Hydrocarbons Detected in Irradiated Shell Eggs During Storage

Keum Taek Hwang*

Department of Food Science and Human Nutrition and Institute for Molecular Biology and Genetics, Chonbuk National University, Chonju, Chonbuk, 561-756, Korea

ABSTRACT: Hydrocarbons produced by γ-radiation of shell eggs were analyzed to determine how irradiation affects their production. Shell eggs were nonirradiated or irradiated at 0.5, 1, and 3 kGy and the stored at 5°C for 8 wk or at 30°C for 10 d. Hydrocarbons were determined by a sequential procedure of lipid extraction by hexane, Florisil column chromatography, and gas chromatography. Hydrocarbons $C_{15:0}$, $C_{14:1}$, $C_{17:0}$, $C_{16:1}$, $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ were detected in shell eggs irradiated at 0.5 kGy or higher, but not in nonirradiated ones except $C_{15:0}$ and $C_{17:0}$. Storage of nonirradiated or irradiated eggs had little effect on detection levels of hydrocarbons. The detection levels in all the samples irradiated at 1 and 3 kGy were in the order of $C_{16:2} + C_{17:1}$, $C_{15:0} + C_{14:1}$, $C_{17:2} + C_{16:3}$, and $C_{17:0} + C_{16:1}$ from the highest to the lowest.

Paper no. J9133 in *JAOCS 76*, 1183–1187 (October 1999).

KEY WORDS: Hydrocarbon, irradiation detection, shell egg, storage.

Fresh shell eggs may contain *Salmonella enterititis*, which causes salmonellosis. One way to control the microorganism is irradiation. Some countries permit irradiation for eggs or egg products: in Croatia for frozen eggs, frozen egg products, and egg powder up to 3 kGy; in France for egg products up to 4 kGy; in Mexico for dehydrated eggs up to 5 kGy; in South Africa for whole and broken eggs and egg powder up to 10 kGy; and in Yugoslavia for egg powder up to 10 kGy (1). A petition was filed in the United States in 1998 proposing that the food additive regulations be amended to provide for the safe use of ionizing radiation for reduction of *Salmonella* in fresh shell eggs (2).

In spite of the benefits from food irradiation, consumers are concerned about how their foods were handled. Therefore, a method is needed to establish whether shell eggs have been postirradiated. One promising method of detecting the postirradiation of lipid-containing foods is the analysis of hydrocarbons, which are produced from lipids by irradiation (3–5). Two types of hydrocarbons are predominantly produced by irradiation of fatty acids in lipids: one is the hydrocarbon that has one carbon less than the parent fatty acid (C_{n-1}), and the other has two carbons less and an additional double bond at position 1 (1-C_{n-2}) (6). Oleic acid is the most abundant lipid found in eggs (35%), followed by palmitic acid (22%), linoleic acid (11%), and stearic acid (8%) (7). Therefore, 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) from oleic acid, *n*-pentadecane ($C_{15:0}$) and 1-tetradecene ($C_{14:1}$) from palmitic acid, 6,9-heptadecadiene ($C_{17:2}$) and 1,7,10-hexadecatriene ($C_{16:3}$) from linoleic acid, and *n*-heptadecane ($C_{17:0}$) and 1-hexadecene ($C_{16:1}$) from stearic acid are expected to be detectable in irradiated eggs. Determination of the presence of hydrocarbons in irradiated liquid eggs and products using irradiated liquid eggs has been studied for identifying the use of irradiated eggs (6,8,9).

In this study, hydrocarbons in irradiated shell eggs were analyzed to determine which hydrocarbons were detected exclusively in irradiated ones so that they could be used as markers for identifying postirradiation of shell eggs. The effects of storage at refrigerated or ambient temperatures on changes in detection of hydrocarbons in nonirradiated and irradiated eggs were also determined.

MATERIALS AND METHODS

Materials and reagents. Shell eggs produced from five different egg producers were purchased from a local market in Chonju, Korea. Sodium sulfate was analytical grade (Pure Chemicals Co., Ltd., Osaka, Japan). *n*-Hexane and *iso*-octane were from Fisher Scientific (Fair Lawn, NJ). The hydrocarbon standards were purchased from Sigma Chemical Co. (St. Louis, MO) or obtained from the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (Berlin, Germany).

