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Analysis of Radiolytic Products of Lipid in Irradiated Dried Squids (*Todarodes pacificus*)

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ABSTRACT

The lipid portion of dried squids (*Todarodes pacificus*) was extracted, and its hydrocarbons and 2-alkylcyclobutanones were separated using a florisil column. Both compounds were identified by gas chromatography and mass spectrometry and used to investigate the production of radiation-induced hydrocarbons and 2-alkylcyclobutanones. Concentrations of the hydrocarbons and 2-alkylcyclobutanones increased linearly with the radiation dosage. The major hydrocarbons in the irradiated dried squids were pentadecane and 1-tetradecene, which originated from palmitic acid. The amount of pentadecane was the highest among the radiation-induced hydrocarbons in the dried squids. The major 2-alkylcyclobutanone in the irradiated dried squids was 2-dodecylcyclobutanone, which was formed from the large amount of palmitic acid. 2-Tetradecylcyclobutanone, which may be produced from stearic acid in sample lipids, was also detected. Radiation-induced hydrocarbons and 2-alkylcyclobutanones were detected at $\geq$0.5 kGy. These compounds were not detected in dried squids that were not irradiated. Radiation-induced hydrocarbons can be used as a detection marker for irradiated dried squids; however, the amount of 2-alkylcyclobutanones produced was not enough to be used as a marker. Radiolytic products of lipids, such as hydrocarbons or 2-alkylcyclobutanones, can be used to monitor food safety for consumers, ensuring proper irradiation labeling in foods and quarantine treatment in international trade.

Among the existing technologies for food preservation, irradiation is recognized as a safe and effective method for a range of specific applications *(3).* An ever-increasing number of countries have approved the irradiation of a long and growing list of different food items, such as spices and grains, fruit and vegetables, meats, poultry, and seafood. If applied properly, irradiation can be an effective way to reduce the incidence of foodborne disease and to treat a variety of potential problems in our food supply *(9, 16).* To facilitate international trade, irradiated food can be evaluated by analytical methods to deterine whether food has been treated with radiation.

Although properly irradiated food is safe and wholesome, consumers should be able to make their own choices between irradiated and nonirradiated food. Thus, adequate product labeling is important *(6).* To check for compliance with existing regulations, suitable methods that can be used to reliably authenticate irradiated foods are needed. A number of methods for detecting irradiated food have been investigated extensively under the auspices of international organizations such as the International Atomic Energy Agency, the United Nations Food and Agriculture Organization, and the World Health Organization. Chemical methods can be used to detect hydrocarbons and 2-alkylcyclobutanones, which are formed from the lipid part of food exposed to radiation. A European standard (EN 1784) based on the formation of radiolytic hydrocarbons has been devised and involves gas chromatography (GC) *(8, 21)* for detecting lipid-derived 2-alkylcyclobutanones after irradiation *(15, 24).* The method is based on the mass spectrometric (MS) detection of hydrocarbons and 2-alkylcyclobutanones after GC separation *(6).*

The radiolysis of fatty acids ($C_{m:n}$, where m is the number of carbon atoms and n is the number of double bonds) leads to the formation of two groups of long-chain hydrocarbons. The first group comprises hydrocarbons with one less carbon than the original fatty acid ($C_{m-1:n}$), and the second group comprises hydrocarbons with two less carbon atoms than the original fatty acid and one additional double bond at position 1 (first carbon storm) ($C_{m-2:n+1}$) created by rupture of the side chain in the α and β positions with respect to the carbonyl group *(1, 4).* The other group is the 2-alkylcylobutanones with a $C_{m-4:n}$ alkyl chain *(13).*

In several investigations, the most appropriate markers appeared to be 1-tetradecene, pentadecane, 1-hexadecene, 1,7-hexadecadiene and heptadecane, 8-heptadecene from the precursor fatty acids, and palmitic, stearic, and oleic acids, which increase with increasing radiation dose *(19, 23).* The precursor fatty acids and palmitic, stearic, and oleic acids give rise to 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, and 2-(5′-tetradecenyl)cyclobutanone, which have been detected in irradiated chicken *(2, 5, 14).*

