Hydrocarbons as Markers for Identifying Postirradiated Peanuts

J.Y. Park[a] and K.T. Hwang[b,*]

Departments of [a]Food Science and Technology and [b]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 peanuts were analyzed to determine the relationship between irradiation and production of hydrocarbons, and the use of hydrocarbons as markers for identifying postirradiated peanuts. Hydrocarbons in peanuts were determined by a sequential procedure of lipid extraction by hexane, Florisil column chromatography, and gas chromatography. Hydrocarbons $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ were easily detected in peanuts irradiated at 0.5 kGy or higher, but not in unirradiated ones. The hydrocarbons were minimally changed by roasting the irradiated peanuts and were not detected in unirradiated roasted peanuts.

Paper no. J8864 in *JAOCS 76,* 125–129 (January 1999).

KEY WORDS: Florisil column chromatography, gas chromatography, hydrocarbons, irradiation detection, peanuts.

Aspergillus flavus producing aflatoxins grows readily on peanuts (1); irradiation is one of the most effective methods to prevent mold growth. At least 10 kGy is needed to control the mold (2). South Africa permits peanuts to be irradiated up to 10 kGy for microbial control (3). Other countries have not specified irradiation permission on peanuts. European countries imported more than 670,000 metric tons of peanuts in 1996, and the United States and Canada imported 140,000 metric tons (4). Although irradiated foods must be properly labeled, there is the possibility of irradiating them without any notice of treatment on the shipment. It is therefore necessary to develop an appropriate method to detect irradiation of imported peanuts in order to apply domestic regulations concerning food irradiation.

It has been suggested that irradiated lipids and lipid-containing foods could be determined by analysis of hydrocarbons (5–9). Since peanuts contain large amounts of lipids, hydrocarbons can be detected in irradiated peanuts. Two types of hydrocarbons are predominantly produced by irradiation of fatty acids: a hydrocarbon which has one carbon less than the parent fatty acid (C_{n-1}) and a hydrocarbon that has two carbons less and an additional double bond at position 1 (C_{n-2}, 1-ene) (6). Meats have been most commonly studied for the presence of hydrocarbons, although plant foods have been studied recently (5,9). A variety of methods to detect radiation-induced hydrocarbons have been developed, including separation of the lipid fraction from foods, separation of the hydrocarbons from the lipids, and gas chromatographic analysis of the hydrocarbons based on food type and lipid composition. The separation of hydrocarbons from lipids is considered to be one of the most critical steps in detecting hydrocarbons. Hydrocarbons have been separated from lipids by cold finger distillation (10), column chromatography (6,7,9), and high-performance liquid chromatography (8,11). Schreiber's group compared high-vacuum cold finger distillation with Florisil column chromatography and concluded that the latter seemed to be more practical for routine application to meats (6,7).

The objectives of the present study were to detect the hydrocarbons exclusively produced by γ-radiation of peanuts by a sequential procedure of lipid extraction by hexane, Florisil column chromatography, and gas chromatography (GC), and to determine how irradiation affects production of hydrocarbons in peanuts. Hydrocarbons produced will then be used as markers for γ-radiation.

MATERIALS AND METHODS

Materials and reagents. Dried peanuts were purchased from a market in Kochang, Korea. Sodium sulfate was analytical grade (Pure Chemicals Co., Ltd., Osaka, Japan). *n*-Hexane and isooctane were from Fisher Scientific (Fairlawn, NJ). The hydrocarbon standards *n*-octane ($C_{8:0}$), *n*-nonane ($C_{9:0}$), *n*-decane ($C_{10:0}$), *n*-dodecane ($C_{12:0}$), *n*-tridecane ($C_{13:0}$), *n*-tetradecane ($C_{14:0}$), *n*-pentadecane ($C_{15:0}$), *n*-hexadecane ($C_{16:0}$), *n*-heptadecane ($C_{17:0}$), *n*-octadecane ($C_{18:0}$), *n*-nonadecane ($C_{19:0}$), *n*-eicosane ($C_{20:0}$), *n*-heneicosane ($C_{21:0}$), *n*-docosane ($C_{22:0}$), 1-hexadecene ($C_{16:1}$), and 1-tetradecene ($C_{14:1}$) were purchased from Sigma Chemical Co. (St. Louis, MO). 8-Heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) were obtained from the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (Berlin, Germany). Linoleic acid was purchased from Nu-Chek-Prep, Inc. (Elysian, MN). Since 6,9-heptadecadiene ($C_{17:2}$) and 1,7,10-hexadecatriene ($C_{16:3}$) standards were not commercially obtainable, linoleic acid was irradiated at 10 kGy, and