Irradiation. Shell eggs were irradiated at 0.5, 1, and 3 kGy using a ^{60}Co γ-radiation source at the Korea Atomic Energy Research Institute (Daejon, Korea).

Storage. Nonirradiated or irradiated shell eggs were stored at 5°C for 8 wk or 30°C for 10 d.

Fat extraction. Fat extraction, separation of hydrocarbons, and gas chromatography (GC) analysis followed the previously reported methods (6,10,11) with minor modifications. Only egg yolk separated from an egg was used for fat extraction. Egg yolk was blended with 80 g anhydrous sodium sulfate (previously heated to 650°C for 5 h) and 200 mL *n*-

*To whom correspondence should be addressed at Department of Food Science and Human Nutrition, College of Home Economics, Chonbuk National University, Chonju, Chonbuk, 561-756, Korea.
E-mail: kthwang@moak.chonbuk.ac.kr

hexane using a homogenizer (M133/1280-0; Biospec Products, Inc., Bartlesville, OK) at full speed for 3 min. The mixture was transferred to Teflon centrifuge tubes (Nalge Co., Rochester, NY) and centrifuged at 2240 × *g* for 20 min using a centrifuge (Union 55R; Hanil Co., Seoul, Korea). The supernatant was collected in a round-bottomed flask. The solvent was evaporated using an Eyela rotary vacuum evaporator (N-N; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 35°C, connected to an Eyela aspirator (A-3S; Tokyo Rikakikai Co., Ltd.). The extracted fat was flushed with nitrogen and stored at 4°C until separated by Florisil column chromatography.

Separation of hydrocarbons by Florisil column chromatography. Hydrocarbons were separated by Florisil column chromatography as previously reported (12).

GC analysis of hydrocarbons. The isolated hydrocarbons were analyzed on a Hewlett-Packard 6980 Series gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with a flame-ionization detector (FID) and a split/splitless injector. Helium was used as the carrier gas at the flow rate of 2.6 mL/min. The column was a DB-5 [(5%-phenyl)-methylpolysiloxane] 0.25 mm i.d. × 30 m column with 0.25 µm film thickness (J&W Scientific, Folsom, CA). The column temperature was initially 50°C for 2 min, and was programmed to rise at 10°C/min to 130°C, 3°C/min to 160°C, and 5°C/min to 200°C, where it was held for 2 min, and 25°C/min to 250°C with a final hold of 5 min. The injector and detector temperatures were 200 and 250°C, respectively. The injector was initially set in splitless mode, pulsed for 2 min and purged at 1.9 min. One microliter of the hexane solution with hydrocarbons was injected. Peaks were identified by a GC–mass spectrometry. All experiments were in triplicate, unless otherwise specified. Data were analyzed with Microsoft Excel 97 (Microsoft Corp., Redmond, WA, 1997) by using linear regressions of hydrocarbons against dose and analysis of variance (ANOVA) among storage conditions.

RESULTS AND DISCUSSION

Hydrocarbons produced from shell eggs by irradiation. Hydrocarbons $C_{15:0}$, $C_{14:1}$, $C_{17:0}$, $C_{16:1}$, $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$, which are expected from the major fatty acids in eggs, were detected in irradiated shell eggs with good resolution. Irradiated samples were clearly distinguishable from nonirradiated samples (Fig. 1). Among the hydrocarbons, $C_{15:0}$ and $C_{17:0}$ were detected in nonirradiated eggs, but $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ were not (Table 1). The eight hydrocarbons were all detected in shell eggs irradiated at 0.5 kGy or higher. They increased with dose, and correlation coefficients (r) of the linear regressions for the hydrocarbons vs. dose were >0.98. The highest detected peaks in each of the irradiated eggs were for $C_{16:2}$.

