Detection of Alkanes and Alkenes for Identifying Irradiated Cereals

Keum Taek Hwang[a,*], Jin Sun Hong[a], Jae Seung Yang[b], Hee Sook Sohn[a], and Curtis L. Weller[c]

[a]Department of Food Science and Human Nutrition, and Institute for Molecular Biology and Genetics, Chonbuk National University, Chonju, Chonbuk, 561-756, Korea, [b]Korea Atomic Energy Research Institute (Detection Lab of Irradiated Food), Daejon, 305-353, Korea, and [c]Department of Biological Systems Engineering, University of Nebraska, Lincoln, Nebraska 68583

ABSTRACT: Alkanes and alkenes can be produced from lipids in maize, brown rice, and wheat by irradiation. The purpose of this study was to determine whether the detection pattern of the hydrocarbons can be used for identifying postirradiation of cereals. Maize, brown rice, and wheat were irradiated at 0.5, 1, and 3 kGy. Oils were extracted from the samples using hexane and anhydrous Na_2SO_4. Hydrocarbon-containing fractions, isolated using Florisil column chromatography, were analyzed using gas chromatography (GC). The first six Florisil column chromatography fractions of 10 mL each were collected. Prominent radiation-induced alkenes 17:1, 16:2, 17:2, and 16:3 appeared mainly in the third, fourth, and fifth fractions. Those three fractions were combined and evaporated to a total volume of 4 mL for GC analyses. Alkenes 17:1, 16:2, 17:2, and 16:3 were detected in all irradiated samples but not in the nonirradiated ones. The levels of the hydrocarbons changed little by roasting the nonirradiated and irradiated cereals.

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KEY WORDS: Brown rice, gas chromatography, hydrocarbon, irradiation detection, maize, oil, roasting, wheat.

Food irradiation has been recognized as a method of preserving food and ensuring its wholesomeness by sterilization or cold pasteurization (1). The safety and wholesomeness of irradiated foods have been approved by international agencies (2,3). Along with traditional food processing and preserving methods, irradiation technology is gaining more and more attention around the world. However, consumers are concerned about how their foods have been treated and demand development of methods for identifying irradiated foods.

Since Nawar's group (4–7) reported that some hydrocarbons are exclusively produced by γ-radiation of lipids and lipid-containing foods, hydrocarbons have been extensively studied as markers to detect irradiation of foods. Analysis and comparison of hydrocarbons in foods containing lipids has been proposed as a promising method to detect food irradiation (8–11). Two types of hydrocarbons are produced predominantly by irradiation of lipids in foods: a hydrocarbon that has one carbon less than the parent fatty acid (Cn-1) and a hydrocarbon that has two carbons less and an additional double bond at the 1-position (Cn-2, 1-ene) (4,9). Also, it has been found that the hydrocarbons detected in irradiated foods clearly depend on the composition of fatty acids in each food commodity (10–16). Studies on hydrocarbons have been carried out mainly with animal foods (5,10,13,14) and plant commodities with high lipid contents (7,11,15,16). Irradiation was permitted for maize, brown rice, and wheat mostly to control insects, with doses ranging from 0.3 to 10 kGy, in 23 countries as of May 2000 (17). Palmitic acid, oleic acid, and linoleic acid are major fatty acids of the lipids found in maize, brown rice, and wheat (18). Therefore, alkane 15:0, alkene 14:1*cis*-1 from palmitic acid, alkenes 17:1*cis*–8 and 16:2*cis*-1,7 from oleic acid, alkenes 16:3*cis*-1,7,10 and 17:2*cis*-6,9 from linoleic acid are expected to be detected in irradiated samples. Fewer studies regarding hydrocarbons in cereals have been conducted because their lipid content is insufficient for detection. A more delicate separation method needs to be developed to detect hydrocarbons in low lipid content foods.

The main objectives of the study were to detect low levels of alkanes and alkenes in maize, brown rice, and wheat after irradiation with minor modification of a previous separation method (13), and to determine whether the detection of the hydrocarbons can be applied to determining postirradiation of the samples and whether the hydrocarbons are retained after roasting.