Because hydrocarbons and 2-alkylcyclobutanones have been used as confirmation markers of irradiation, the development of faster extraction methods for this group of compounds would be of value for routine detection of irradiated foods *(12, 20).* More studies on chemical reactions induced by irradiation in different media are being undertaken. Research subjects include losses of reactions under

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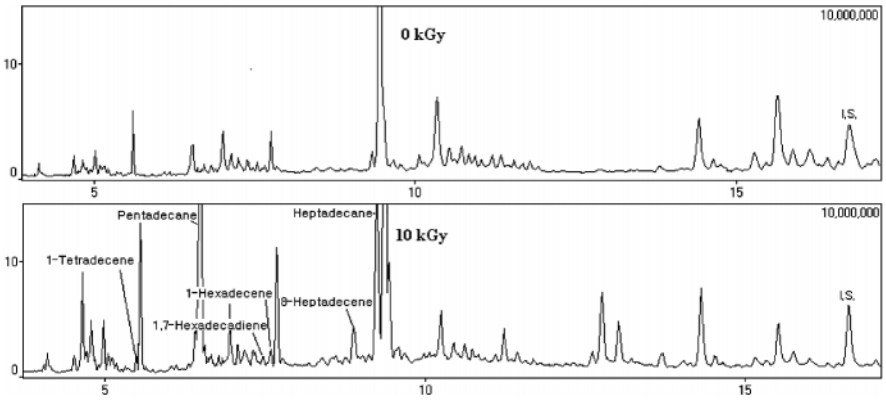

FIGURE 1. *Chromatograms for the hydrocarbons from nonirradiated and irradiated (10 kGy) dried squids.*

various conditions of irradiation and storage, combination methods (radiation plus heat, water activity, pH, packaging atmosphere, etc.), the suitability of various packaging materials for irradiated foods, and the effect of radiation conditions on the sensory quality of foodstuffs *(7).*

The present study was conducted to identify and quantify hydrocarbons and 2-alkylcyclobutanones formed in irradiated dried squids. This information was used to determine whether dried squids were irradiated and to provide labeling information to enhance consumer confidence.

MATERIALS AND METHODS

Materials. Dried squids (*Todarodes pacificus*) were purchased from a local supermarket and irradiated at 0.5, 1, 3, 5, and 10 kGy at 12 ± 1°C using a ^{60}Co γ-irradiator at the Korea Energy Research Institute. The dose rate was 2.5 kGy/h, with a dose rate error of ±0.02 kGy. The irradiated sample and nonirradiated control were stored at −18°C until used for the experiments.

Regents. Standards for hydrocarbons and 2-alkylcyclobutanones were purchased from TeLA Co. (Berlin, Germany). High-pressure liquid chromatography grade solvents (*n*-hexane and diethyl ether), were purchased from Fisher Scientific (Pittsburg, Pa.) and further distilled before use with a spiral packed double-distilling apparatus. The florisil (60 to 100 mesh, Fisher) was heated overnight at 550°C to remove possible contaminants. Before use, the florisil was heated for at least 5 h at 130°C in a dry oven and then cooled in a desiccator. Cooling was followed by the addition of 3% water (wt/wt) to separate the hydrocarbons or 20% water (wt/wt) to separate the 2-alkylcyclobutanones. Solutions were shaken for at least 20 min and then stored at room temperature for 10 to 12 h.

Extraction of fat from dried squid. A Soxtec HT2 (Foss, France) apparatus was used to extract the fat from the sample. Five grams each of homogenized dried squid and anhydrous sodium sulfate were mixed, the mixture was weighed into an extraction thimble, and the thimble was inserted into the extraction unit. The soluble lipids were extracted automatically into the solvent in two stages (rinsing and boiling), follwed by recovery of the solvent. The sample was boiled for 60 min at 115°C and then rinsed for 15 min. The extracted fat was placed in vials with nitrogen and stored at −20°C.