Hydrocarbons in shell eggs during storage. When nonirradiated or irradiated shell eggs were stored at 30°C for 10 d or at 5°C for 8 wk, the patterns of the detected hydrocarbons were little different from those of day 0 (Table 1). ANOVA tests among the five storage conditions on each hydrocarbon at each dose showed $P > 0.05$ except for $C_{15:0}$ at 0, 0.5, 1, and 3 kGy, and $C_{17:0}$ at 0, 0.5, and 1 kGy. How storage conditions affect detection levels of $C_{15:0}$ and $C_{17:0}$ is uncertain. Since they were detected in all the nonirradiated samples, when we consider only the hydrocarbons induced from egg lipids exclusively by irradiation, it could be suggested that the hydrocarbons in eggs are not produced nor degraded during storage at ambient or refrigerated temperatures where eggs are usually handled.

Schulzki *et al.* (8) reported $C_{16:2}$ and $C_{17:2}$ were detected at concentations of about 2.5 and 1 µg/g fat, respectively, in liquid egg irradiated at 3 kGy. These detected amounts are a little higher than in this study. They also detected $C_{14:1}$, $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ in sponge cake prepared with irradiated liquid eggs, but not in that with nonirradiated eggs. It could therefore be concluded that irradiated eggs which are in the shell, then broken, stored at ambient or refrigerated temperatures, or prepared for a product with heat treatment could be identified by detection of hydrocarbons.

Pattern of hydrocarbons detected in irradiated eggs. Hydrocarbons $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ could be markers for identifying postirradiation of shell eggs. However, detection of one or two kinds of hydrocarbons, which may result from contamination during the analytical process,

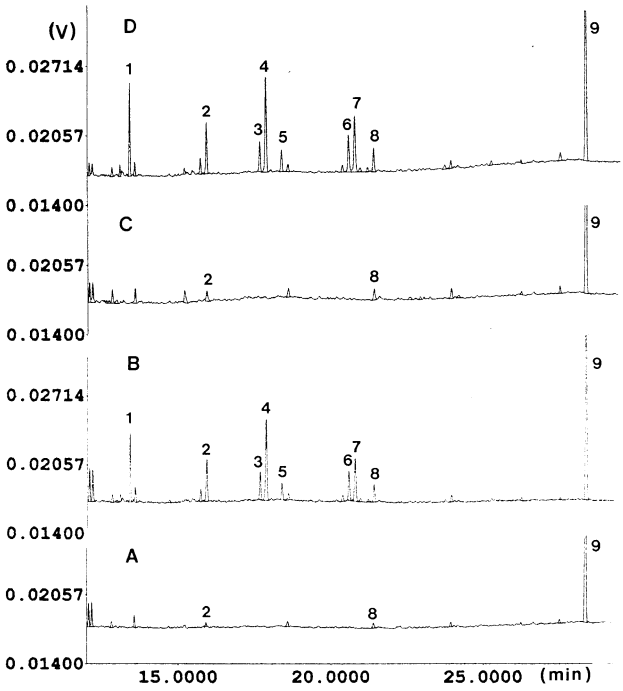

FIG. 1. Gas chromatograms of the hydrocarbons in shell eggs. (A) Nonirradiated (day 0); (B) irradiated at 3 kGy (day 0); (C) nonirradiated and stored at 30°C for 10 d; and (D) irradiated at 3 kGy and stored at 30°C for 10 d. Peak (1) 14:1; (2) 15:0; (3) 16:3; (4) 16:2; (5) 16:1; (6) 17:2; (7) 17:1; (8) 17:0; and (9) 20:0 (internal standard). Column: DB-5 [J&W Scientific, Folsom, CA; (5%-phenyl)-methylpolysiloxane]; 0.25 mm i.d. × 30 m, 0.25 µm. Oven: 50°C for 2 min; 10°C/min to 130°C; 3°C/min to 160°C; 5°C/min to 200°C with a hold of 2 min; and 25°C/min to 250°C with a hold of 5 min. Injector: split/splitless, 200°C. Detector: flame-ionization, 250°C.