*To whom correspondence should be addressed at the 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

the two largest GC peaks were identified as the two hydrocarbons by GC–mass spectroscopy (MS).

Irradiation. Five hundred grams of peanuts were irradiated at 0.5, 1, 3, or 6 kGy using a ^{60}Co radiation source at the Korea Atomic Energy Research Institute (Daejon, Korea). Linoleic acid was irradiated at 10 kGy.

Roasting. Thirty grams of unirradiated and irradiated peanuts were roasted for 8 min on an electric frying pan (temperature setting: 400; Sunbeam Appliance Co., Oak Brook, IL). All samples were kept at −80°C before oil extraction.

Oil extraction. Oil extraction, separation of hydrocarbons, and GC analysis followed the previously reported methods (6,9) with minor modification. Peanuts (10 g) were ground in a mixer (FM680T; Hanil Electronic, Seoul, Korea) with 10 g of anhydrous sodium sulfate (previously heated to 650°C for 5 h). After that, 150 mL *n*-hexane was added and the mixture was homogenized thoroughly with a homogenizer (M133/1280-0; Biospec Products, Inc., Bartlesville, OK). The mixture was transferred to Teflon centrifuge tubes (Nalge Co., Rochester, NY) and centrifuged at 3400 rpm for 20 min using a Union 55R (Hanil Co.) centrifuge. 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., Japan) at 34°C, connected to an Eyela aspirator (A-3S; Tokyo Rikakikai Co., Ltd.). The extracted oil was flushed with nitrogen and stored at 4°C until separated by Florisil column chromatography.

Separation of hydrocarbons by Florisil column chromatography. Florisil (60–100 mesh, F100-3; Fisher Scientific) was heated at 550°C overnight. Just prior to packing the column, it was heated again to 130°C for 5 h and cooled down to room temperature. It was then deactivated by the addition of 3% water. The glass column (2.3 cm i.d.) with a Teflon stopcock was rinsed with hexane and filled with 20 g Florisil. Oil sample (1 g) mixed with 1 mL of hexane containing 8 g/mL *n*-eicosane as an internal standard was applied to the column, followed by 60 mL hexane, and the mixture was eluted at 3 mL/min. Isooctane (0.5 mL) was added to the eluate, and the eluate was concentrated to a volume of about 4 mL under a nitrogen stream. The concentrated sample was filtered through a Nylon membrane (13 mm diameter, 0.2 µm pore; Whatman International Ltd., Maidstone, England) contained in a 13 mm syringe holder (Nucleopore Corp., Pleasanton, CA), which was connected to a 10 mL Luer-lock syringe (Popper & Sons, Inc., New Hyde Park, NY). The filtrate was concentrated to 0.5 mL under nitrogen and transferred into a GC vial. Hydrocarbons from linoleic acid, unirradiated or irradiated, were separated in the same way.

GC analysis of hydrocarbons. The isolated hydrocarbons were analyzed on a Younglin gas chromatograph 600 D (Younglin Instruments, Seoul, Korea) equipped with a flame-ionization detector (FID) and a split/splitless injector. Helium was used as the carrier gas. The column was a 0.25 mm i.d. × 30 m column with 0.25 µm film thickness {DB-5 [(5%-phenyl)-methylpolysiloxane]; J&W Scientific, Folsom, CA}. The initial column temperature was held at 50°C for 2 min, then programmed at 10°C/min to 130°C and 5°C/min to 200°C where it was held for 2 min, then 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 set in splitless mode for 2 min and then in split mode. Sample (1 µL) was injected. Initially unidentified peaks were identified by GC–MS. All experiments were in duplicate unless otherwise stated.