Separation of hydrocarbons. Separation of hydrocarbons was performed on florisil resin, according to the method of Kim et al. *(11).* Twenty-five grams of deactivated florisil was packaged into a glass column (200 by 20 mm). A 1-cm layer of anhydrous sodium sulfate was added to the top of the column. One gram of extracted fat was mixed with an internal standard (1 ml of *n*-eicosane, 4 μg/ml in hexane), applied to the column, and eluted with 80 ml of *n*-hexane at a flow rate of 3 ml/min. The hexane eluent was concentrated to a volume of 2 ml in a rotary vacuum evaporator and further concentrated to a volume of 0.5 ml using nitrogen.

Separation of 2-alkylcyclobutanones. The florisil column was prepared as previously described. Extracted fat (0.2 g) was mixed with 1 ml of 2-cyclohexylcyclohexanone (1 μg/ml in hexane) as the internal standard, and the mixture was applied to the column and eluted with 150 ml of *n*-hexane followed by 120 ml of diethylether-hexane (2:98, vol/vol) at a flow rate of 3 ml/min. The latter hexane fraction was concentrated to a volume of 2 ml in a rotary vacuum evaporator and further concentrated to a volume of 0.2 ml using nitrogen.

GC-MS analysis of hydrocarbons. The GC-MS analyses were carried out on a Shimadzu GC/MS QP-5050 spectrometer (Kyoto, Japan) in the EI mode employing a DB-5 column (inside diameter 30 m by 0.32 mm, 0.25-μm film thickness; J & W Scientific, Folsom, Calif.). The ionization voltage was set at 70 eV,

TABLE 1. *Concentrations of radiation-induced hydrocarbons in dried squids*[a]

Irradiation dose (kGy)	Palmitic acid		Stearic acid		Oleic acid	
	Pentadecane	1-Tetradecene	Heptadecane	1-Hexadecene	8-Heptadecene	1,7-Hexadecadiene
0	0	0	0	0	0	0
0.5	1.43 ± 0.36	0.136 ± 0.007	2.209 ± 0.098	0.367 ± 0.007	0.327 ± 0.061	0.243 ± 0.062
1	1.693 ± 0.175	0.151 ± 0.049	2.502 ± 0.065	0.425 ± 0.059	0.592 ± 0.064	0.304 ± 0.083
3	6.601 ± 0.098	0.27 ± 0.021	6.113 ± 0.050	0.722 ± 0.001	1.69 ± 0.027	0.538 ± 0.103
5	7.304 ± 1.148	0.351 ± 0.048	7.681 ± 1.054	1.43 ± 0.109	2.792 ± 0.220	0.777 ± 0.105
10	9.809 ± 1.250	0.397 ± 0.024	9.83 ± 0.565	1.576 ± 0.095	3.596 ± 0.408	0.813 ± 0.136

[a] Values (in μg/g fat) are means ± standard deviations.

TABLE 2. *Concentrations of radiation-induced 2-alkylcyclobutanones from dried squids*[a]

Irradiation dose (kGy)	Palmitic acid (2-dodecylcyclobutanone)	Stearic acid (2-tetradecylcyclobutanone)
0	0	0
0.5	0.007 ± 0.001	0.001 ± 0.001
1	0.008 ± 0.004	0.002 ± 0.000
3	0.019 ± 0.003	0.005 ± 0.001
5	0.024 ± 0.004	0.006 ± 0.001
10	0.029 ± 0.003	0.009 ± 0.004

[a] Values (in μg/g fat) are means ± standard deviations.

and the injector and ion source temperatures were kept at 250°C. The oven temperature was programmed as follows: 60 to 170°C at 25°C/min, to 205°C at 2°C/min, and finally to 270°C at 10°C/min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. A 1-μl sample was injected in the splitless mode for 2 min and then in a split mode (20:1). The hydrocarbons were identified by comparing the retention time and mass spectrum of peaks, as shown in the total ion chromatogram, with that of an authentic hydrocarbon standard. The concentration of each hydrocarbon in the fat was determined using *n*-eicosane (4 μg/ml) as an internal standard.