TABLE 1
Hydrocarbons Detected in Irradiated Shell Eggs During Storage (μg/g fat)[a]

Day (Temperature)	Hydrocarbon	Dose (kGy) 0	0.5	1	3
Day 0	15:0	0.08 ± 0.038	0.14 ± 0.021	0.23 ± 0.009	0.72 ± 0.036
	14:1	0	0.15 ± 0.014	0.31 ± 0.008	1.00 ± 0.059
	17:0	0.11 ± 0.031	0.13 ± 0.011	0.17 ± 0.013	0.36 ± 0.049
	16:1	0	0.05 ± 0.014	0.10 ± 0.012	0.35 ± 0.040
	17:1	0	0.12 ± 0.008	0.28 ± 0.036	1.00 ± 0.133
	16:2	0	0.24 ± 0.017	0.52 ± 0.049	1.62 ± 0.139
	17:2	0	0.08 ± 0.001	0.17 ± 0.025	0.65 ± 0.118
	16:3	0	0.07 ± 0.023	0.12 ± 0.030	0.50 ± 0.069
Day 5 (30°C)	15:0	0.12 ± 0.049	0.18 ± 0.027	0.25 ± 0.032	0.83 ± 0.039
	14:1	0	0.18 ± 0.007	0.46 ± 0.202	1.14 ± 0.149
	17:0	0.14 ± 0.031	0.18 ± 0.030	0.18 ± 0.013	0.41 ± 0.085
	16:1	0	0.06 ± 0.003	0.12 ± 0.017	0.39 ± 0.050
	17:1	0	0.13 ± 0.016	0.31 ± 0.031	1.20 ± 0.019
	16:2	0	0.29 ± 0.023	0.56 ± 0.055	1.79 ± 0.177
	17:2	0	0.11 ± 0.009	0.21 ± 0.020	0.79 ± 0.054
	16:3	0	0.08 ± 0.027	0.16 ± 0.035	0.59 ± 0.041
Day 10 (30 °C)	15:0	0.13 ± 0.052	0.21 ± 0.039	0.34 ± 0.017	0.87 ± 0.097
	14:1	0	0.15 ± 0.023	0.38 ± 0.027	1.22 ± 0.089
	17:0	0.17 ± 0.026	0.18 ± 0.014	0.26 ± 0.050	0.42 ± 0.011
	16:1	0	0.04 ± 0.010	0.12 ± 0.024	0.37 ± 0.006
	17:1	0	0.12 ± 0.014	0.34 ± 0.014	1.20 ± 0.085
	16:2	0	0.26 ± 0.010	0.61 ± 0.038	1.89 ± 0.235
	17:2	0	0.08 ± 0.005	0.22 ± 0.027	0.77 ± 0.060
	16:3	0	0.07 ± 0.002	0.18 ± 0.036	0.56 ± 0.060
Day 28 (5°C)	15:0	0.21 ± 0.063	0.28 ± 0.153	0.33 ± 0.034	0.75 ± 0.036
	14:1	0	0.18 ± 0.070	0.39 ± 0.029	1.08 ± 0.148
	17:0	0.22 ± 0.027	0.26 ± 0.064	0.26 ± 0.007	0.36 ± 0.017
	16:1	0	0.09 ± 0.040	0.12 ± 0.021	0.40 ± 0.046
	17:1	0	0.14 ± 0.055	0.31 ± 0.028	1.13 ± 0.086
	16:2	0	0.29 ± 0.103	0.61 ± 0.079	1.85 ± 0.262
	17:2	0	0.10 ± 0.045	0.18 ± 0.010	0.73 ± 0.053
	16:3	0	0.08 ± 0.014	0.17 ± 0.007	0.54 ± 0.019
Day 56 (5°C)	15:0	0.09 ± 0.032	0.15 ± 0.024	0.29 ± 0.007	0.77 ± 0.013
	14:1	0	0.15 ± 0.016	0.38 ± 0.046	1.12 ± 0.010
	17:0	0.08 ± 0.008	0.12 ± 0.012	0.16 ± 0.029	0.35 ± 0.009
	16:1	0	0.06 ± 0.009	0.12 ± 0.055	0.40 ± 0.015
	17:1	0	0.11 ± 0.004	0.30 ± 0.041	1.13 ± 0.030
	16:2	0	0.25 ± 0.011	0.62 ± 0.112	1.91 ± 0.115
	17:2	0	0.06 ± 0.012	0.17 ± 0.012	0.78 ± 0.036
	16:3	0	0.07 ± 0.013	0.17 ± 0.013	0.56 ± 0.069