Heated peanut oil. Peanut oil (1 g) extracted as above was placed in a test tube and heated in an oven (Model H080; Han Won Testing Co., Korea) at 150°C. The test tube with the oil was taken out every 5 min and cooled at room temperature. Hydrocarbons were separated and analyzed as described above.

RESULTS AND DISCUSSION

Hydrocarbons produced by irradiation. The irradiated peanuts, even at 6 kGy, were hardly distinguishable from the unirradiated ones by appearance or flavor. The peanut samples in the study contained 48.9% fat and 6.8% moisture and, after roasting, fat and moisture contents were 49.8 and 2.0%, respectively; therefore, less sample was needed than for meats or fruits. Therefore, less sodium sulfate was used to remove moisture.

No unsaturated hydrocarbons were detected in the oil extracted from the unirradiated peanuts (Fig. 1), but saturated hydrocarbons $C_{14:0}$, $C_{15:0}$, and $C_{16:0}$ were detected (Table 1). However, it cannot be concluded that these are naturally present in peanuts, because the hexane used in the experiment might contain a minute amount of longer hydrocarbons and these may have been concentrated to detection levels during the solvent evaporation processes. $C_{14:1}$, one of the hydrocarbons possibly produced from palmitic acid, was not detected in unirradiated peanuts, but was detected in the peanuts irradiated at 0.5 kGy or higher. Hydrocarbons $C_{17:0}$ and $C_{16:1}$, possibly from stearic acid, were not detected in unirradiated peanuts but were detected in those irradiated at 0.5 kGy or higher, although in small amounts. The prominent radiation-induced unsaturated hydrocarbons $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ were detected at fairly high levels in the peanuts irradiated at 0.5 kGy or higher. Among them $C_{16:2}$ was the most abundant. Since oleic and linoleic acids constitute about 50 and 26% of the total fatty acids in peanut oils, respectively (12), this result could be expected.

Roasted peanuts. It was determined whether the prominent radiation-induced hydrocarbons in the irradiated peanuts were retained after roasting and whether the hydrocarbons were detected in edible roasted unirradiated peanuts. Roasting the irradiated peanuts had little effect on the detected amounts of the prominent radiation-induced hydrocarbons (Table 2). When the unirradiated peanuts were roasted, the irradiation-inducible hydrocarbons were not detected. The roasted peanuts purchased in the marketplace did not contain any marker hydrocarbons. Therefore, peanuts, once irradiated, could be detected by analyzing hydrocarbons even after roasting.