MATERIALS AND METHODS

Materials and reagents. Whole maize kernels, dehulled brown rice, and whole wheat kernels were purchased from a local market in Wanju, Chonbuk, Korea. Certified American Chemical Society grade hexane was purchased from Fisher Scientific (assay ≥98.5%: 86.1% *n*-hexane; 9.7% methylcyclopentane; 4.2% various methylpentanes; Fair Lawn, NJ). Ethyl ether was purchased from Samchun Pure Chemical Co., Ltd. (Pyongtaek, Korea). NaOH was purchased from Jin Chemical Co., Ltd. (Shehyung, Korea). BF_3/methanol (125 g BF_3/L methanol) was purchased from BDH Laboratory

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

(Poole, England). Chloroform, methanol, KCl, and Na_2SO_4 were purchased from Oriental Chemical Inc. (Seoul, Korea). The standards of fatty acid methyl ester (FAME) were purchased from Nu-Chek-Prep, Inc. (Elysian, MN), and hydrocarbon standards were purchased from Sigma Chemical Co. (St. Louis, MO).

Sample preparation and irradiation. Maize, brown rice, and wheat were irradiated at 0.5, 1, and 3 kGy with a ^{60}Co γ-radiation source at the Korea Atomic Energy Research Institute (Daejon, Korea). Some irradiated and nonirradiated samples were roasted for 8 min in an open electric pan (temperature setting: 400°F; Sunbeam Appliance Co., Oak Brook, IL). All samples were kept at –18°C until subsequent oil extraction.

Oil extraction for fatty acid composition analysis. Oil extraction followed the Bligh and Dyer method (19).

Methylation. Methylation of the oils was carried out using AOCS Official Method Ce 2-66 (20).

Gas chromatography (GC) analysis of fatty acid composition. The FAME were analyzed on a Hewlett-Packard 6890 Series gas chromatograph (Hewlett-Packard Co., Wilmington, DE), equipped with a flame-ionization detector (FID). The column was an HP-23 *cis/trans* FAME column (30 m × 0.25 mm × 0.25 μm; Hewlett-Packard Co.). Helium was used as the carrier gas. One microliter of each sample was injected. The initial flow rate of the carrier gas was 1 mL/min for 12 min and then increased incrementally by 0.2 mL/min up to 2 mL/min. The injector and detector temperatures were 250 and 270°C, respectively. The injector was set in a split ratio of 100:1. Initial column temperature was 150°C for 2 min, programmed to increase at 3.5°C/min up to 170°C, at which point it was held for 10 min followed by increases of 4°C/min to 210°C and then 25°C/min to 250°C with a final hold for 5 min.

Oil extraction for hydrocarbon separation. Maize (100 g), brown rice (130 g), and wheat (240 g) were ground in a blender with 60, 80, and 160 g anhydrous Na_2SO_4, respectively. Hexane (300, 300, and 500 mL, respectively) was added and the contents were homogenized thoroughly using a homogenizer (M133/1281-1; BioSpec Products, Inc., Bartlesville, OK). The rest of the oil extraction procedure was the same as in a previous report (13).

Separation of hydrocarbons by Florisil column chromatography. Hydrocarbons were separated from the oil samples as described in a previous report (13), with the following minor modification. The first 60 mL of hexane eluate was collected at 3 mL/min, as in previous papers (14,16). Separately, oils extracted from nonirradiated samples were spiked with hydrocarbon standards. The first six fractions of 10 mL each were collected from the Florisil column. Only the third, fourth, and sixth fractions, in which prominent radiation-induced alkenes (17:2, 16:3, 17:1, and 16:2) appeared, were combined and evaporated to a total volume of 4 mL under nitrogen flow for GC analysis.

GC analysis of hydrocarbons. Hydrocarbons were analyzed using GC, and peaks were identified as described in a previous report (13). All the experiments were done in triplicate. Data were compared with a one-tailed *t*-test [level of significance (α) = 0.05].

