)	0.448 (±0.09)	0.102 (±0.08)	0.076 (±0.03)	0.794 (±0.19)	1.338 (±0.19)	0.748 (±0.12)	0.857 (±0.15)
5	0.769 (±0.12)	0.706 (±0.14)	0 226 (±0.07)	0.175 (±0.05)	2.224 (±0.52)	2.600 (±0.31)	2.401 (±0.29)	1.945 (±0.18)
10	1.856 (±0.11)	2.025 (±0.11)	0.593 (±0.08)	0 455 (±0.04)	5.704 (±0.62)	6.265 (±0.44)	5.344 (±0 32)	4 807 (±0.35)

[1)]Mean±standard deviation

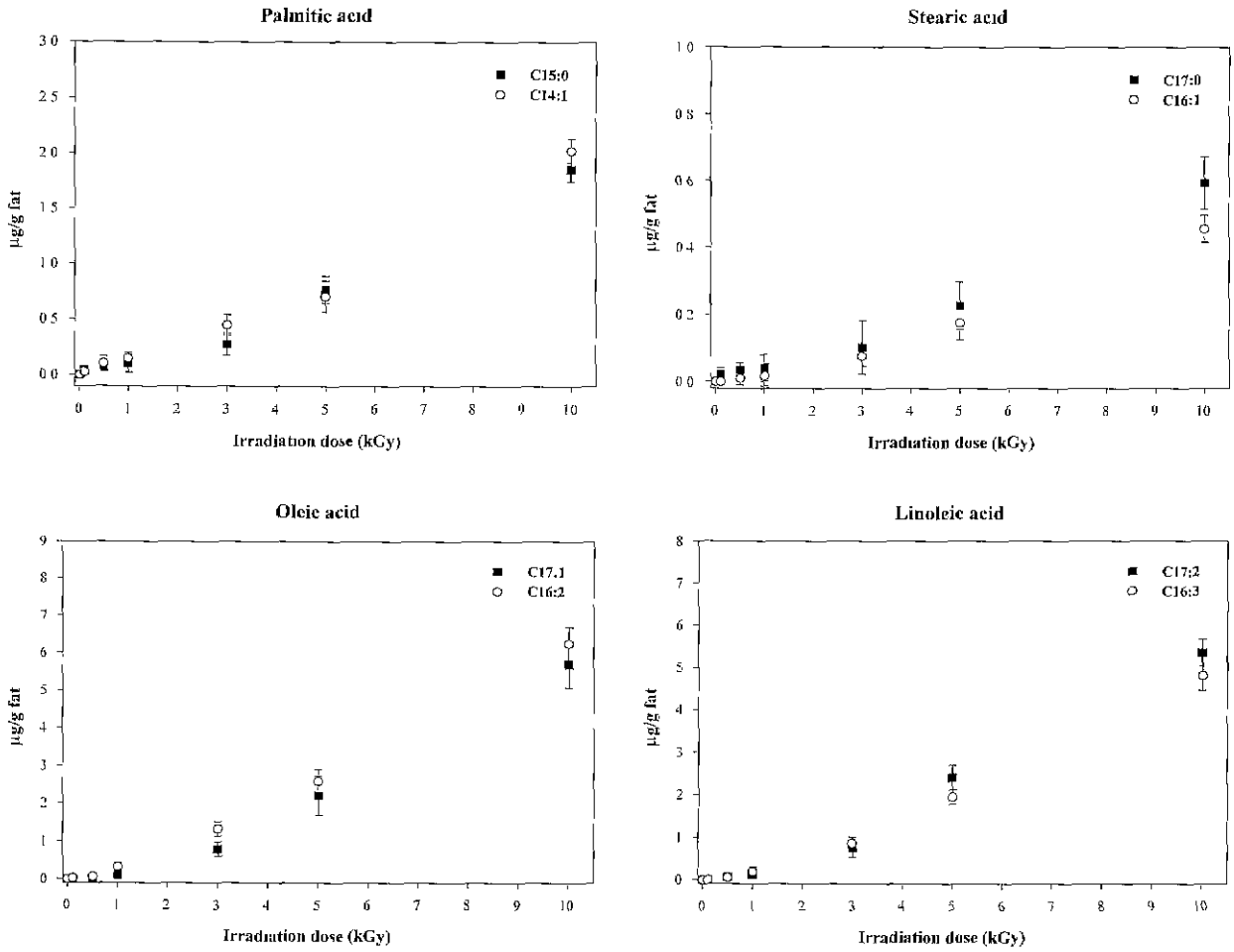

Fig. 2. Effect of irradiation doses on the radiation-induced hydrocarbons of peanuts.

needed to be irradiated with dose levels of 0.5~10 kGy.

Fig. 3. shows the chromatograms of 2-alkylcyclobutanones of unirradiated and 10 kGy irradiated peanuts in SIM mode. Quantitative data of 2-alkylcyclobutanone by SIM method suggested that the concentrations of these compounds increase with their radiation dose (Table 2).

2-Dodecylcyclobutanone had a higher concentration than 2-tetradecylcyclobutanone as palmitic acid has a greater presence compared with stearic acid in peanuts. This result is similar to a report on the use of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone as an irradiation marker in liquid whole egg (19). Among 2-alkylcyclobutanones, 2-(5′-tetradecenyl)cyclobutanone originating from oleic acid had the highest concentration.