[a]Means ± standard deviations (n = 3).

may lead to a wrong judgment on irradiation. Therefore, multiple hydrocarbons should be used as markers. The composition of the detected hydrocarbons would also provide clearer information by comparison with fatty acid compositions of the samples. By analyzing the compositions of fatty acids in egg lipids and hydrocarbon pairs induced from the parent fatty acids at each dose, the hydrocarbon composition was found to be not exactly the same as the fatty acid; however, the higher the dose, the closer the hydrocarbon composition was to the fatty acid composition (Fig. 2). (Hydrocarbon composition data were the means of all the samples.) The reason the hydrocarbon composition differed more from the fatty acid composition at the lower doses than the higher ones was probably because $C_{15:0}$ and $C_{17:0}$ were also detected in nonirradiated samples. When the amounts of $C_{15:0}$ and $C_{17:0}$ detected in nonirradiated samples were subtracted from the values detected in the irradiated samples, the compositions between fatty acids and their corresponding hydrocarbons produced by irradiation appeared more similar (Fig. 3), suggesting the detection amounts of hydrocarbon pairs produced by irradiation of lipids may be highly predicted by the composition of the fatty acids in lipids.

Ratio of 1-C_{n-2} and C_{n-1}. Hydrocarbons 1-C_{n-2} derived from palmitic, stearic, and oleic acids were detected in higher quantities than C_{n-1} in irradiated eggs, while C_{n-1} derived from linoleic acid was higher than 1-C_{n-2} (Table 2). In egg triacylglycerols, about 5% of total palmitic acid, 14% of stearic acid, and 43% of oleic acid are in position *sn*-2, while about 90% of total linoleic acid is in position *sn*-2 (13). A certain factor might cause a fatty acid in position *sn*-2 to be more

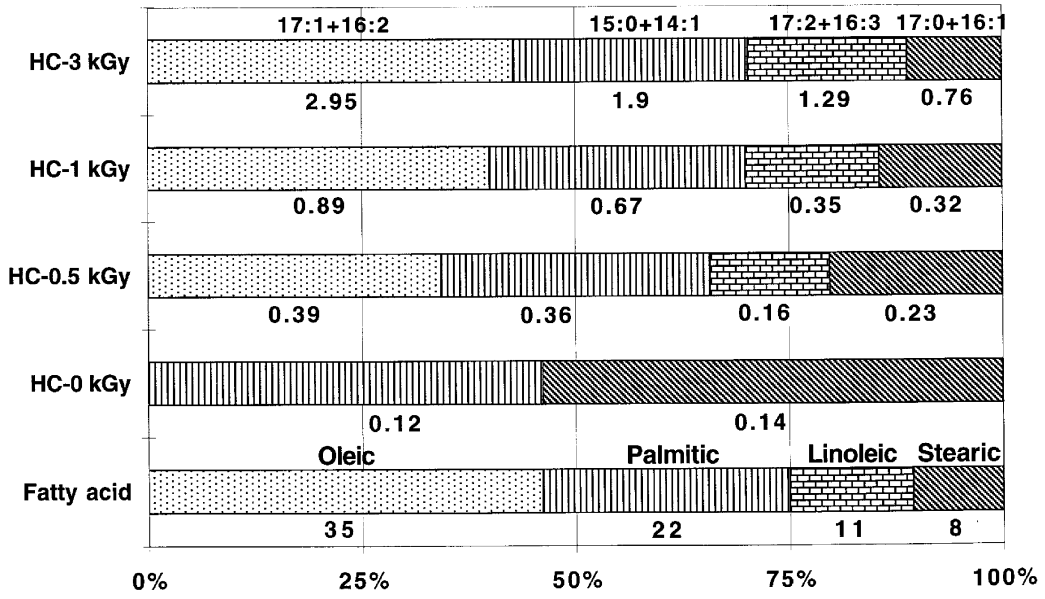

FIG. 2. Compositions of major fatty acids and hydrocarbon pairs induced from their parent fatty acids; hydrocarbon data were based on the actual detection values (n = 15). HC: hydrocarbon; units: fatty acids; percentage of total fatty acids: hydrocarbons, μg/g fat.

vulnerable to cleavage by irradiation at carbon number 1 than number 2 of the fatty acid. Schreiber *et al.* (14) also reported that $C_{14:1}/C_{15:0}$ and $C_{16:2}/C_{17:1}$ in irradiated pork were 0.4 and 2.6, respectively. Considering that 88% of total palmitic acid and 9% of oleic acid in pork lipids are in position *sn*-2 (13), it could be postulated that the ratio of 1-C_{n-2} and C_{n-1} detected in irradiated lipid foods might be affected to some extent by the positional distribution of fatty acids in triacylglycerols.