RESULTS AND DISCUSSION

Fatty acid compositions of nonirradiated maize, brown rice, and wheat. Maize, brown rice and wheat contained 13.0, 10.9 and 12.3% (w/w, wet basis), moisture and 3.36, 2.55, and 1.36% crude lipids, respectively. Linoleic acid was the most abundant fatty acid in the oils present in maize and wheat, consisting of 50 and 56%, respectively (Table 1). In maize, oleic acid (30%) and palmitic acid (14%) were the next-most abundant fatty acids. In wheat, palmitic acid content (19%) was greater than oleic acid content (16%). But oleic acid was the most abundant fatty acid in the oils extracted from brown rice, consisting of 40%, followed by linoleic acid (36%) and palmitic acid (18%) (Table 1). The compositions of fatty acids in the cereals, presented by Sonntag (18), were similar to our data. Thus, the major hydrocarbons induced by irradiation were expected to be alkenes 17:2, 16:3, 17:1, and 16:2, as described above.

Isolation of hydrocarbons by Florisil column chromatography. The first 60 mL hexane eluate from Florisil column chromatography was used for GC analyses, as in the experiments with pork and peanuts in previous reports (14,16). Many small peaks appeared around hydrocarbons 17:2, 16:3, 17:1, 16:2, 15:0, and 14:1 peaks in all the cereal samples. It was difficult to identify the hydrocarbons induced solely by irradiation with the cereals in this study.

Extracted lipids from maize (100 g), brown rice (130 g), and wheat (240 g) used in this study were 2.3–3.0, 1.4–2.2, and 0.8–1.0% (w/w, wet basis), respectively. The extracted oil may have contained minor components from cereals and extractant (300–500 mL hexane). Relative to pork or peanuts (14,16), the minor components of nonlipid material and extractant were more likely to produce GC peaks for cereals because of the much smaller yields of oil for the latter.

To see which fractions from the column chromatography contained most of the irradiation-induced hydrocarbons, hydrocarbon standards were spiked into nonirradiated cereal samples. Oils were extracted and subjected to Florisil column chromatography to collect six fractions of 10 mL each. The alkenes (17:2, 16:3, 17:1, and 16:2) were mostly eluted in the third, fourth, and fifth fractions. Therefore, for all the samples, the three fractions were combined, concentrated 7.5-fold

TABLE 1
Fatty Acid Compositions (%, w/w) of Lipids Extracted from Selected Cereals Prior to Irradiation[a]

Fatty acid	Maize	Brown rice	Wheat
Myristic acid	0.04 (0.00)	0.26 (0.01)	0.09 (0.00)
Palmitic acid	14.5 (0.41)	17.8 (0.39)	18.6 (0.06)
Palmitoleic acid	0.12 (0.06)	0.16 (0.00)	0.16 (0.00)
Margaric acid	0.08 (0.00)	0.06 (0.00)	0.10 (0.00)
Stearic acid	0.93 (0.01)	0.58 (0.03)	0.02 (0.01)
Oleic acid	29.9 (0.11)	39.7 (0.35)	16.0 (0.11)
Linoleic acid	50.3 (0.25)	35.7 (0.24)	56.4 (0.44)
Linolenic acid	0.98 (0.01)	0.43 (0.01)	0.92 (0.02)
Arachidic acid	0.54 (0.01)	0.55 (0.02)	0.20 (0.00)
Eicosenoic acid	0.28 (0.01)	0.55 (0.02)	0.26 (0.02)

[a]Mean (standard deviation) of three values.