When the concentrations of radiation-induced 2-alkylcyclobutanones were plotted versus the amounts of parent fatty acids, the linear response with the radiation dose is demonstrated (Fig. 4). 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 over. Therefore, 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone and 2-(5′-tetradecenyl)cyclobutanone in peanuts were notably detected at doses of 1 kGy and higher and were

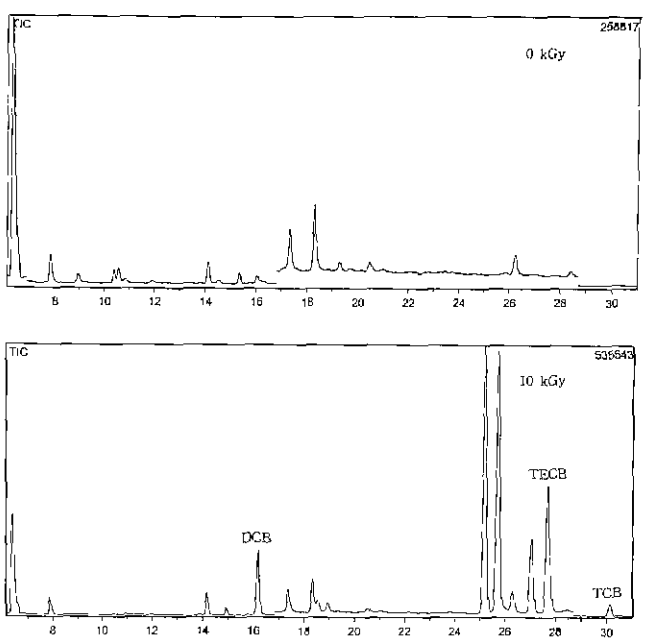

Fig. 3. Gas chromatograms of radiation-induced 2-alkylcyclobutanones of unirradiated and 10 kGy irradiated peanuts in SIM mode. DCB: 2-dodecylcyclobutanone, TECB: 2-(5′-tetradecenyl)cyclobutanone, TCB: 2-tetradecylcyclobutanone.

Table 2. Concentrations of radiation-induced 2-alkylcyclobutanones in peanuts (μg/g fat)

Irradiation dose (kGy)	Palmitic acid	Oleic acid	Stearic acid
	2-Dodecylcyclobutanone	2-(5'-Tetradecenyl)-cyclobutanone	2-Tetradecylcyclobutanone
0	-	-	-
0.5	0.025 (±0.006)[1)]	0.024 (±0.010)	t[2)]
1	0.060 (±0.010)	0.072 (±0.035)	0.010 (±0.005)
3	0.130 (±0.019)	0.664 (±0.153)	0.040 (±0.012)
5	0.305 (±0.022)	1.764 (±0.231)	0.081 (±0.018)
10	0.806 (±0.036)	5.796 (±0.382)	0.265 (±0.032)

[1)]Mean±standard deviation
[2)]Trace

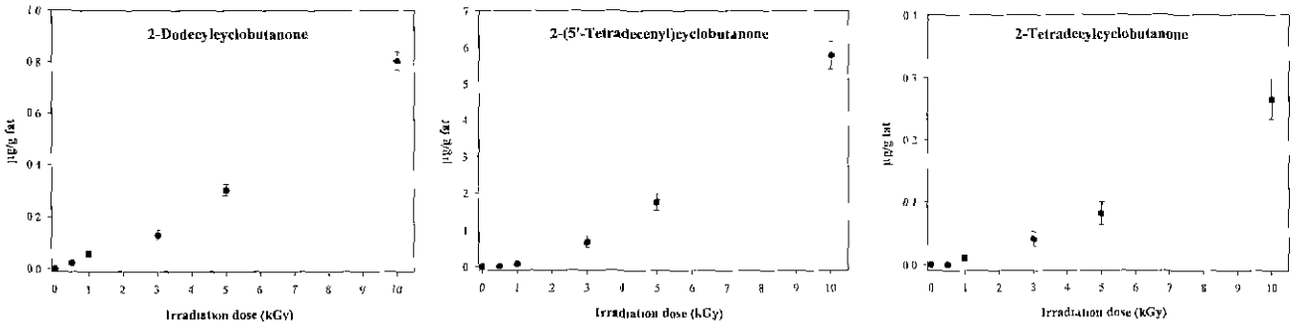

Fig. 4. Effect of radiation doses on the radiation-induced 2-alkylcyclobutanones of peanuts.

not confirmed in unirradiated samples.

Finally, hydrocarbons and 2-alkylcyclobutanones formed during the irradiation of peanuts proportionally increased with the irradiation dose. Hydrocarbons could be detected at doses of 0.5 kGy and over, 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, and one of 2-alkylcyclobutanones, 2-(5'-tetradecenyl)cyclobutanone had the highest concentration in the peanuts. These major hydrocarbons and 2-alkylcyclobutanone would be available for the detection of radiation in peanuts. These studies should be applied to a variety of foods since concentrations of radiation-induced hydrocarbons and 2-alkylcyclobutanones in various foods are different.

ACKNOWLEDGEMENTS

This work was supported by the Research Project Fund of Nuclear Energy Activities from the Ministry of Science and Technology, Korea.

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(Received September 27, 1999)