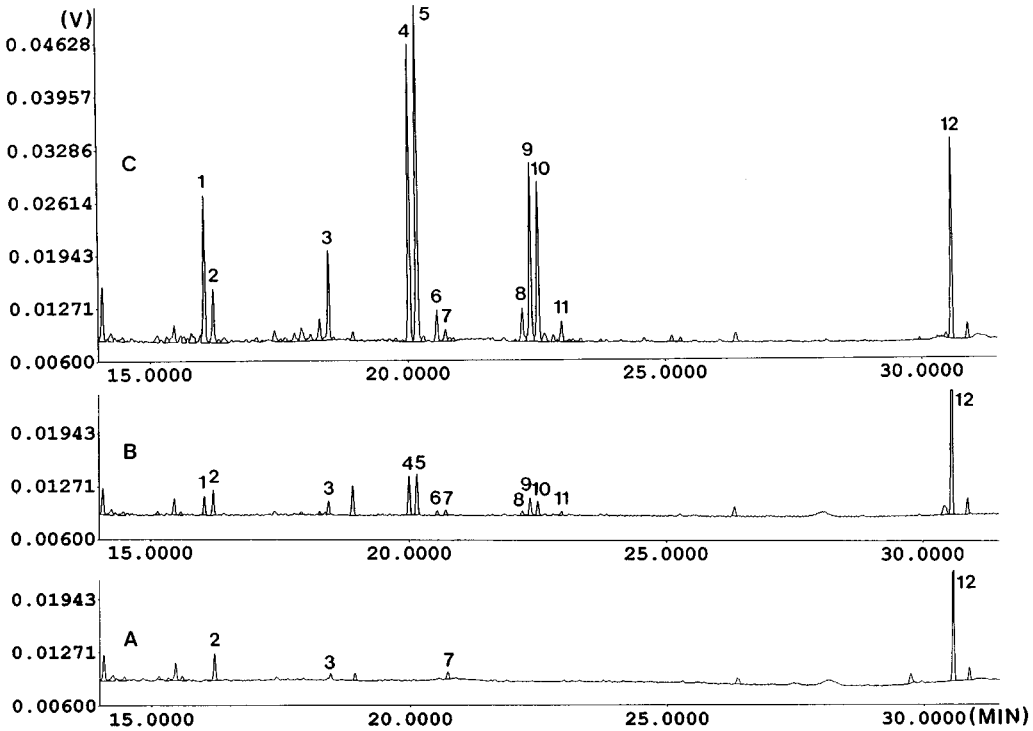

FIG. 1. Gas chromatograms of the hydrocarbons in irradiated peanuts. (A) Unirradiated; (B) irradiated at 1 kGy; and (C) irradiated at 6 kGy. (1) 14:1; (2) 14:0; (3) 15:0; (4) 16:3; (5) 16:2; (6) 16:1; (7) 16:0; (8) 17:3(?); (9) 17:2; (10) 17:1; (11) 17:0; and (12) 20:0 (internal standard). Column: DB-5 [(5%-phenyl)-methylpolysiloxane]; 0.25 mm i.d. 30 m, 0.25 m. Oven: 50°C for 2 min; 50–130°C at 10°C/min; 130–200°C at 5°C/min; 200°C for 2 min; 200–250°C at 25°C/min, and 250°C for 5 min. Injector: splitless, 200°C. Detection: flame-ionization detector, 250°C.

Heated peanut oil. Peanut oil was heated at 150°C in the present study to determine whether hydrocarbons $C_{17:1}$ and $C_{17:2}$, which were reported by Lesgards *et al.* (5) to be detected at a fairly high level in unirradiated peanut oil, are produced in the oil by heating. Hydrocarbons $C_{17:1}$ and $C_{17:2}$ were first detected at 0.053 and 0.046 µg/g oil, respectively, in the peanut oil heated for 30 min, when checked every 5 min (Fig. 2). They increased with heating time. Other radiation-inducible hydrocarbons were also detected in oil heated for longer times, although in smaller amounts compared to $C_{17:1}$ and $C_{17:2}$ ($C_{17:1}$, 0.56, $C_{17:2}$, 0.53; and $C_{16:2}$, 0.04 µg/g oil heated for 6 h; $C_{17:1}$, 6.9, $C_{17:2}$, 4.3, $C_{16:1}$, 0.18; and $C_{16:2}$, 0.34 µg/g oil for 24 h). $C_{16:3}$ was not detected even in the oil heated for 24 h.

Lesgards *et al.* (5) reported that hydrocarbons $C_{17:1}$ and $C_{17:2}$ were detected at 0.4 and 0.2 µg/g oil, respectively, in an unirradiated peanut oil. These two hydrocarbons were found at 15.4 and 7.55 µg/g oil, respectively, in peanut oil heated at

TABLE 1
Hydrocarbons in Irradiated Peanuts (µg/g oil)[a]

	Dose (kGy)				
Hydrocarbon	0	0.5	1	3	6
14:1	N.D.[b]	0.15 (0.06)	0.15 (0.10)	0.72 (0.31)	1.72 (0.77)
14:0	0.37 (0.05)	0.40 (0.19)	0.19 (0.09)	0.33 (0.11)	0.79 (0.02)
15:0	0.04 (0.05)	0.17 (0.05)	0.11 (0.07)	0.45 (0.07)	1.08 (0.53)
16:3	N.D.	0.31 (0.08)	0.41 (0.17)	1.82 (0.22)	4.05 (1.86)
16:2	N.D.	0.41 (0.12)	0.47 (0.09)	2.31 (0.67)	6.38 (1.03)
16:1	N.D.	0.07 (0.02)	0.05 (0.04)	0.25 (0.08)	0.38 (0.36)
16:0	0.05 (0.07)	0.17 (0.07)	0.06 (0.04)	0.12 (0.05)	0.39 (0.36)
17:2	N.D.	0.18 (0.09)	0.27 (0.06)	1.43 (0.18)	3.38 (1.11)
17:1	N.D.	0.13 (0.04)	0.21 (0.10)	1.24 (0.32)	3.78 (0.15)
17:0	N.D.	0.11 (0.07)	0.05 (0.07)	0.25 (0.11)	0.28 (0.10)