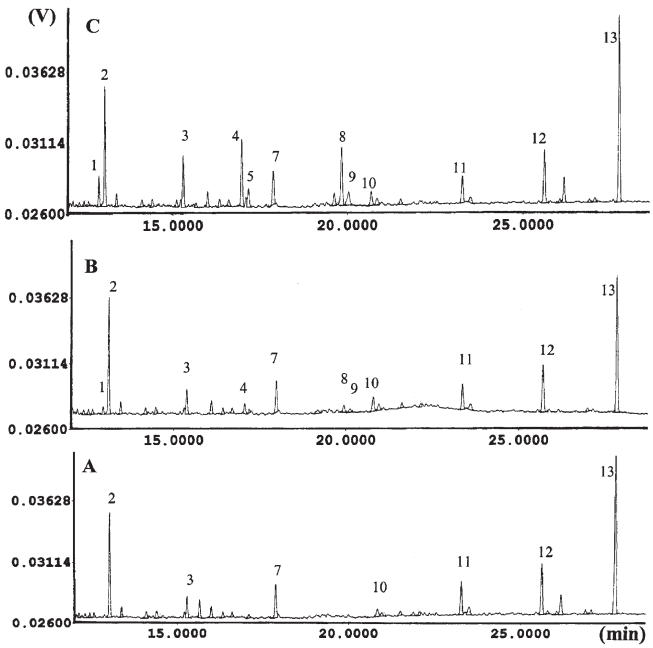

FIG. 1. Gas chromatograms of hydrocarbons in wheat: (A) nonirradiated; (B) irradiated at 0.5 kGy, and (C) irradiated at 3 kGy. (1) 14:1, (2) 14:0, (3) 15:0; (4) 16:3, (5) 16:2, (7) 16:0, (8) 17:2, (9) 17:1, (10) 17:0, (11) 18:0, (12) 19:0, and (13) 20:0 (internal standard). Column: DB-5 [(5%-phenyl)-methylpolysiloxane], 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 detector, 250°C.

by solvent evaporation, and then used for GC analysis. High-quality GC chromatograms were obtained with a distinctive appearance of the prominent hydrocarbons (Fig. 1).

Hydrocarbons detected in irradiated cereals. Analyzed by the minor modified method described above, hydrocarbons 17:2*cis*-6,9, 16:3*cis*-1,7,10, 17:1*cis*-8, 16:2*cis*-1,7, 15:0, and 14:1*cis*-1 were detected in the samples irradiated at 0.5 kGy or higher (Table 2), as expected. However, hydrocarbons 14:1 and 15:0 could not be used as markers for determining post-irradiation of the cereals, because those hydrocarbons were detected in nonirradiated samples. It was not clear whether these hydrocarbons had originated from hexane or cereal samples. Alkanes such as 14:0, 15:0, or 16:0 were detected from other commodities in previous papers (13–16).

In maize and wheat, hydrocarbons 17:2 and 16:3 derived from linoleic acid by irradiation were detected at fairly high levels in the irradiated samples but were not detected in non-irradiated ones (Table 2). Since oleic acid content was greater than linoleic acid content in brown rice, the pair of alkenes, 17:1 and 16:2, which were mainly induced from oleic acid by irradiation, were detected in higher amounts than the pair of 17:2 and 16:3, which were mainly induced from linoleic acid. The prominent radiation-induced hydrocarbons increased with dose, whereas the prominent hydrocarbons were not detected in nonirradiated samples. The patterns of hydrocarbons induced from cereal lipids by irradiation were consistently related to fatty acid composition, as were those for the oils of meats (14) and other high-lipid plant commodities (11,15,16) in previous papers.

Hydrocarbons in roasted cereals. To see how minor heat treatment affected hydrocarbons in cereals, nonirradiated and irradiated cereals were roasted, and their hydrocarbons were analyzed. The detection pattern of the roasted cereal samples showed little difference from the untreated ones; that is, the prominent radiation-induced hydrocarbons were not detected

TABLE 2
Hydrocarbons in Nonirradiated and Irradiated Cereals (μg/g oil)[a]

Parent fatty acid	Hydrocarbon	Dose (kGy)											
		Maize				Brown rice				Wheat			
		0	0.5	1	3	0	0.5	1	3	0	0.5	1	3
16:0	15:0	0.46 (0.17)	0.62 (0.03)	0.39 (0.04)	0.62 (0.14)	0.59 (0.09)	0.80 (0.03)	0.86 (0.08)	1.22 (0.05)	0.51 (0.03)	0.58 (0.11)	0.62 (0.18)	0.96 (0.10)
	14:1	—[b]	0.08 (0.11)	0.12 (0.01)	0.42 (0.08)	0.16 (0.03)	0.23 (0.01)	0.31 (0.03)	0.54 (0.01)	0.03 (0.05)	0.15 (0.04)	0.22 (0.06)	0.47 (0.04)
18:0	17.0	0.30 (0.05)	0.26 (0.02)	0.29 (0.01)	0.23 (0.05)	0.39 (0.06)	0.38 (0.06)	0.38 (0.02)	0.42 (0.03)	0.50 (0.01)	0.48 (0.06)	0.29 (0.28)	0.36 (0.02)
	16:1	—	—	—	0.10 (0.07)	—	—	0.15 (0.09)	0.95 (1.11)	—	—	—	—
18:1	17:1	—	— (0.06)	—	0.42 (0.03)	— (0.01)	0.29 (0.03)	0.38	0.77 (0.09)	— (0.13)	0.15 (0.04)	0.20	0.48
	16:2	—	0.19 (0.01)	0.41 (0.01)	0.91 (0.06)	—	0.19 (0.02)	0.37 (0.04)	1.07 (0.01)	—	—	0.12 (0.11)	0.43 (0.03)
18:2	17:2	—	0.16 (0.01)	0.34 (0.00)	0.73 (0.09)	—	0.22 (0.01)	0.32 (0.02)	0.76 (0.06)	—	0.21 (0.04)	0.41 (0.08)	1.44 (0.08)
	16:3	—	0.26 (0.00)	0.54 (0.00)	1.25 (0.16)	—	0.21 (0.04)	0.38 (0.06)	0.98 (0.00)	—	0.22 (0.08)	0.42 (0.14)	1.30 (0.20)