In conclusion, detection of hydrocarbons and comparison of their composition and fatty acid composition in egg lipids could clearly identify postirradiation of shell eggs, irradiated even at 0.5 kGy, regardless of their storage conditions.

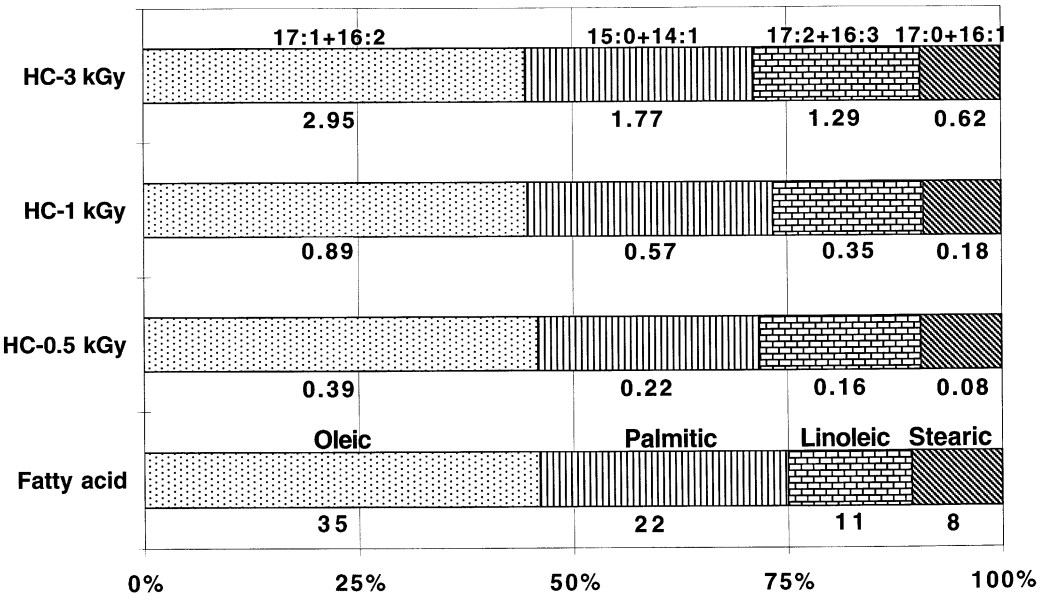

FIG. 3. Compositions of major fatty acids and hydrocarbon pairs induced from their parent fatty acids; hydrocarbon data were actual detection values minus values of nonirradiated samples (n = 15). For abbreviation and units see Figure 2.

TABLE 2
Ratio of Hydrocarbons 1-C_{n-2} and C_{n-1} Induced from Major Fatty Acids in Shell Eggs[a]

Parent fatty acid	Hydrocarbons (μg/g fat) and their ratio	Dose (kGy)		
		0.5	1	3
Palmitic acid	C14:1	0.16 ± 0.03	0.38 ± 0.09	1.11 ± 0.12
	C15:0	0.06 ± 0.08	0.16 ± 0.05	0.66 ± 0.09
		(0.19 ± 0.08)	(0.29 ± 0.05)	(0.79 ± 0.07)
	C14:1/C15:0	2.58	2.42	1.69
		(0.84)	(1.33)	(1.41)
Stearic acid	C16:1	0.06 ± 0.02	0.12 ± 0.03	0.39 ± 0.04
	C17:0	0.02 ± 0.04	0.06 ± 0.04	0.23 ± 0.06
		(0.17 ± 0.06)	(0.20 ± 0.05)	(0.38 ± 0.05)
	C16:1/C17:0	2.71	2.10	1.65
		(0.35)	(0.57)	(1.00)
Oleic acid	C16:2	0.27 ± 0.04	0.58 ± 0.07	1.81 ± 0.20
	C17:1	0.12 ± 0.02	0.31 ± 0.03	1.13 ± 0.10
	C16:2/C17:1	2.14	1.90	1.60
Linoleic acid	C16:3	0.07 ± 0.02	0.16 ± 0.03	0.55 ± 0.06
	C17:2	0.08 ± 0.03	0.19 ± 0.03	0.74 ± 0.08
	C16:3/C17:2	0.85	0.84	0.74