[a]Mean (standard deviation) of duplicate.
[b]N.D., not detected.

TABLE 2
Hydrocarbons in Roasted Peanuts (μg/g oil)[a]

Hydrocarbon	Roasted in the lab		Roasted—purchased from market
	Unirradiated	Irradiated at 6 kGy	
14:1	N.D.[b]	1.71 (0.02)	N.D.
14:0	0.41 (0.06)	1.74 (0.19)	1.01 (0.06)
15:0	0.14 (0.06)	1.84 (0.73)	0.30 (0.07)
16:3	N.D.	4.61 (0.03)	N.D.
16:2	N.D.	5.46 (0.10)	N.D.
16:1	N.D.	0.49 (0.00)	N.D.
16:0	N.D.	0.43 (0.21)	0.16 (0.05)
17:2	N.D.	4.11 (0.06)	N.D.
17:1	N.D.	3.77 (0.09)	N.D.
17:0	N.D.	0.58 (0.01)	0.07 (0.06)

[a]Mean (standard deviation) of duplicate.
[b]N.D., not detected.

180°C with oxygen, while 10.5 and 5.09 μg/g oil, respectively, were found in the oil irradiated at 10 kGy. These hydrocarbons were even detected at 2.79 and 1.31 μg/g oil, respectively, in the oil heated without oxygen. The researchers also noted that long-chain unsaturated fatty acids decreased significantly after heating, while the fatty acid composition of the oils did not change significantly after irradiation. It should be noted that they did not separate the hydrocarbons from cold oil; instead, they employed a desorption oven where oil was heated at 150°C to evaporate volatiles including hydrocarbons, followed by cold-trap concentration before GC injection. The small amounts of hydrocarbons $C_{17:1}$ and $C_{17:2}$ detected in the unirradiated peanut oil in their report might have been induced during the heating of the oil in the desorption oven. Since all the procedures to separate hydrocarbons from peanuts in the present study were performed below 40°C, any hydrocarbons possibly induced by heating could be excluded.

If peanuts were irradiated, it would be mostly for the purpose of microbial control, for which at least 10 kGy would be applied. The hydrocarbons $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ were not detected in unirradiated peanuts, while they were detected at fairly high levels in the peanuts irradiated at 10 kGy. Even in peanuts irradiated at 1 kGy, as usually applied for disinfestation, these hydrocarbons were detected. Thus, the detection of hydrocarbons by the manner shown in this study would permit detection of irradiated peanuts. Therefore, it can be concluded that the methodology applied in this study can clearly differentiate irradiated from unirradiated peanuts by detecting the four prominent hydrocarbons. However, the dose vs. quantity of marker response was not always indicative of the irradiation dose, probably because of the variations in the serial procedure which requires delicate handling. Thus, this methodology could be limited for detecting of irradiation dose levels.

ACKNOWLEDGMENTS

This research was supported by a grant from the Health Science and Technology Research Project (HMP-96-F-1-1007) of the Korea Institute of Health Services Management—Health Science and Technology Planning and Evaluation Board. The authors gratefully acknowledge the Department of Food Irradiation, Korea Atomic Energy Research Institute for irradiating the samples for us.