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

The 2-alkylcyclobutanones were analyzed by GC-MS using the selected ion monitoring mode (SIM). The SIM for the 2-alkylcyclobutanones was set for 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone using the *m*/*z* 98 and *m*/*z* 112 ions and for 2-(5′-tetradecenyl)cyclobutanone using the *m*/*z* 67, *m*/*z* 98, and *m*/*z* 109 ions. The peaks produced and the retention times and ion ratios were compared with those of the 2-alkylcyclobutanone standards. Mass spectra of the 2-alkylcyclobutanones were confirmed by GC-MS in the full scan mode. The concentration of each 2-alkylcyclobutanone in the fat was determined using 2-cyclohexylcyclohexanone (1 μg/ml) as an internal standard.

RESULTS AND DISCUSSION

Radiation-induced hydrocarbons from irradiated dried squids. The major fatty acids in the dried squids were docosahexaenoic acid (32.8 to 41.1%), palmitic acid (22.3 to 24.0%), eicosapentaenoic acid (14.3 to 14.7%), stearic acid (5.3 to 8.7%), and oleic acid (1.6 to 4.0%) *(17).* According to the fatty acid composition, the major radiation-induced hydrocarbons were expected to be from docosahexaenoic acid, palmitic acid, and eicosapentaenoic acid. However, the radiation-induced hydrocarbons from docosahexaenoic acid and eicosapentaenoic acid could not be confirmed because there are no hydrocarbon standards for these fatty acids. Therefore, we examined the radiolytic hydrocarbons of palmitic, stearic, and oleic acids and compared their amounts.

When these fatty acids are irradiated, two types of hydrocarbons are formed as a result of the loss of the carboxyl group. The types are those hydrocarbons that have one or two less carbon atoms than their parent fatty acids (C_{n-1}) or (C_{n-2}) with an additional double bond at the C_1 position *(10, 22).* 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, and 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) from oleic acid were formed and confirmed.

Figure 1 shows the GC-MS chromatograms of the radiation-induced hydrocarbons of the nonirradiated and irradiated (10 kGy) dried squids. The results agreed with the irradiation lipid degradation patterns proposed by Nawar *(18),* and a number of radiolytic hydrocarbons were detected. The data from the quantitative analysis for the radiation-induced hydrocarbons of the dried squid samples are shown in Table 1; the concentrations of the hydrocarbons increased with irradiation dose.

Pentadecane and 1-tetradecene were formed from palmitic acid, and pentadecane was detected in relatively large amounts compared with 1-tetradecene at all radiation doses. Because of the composition of fatty acids in the dried squid, these hydrocarbons were present in higher concentrations than were the other radiolytic hydrocarbons. Heptadecane and 1-hexadecene were formed from stearic acid, and heptadecane was detected in relatively large amounts compared with 1-hexadecene. Amounts of these hydrocarbons in-

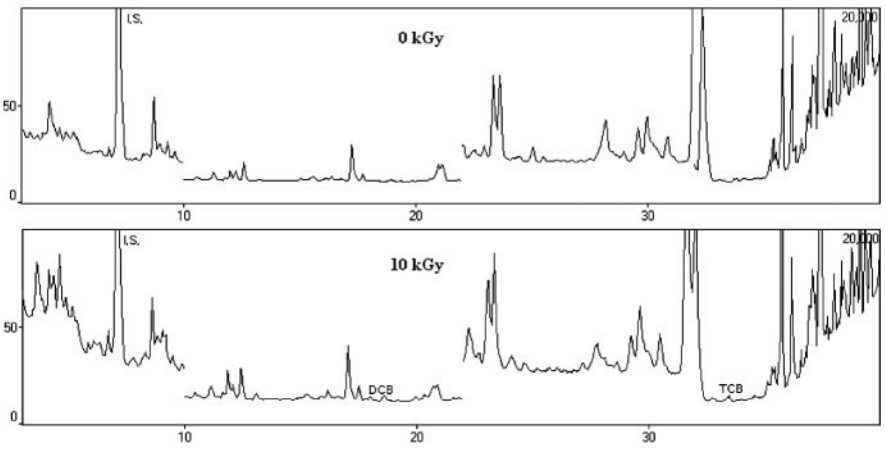

FIGURE 2. *Chromatograms of 2-alkylcyclobutanones from nonirradiated and irradiated (10 kGy) dried squids. DCB, 2-dodecylcyclobutanone; TCB, 2-tetradecylcyclobutanone.*