[a]Means ± standard deviations (n = 15); based on the values obtained after having subtracted the values of nonirradiated samples; values in parentheses: based on actual detection values.

REFERENCES

1. International Atomic Energy Agency, *Food and Environmental Protection Newsletter (Supplement) 1 (2)*:1–17 (1998).
2. Josephson, E.S., Filing of Food Additive Petition (Notice) in Food and Drug Administration, *Federal Register 63 (54)*:13675 (1998).
3. Champagne, J.R., and W.W. Nawar, The Volatile Components of Irradiated Beef and Pork Fats, *J. Food Sci. 34*:335–339 (1969).
4. Kavalam, J.R., and W.W. Nawar, Effects of Ionizing Radiation on Some Vegetable Fats, *J. Am. Oil Chem. Soc. 46*:387–390 (1969).
5. Dubravcic, M.F., and W.W. Nawar, Effects of High-Energy Radiation on the Lipids of Fish, *J. Agr. Food Chem. 17*:639–644 (1969).
6. Spiegelberg, A., G. Schulzki, N. Helle, K.W. Bögl, and G.A. Schreiber, Methods for Routine Control of Irradiated Food: Optimization of a Method for Detection of Radiation-Induced Hydrocarbons and Its Application to Various Foods, *Radiat. Phys. Chem. 43*:433–444 (1994).
7. Hands, E.S., Lipid Composition of Selected Foods, in *Bailey's Industrial Oil and Fat Products: Vol. 1. Edible Oil and Fat Products: General Applications*, 5th edn., edited by Y.H. Hui, John Wiley & Sons, Inc., New York, 1996, pp. 441–505.
8. Schulzki, G., A. Spiegelberg, K.W. Bögl, and G.A. Schreiber, Detection of Radiation-Induced Hydrocarbons in Baked Sponge Cake Prepared with Irradiated Liquid Egg, *Radiat. Phys. Chem. 46*:765–769 (1995).
9. Helle, N., G. Schulzki, B. Linke, A. Spiegelberg, K.W. Bögl, G.A. Schreiber, H.U. von Grabowski, J. Pfordt, U. Mauermann, S. Jülicher, C. Bischoff, N. Vater, and M. Heitmann, Identification of Irradiated Pasteurized Egg Products—A Combined Method to Use in Routine Control [German], *Z. Lebensm. Unters. Forsch. 197*:321–31 (1993).
10. Choi, C.R., and K.T. Hwang, Detection of Hydrocarbons in Irradiated and Roasted Sesame Seeds, *J. Am. Oil Chem. Soc. 74*: 469–472 (1997).
11. Park, J.Y., and K.T. Hwang, Hydrocarbons as Markers for Identifying Post-Irradiated Peanuts, *Ibid. 76*:125–129 (1999).
12. Hwang, K.T., Hydrocarbons Detected in Irradiated Pork, Bacon and Ham, *Food Res. Internat.* (in press).
13. Christie, W.W., The Positional Distributions of Fatty Acids in Triglycerides, in *Analysis of Oils and Fats*, edited by R.J. Hamilton and J.B. Rossell, Elsevier Applied Science, London, 1986, pp. 313-339.
14. Schreiber, G.A., G. Schulzki, A. Spiegelberg, N. Helle, and K.W. Bögl, Evaluation of a Gas Chromatographic Method to Identify Irradiated Chicken, Pork, and Beef by Detection of Volatile Hydrocarbons, *J. Assoc. Off. Anal. Chem. Int. 77*: 1202–1217 (1994).

[Received February 1, 1999; accepted March 29, 1999]