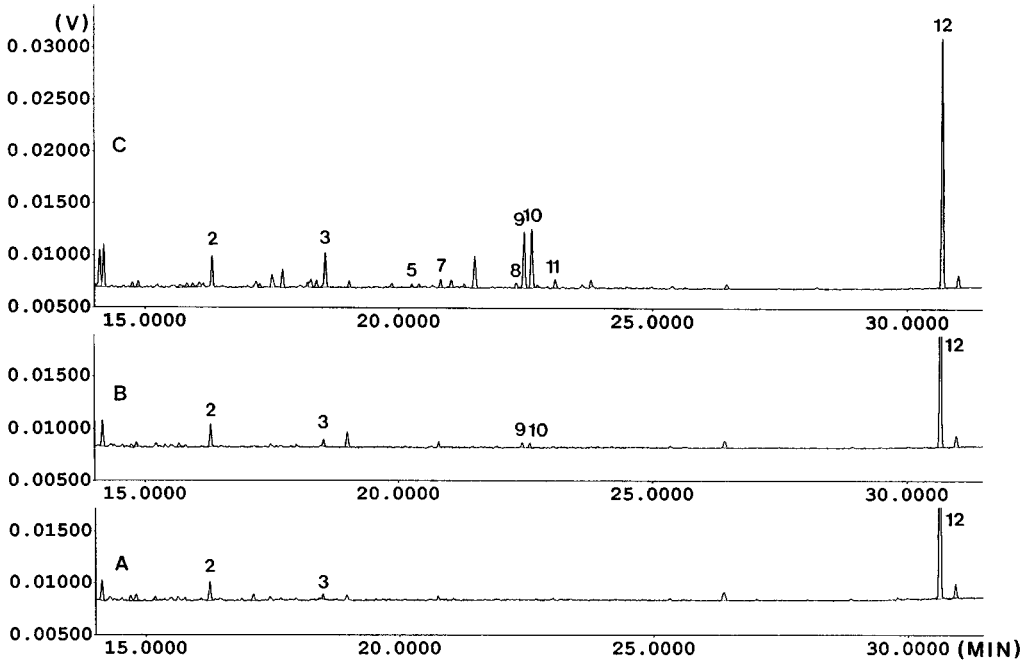

FIG. 2. Gas chromatograms of the hydrocarbons in heated peanut oil. (A) Untreated; (B) heated for 30 min at 150°C; and (C) heated for 6 h at 150°C. See Figure 1 for further information.

REFERENCES

1. Shibamoto, T., and L.F. Bjeldanes, Fungal Toxins Occurring in Foods, in *Introduction to Food Toxicology*, Academic Press, San Diego, 1993, pp. 97–116.
2. Diel, J.F., Potential and Actual Applications of Irradiated Foods, in *Safety of Irradiated Foods,* Marcel Dekker, Inc., New York, 1990, pp. 217–253.
3. International Atomic Energy Agency, *Food Irradiation Newsletter (Supplement) 19(2):*1–35 (1995).
4. *FAOSTAT Agriculture Data,* Food and Agricultural Organization of the United Nations, Rome, Italy (http://apps.fao.org) (1998).
5. Lesgards, G., J. Raffi, I. Pouliquen, A.A. Chaouch, P. Giamarchi, and M. Prost, Use of Radiation-Induced Alkanes and Alkenes to Detect Irradiated Food Containing Lipids, *J. Am. Oil Chem. Soc. 70:*179–185 (1993).
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. 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, *JAOAC International 77:*1202–1217 (1994).
8. Schulzki, G., A. Spiegelberg, K.W. Bögl, and G.A. Schreiber, Detection of Radiation-Induced Hydrocarbons in Camembert Irradiated Before and After the Maturing Process—Comparison of Florisil Column Chromatography as Well as On-line Coupled Liquid Chromatography–Gas Chromatography, *J. Agric. Food Chem. 43:*372–376 (1995).
9. 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).
10. Nawar, W.W., Z.R. Zhu, and Y.J. Yoo, Radiolytic Products of Lipids as Marker for the Detection of Irradiated Meat, in *Food Irradiation and the Chemist,* edited by D.E. Johnston and M.H. Stevenson, The Royal Society of Chemistry, London, 1990, pp. 13–24.
11. 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).
12. 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.

[Received May 4, 1998; accepted September 3, 1998]