creased with increased radiation dose. 8-Heptadecene and 1,7-hexadecadiene from oleic acid were detected, with the same radiolysis pattern; the C_{n-1} hydrocarbons were formed in higher concentrations than were the $C_{n-2:1}$ hydrocarbons. Similar to other hydrocarbons, the concentration of 8-heptadecene and 1,7-hexadecadiene in irradiated dried squids increased linearly with the radiation dose.

The major hydrocarbons formed in dried squids, based on the composition of fatty acids and their degradation mechanisms, were pentadecane, 1-tetradecene, heptadecane, 1-hexadecene, 8-heptadecene, and 1,7-hexadecadiene. The C_{n-1} hydrocarbons (pentadecane, heptadecane, and 1-hexadecene) were detected in higher concentrations than were the $C_{n-2:1}$ hydrocarbons (1-tetradecene, 8-heptadecene, and 1,7-hexadecene).

Radiation-induced hydrocarbons were detected at radiation doses of ≥0.5 kGy but were not detected in nonirradiated dried squids.

Radiation-induced 2-alkylcyclobutanones from irradiated dried squids. The 2-alkylcyclobutaonoes are cyclic compounds that have the same number of carbon atoms as their precursor fatty acids with the addition of an alkyl group. When palmitic, stearic, and oleic acids are exposed to radiation, 2-dodecylcyclobutnone, 2-tetradecylcylobutanone, and 2-(5′-tetradecenyl)cyclobutanone, respectively, are formed. These compounds are cyclic compounds formed by the loss of an electron from the oxygen atom on the carbonyl of a fatty acid or triglyceride, followed by a rearrangement process to produce 2-alkylcyclobutanones specific to their parent fatty acids *(13).*

Based on this process, the major fatty acids in irradiated dried squids can be degraded to the corresponding 2-alkylcyclobutanones (Table 2). Figure 2 shows the chromatograms of the 2-alkylcyclobutanones of the nonirradiated and irradiated (10 kGy) dried squids. The concentrations of 2-alkylcyclobutanones increased with radiation dose as a marker of radiation-induced hydrocarbons, as confirmed by LeTellier and Nawar *(13).*

Of the 2-alkylcyclobutanones, 2-dodecylcyclobutanone was formed from palmitic acid and had the highest concentration per amount of precursor fatty acid in the dried squids. The concentration of 2-tetradecylcyclobutanone was higher than that of the 2-(5′-tetradecenyl)cyclobutanone because more stearic acid than oleic acid was present in the squids. However 2-(5′-tetradecenyl)cyclobutanone was not of value as an irradiation marker because of its relatively low levels. Therefore, the radiolytic 2-alkylcyclobutanones of palmitic and stearic acids were determined, and the amount formed was compared. These results were similar to those found by McMurray et al. *(15)* in a study on irradiation markers in irradiated prawns.

When the concentrations of radiation-induced 2-alkylcyclobutanones were plotted against the amount of fats, a linear correlation with the radiation dose was observed. Radiation-induced 2-alkylcyclobutanones 2-dodecylcyclobutanone and 2-tetradecyclcyclobutanone were detected at doses of ≥0.5 kGy but not in the nonirradiated samples.

The hydrocarbons and 2-alkylcyclobutanones formed by irradiation of dried squids increased in proportion to the radiation dose and could be detected at doses of ≥0.5 kGy. The major hydrocarbons detected were pentadecane, 1-tetradecene, heptadecane, and 1-hexadecene. One of the 2-alkylcyclobutanones, 2-dodecylcyclobutanone had the highest concentration in the irradiated dried squids. These major hydrocarbons and 2-alkylcyclobutanones could be used for the detection of irradiated dried squids. The results of this study can be applied to a variety of foods, because concentrations of the radiation-induced hydrocarbons and 2-alkylcyclobutanones differ in various foods.

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