[a]Mean (standard deviation) of three values.
[b]Not detected.

TABLE 3
Hydrocarbons in Roasted Cereals (μg/g oil)[a]

Parent fatty acid	Hydrocarbon	Dose (kGy)											
		Maize				Brown rice				Wheat			
		0	0.5	1	3	0	0.5	1	3	0	0.5	1	3
16:0	15:0	0.39 (0.10)	0.97 (0.95)	0.45 (0.02)	0.55 (0.02)	0.46 (0.06)	0.60 (0.11)	—	—	—	—	—	—
	14:1	—[b]	0.08 (0.00)	0.14 (0.01)	0.40 (0.01)	0.10 (0.01)	0.10 (0.08)	0.18 (0.01)	0.46 (0.09)	0.06 (0.00)	0.16 (0.05)	0.26 (0.01)	0.55 (0.09)
18:0	17.0	0.22 (0.02)	0.10 (0.01)	0.24 (0.03)	0.24 (0.03)	0.29 (0.04)	0.35 (0.05)	0.30 (0.07)	0.46 (0.05)	0.22 (0.06)	0.31 (0.12)	0.43 (0.03)	0.45 (0.01)
	16:1	—	—	—	—	—	—	—	0.12 (0.07)	—	—	—	—
18:1	17:1	—	—	0.19 (0.00)	0.19 (0.00)	—	0.32 (0.05)	0.40 (0.10)	0.82 (0.15)	—	0.21 (0.10)	0.37 (0.07)	0.73 (0.00)
	16:2	—	0.10 (0.01)	0.27 (0.00)	0.27 (0.00)	—	0.10 (0.095)	0.27 (0.05)	1.02 (0.13)	—	—	0.38 (0.02)	0.81 (0.01)
18:2	17:2	—	0.16 (0.01)	0.28 (0.11)	0.28 (0.11)	—	0.40 (0.07)	0.43 (0.01)	0.97 (0.13)	—	0.78 (0.04)	0.16 (0.01)	0.06 (0.02)
	16:3	—	0.17 (0.00)	0.36 (0.07)	0.36 (0.07)	—	0.16 (0.03)	0.22 (0.00)	0.92 (0.15)	—	0.12 (0.00)	0.42 (0.00)	0.43 (0.01)

[a]Mean (standard deviation) of three values. Roasted for 8 min in an open electric pan
[b]Not detected.

in roasted nonirradiated samples, whereas they were detected in the roasted irradiated ones (Table 3). The amounts of hydrocarbons detected in the irradiated maize did not significantly change by roasting ($\alpha = 0.05$). The hydrocarbons in the irradiated brown rice and wheat minimally increased because of roasting. This result is consistent with previous reports for sesame seeds and peanuts (15,16). According to the previous studies (11,16,21), severe heating produced some hydrocarbons, although its pattern was totally different from that of irradiation.

Implications. Detection of prominent radiolytic hydrocarbons in maize, brown rice, and wheat using GC analysis of nonpolar fractions resulting from column chromatography, as suggested in this study, makes it possible to identify whether the grains were irradiated previously. This method also can be applied to roasted samples. The method employed herein also may be used for identifying irradiation of other food commodities with low amounts of lipids.

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