# Detection of Hydrocarbons in Irradiated and Roasted Sesame Seeds

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**ABSTRACT:** Hydrocarbons produced by γ-radiation of sesame seed were analyzed to determine the relation of irradiation dose to the production of hydrocarbons and to eventually use them as markers for identifying post-irradiated sesame seed. Hydrocarbons in sesame seed were determined by a sequential procedure of lipid extraction by hexane, Florisil column chromatography, and gas chromatography. 16:2, 16:3, 17:1, and 17:2 were prominently detected in irradiated sesame seed. 17:2 was detected in seed irradiated at 0.1 kGy or higher, and the others were detected at 0.5 kGy or higher. These hydrocarbons were not detected in unirradiated sesame seeds that were raw, roasted, whole, ground, or stored.
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**KEY WORDS:** Detection of post-irradiation, Florisil column chromatography, gas chromatography, hydrocarbon(s), irradiation, sesame oil, sesame seed.

Sesame seed is one of the most important condiments for Korean foods. In Korea, a large amount of sesame seed is imported and may become infested with insects during shipping. Sesame seed is not permitted to be irradiated in any country except Cuba, which permits sesame seed to be irradiated up to 2 kGy for disinfestation (1). Sesame seed belongs to the spices and seasonings, for which irradiation is permitted to control insects, with doses ranging from 1 to 5 kGy in several countries (1). Although irradiated foods must be properly labeled, there is the possibility of irradiating them without any notification on the shipment. It is, therefore, necessary to develop an appropriate method to detect irradiation of imported sesame seed.

Since Nawar's group (2–4) reported that some hydrocarbons are exclusively produced by γ-radiation of lipids and lipid-containing foods, hydrocarbons have been extensively studied as a marker to detect irradiation of foods (5–9). Two types of hydrocarbons are predominantly produced by irradiation of fatty acids in lipids: one has one carbon less than the parent fatty acid (n-1), and the other has two carbons less and an additional double bond at position 1 (n-2, 1-ene) (10). Many analyses of hydrocarbons have concentrated on meats, although other foods have recently been included (9,11). A variety of methods to detect the radiation-induced hydrocarbons has been developed, including separation of the lipid fraction from foods, separation of the hydrocarbons from lipids, and gas chromatographic analysis of the hydrocarbons in accordance with food types and lipid composition. The separation of hydrocarbons from lipids is considered to be the most critical step in detecting hydrocarbons. Hydrocarbons have been separated from lipid fractions by cold-finger distillation (6), column chromatography (5,7,8,10), or high-performance liquid chromatography (12–14). Schreiber's group compared high-vacuum cold-finger distillation and Florisil column chromatography and concluded that the latter seemed to be more practical for routine application to meats (10,15). They also applied the Florisil chromatography method to some fresh fruits and obtained satisfactory results (11,16).

The objective of the present study is to determine whether a sequential procedure of lipid extraction by hexane, Florisil column chromatography, and gas chromatography (GC) is suitable for (i) detecting the hydrocarbons that are exclusively produced by γ-radiation of sesame seeds, (ii) examining how dose relates to the production of hydrocarbons in sesame seeds, and (iii) whether determining the hydrocarbons can be linked to irradiation of sesame seeds.

## MATERIALS AND METHODS

*Materials and reagents.* Sesame seeds were purchased from a farmer in Kimje, Korea. Sodium sulfate was of analytical grade (Pure Chemicals Co., Ltd., Osaka, Japan). *n*-Hexane was from J.T. Baker Inc. (Phillipsburg, NJ). The hydrocarbon standards [*n*-octane (8:0), *n*-nonane (9:0), *n*-decane (10:0), *n*-dodecane (12:0), *n*-tridecane (13:0), *n*-tetradecane (14:0), *n*-pentadecane (15:0), *n*-hexadecane (16:0), *n*-heptadecane (17:0), *n*-octadecane (18:0), *n*-nonadecane (19:0), *n*-eicosane (20:0), *n*-heneicosane (21:0), and *n*-docosane (22:0)] were purchased from Sigma Chemical Co. (St. Louis, MO). 1-Hexadecene (16:1) and 1-tetradecene (14:1) were also from Sigma Chemical Co. Oleic and linoleic acids were purchased from Nu-Chek-Prep, Inc. (Elysian, MN).

*Sample preparation and irradiation*. Sesame seeds were irradiated at 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 kGy with a com-

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mercial $^{60}Co$ source (Greenpia Technology Inc., Yoju, Korea). Oleic and linoleic acids, which are the predominant fatty acids in sesame oil, were irradiated at 41 kGy. Some irradiated sesame seeds were roasted for 10 min in an electric frying pan (temperature setting: 400°F; Sunbeam Appliance Co., Oak Brook, IL). A part of the roasted seeds was ground in a mortar. Untreated sesame seeds were also included as the control. All samples were kept at –40°C until the subsequent fat extraction.

*Fat extraction.* Fat extraction, separation of hydrocarbons, and GC analysis followed Schreiber's group's method (10,11) with minor modification. Three grams of sample were ground in a mortar with 3 g of anhydrous sodium sulfate (previously heated at 650°C for 5 h). After that, 100 mL *n*-hexane was added, and the content was homogenized thoroughly with a homogenizer (Nissei AM-3; Nihonseiki Kaisha, Ltd., Tokyo, Japan) for 2 min. The mixture was transferred to Teflon centrifuge tubes (Nalge Co., Rochester, NY) and centrifuged at 3400 rpm for 20 min in a VS 5500 centrifuge (Vision Scientific Co., Ltd., Seoul, Korea). The supernatant was collected in a round-bottomed flask. The solvent was evaporated under a nitrogen stream. The extracted fat was stored at 4°C until subsequent Florisil column chromatography.

*Separation of hydrocarbons by Florisil column chromatography*. Florisil (60–100 mesh, F100-3; Fisher Scientific, Fairlawn, NJ) was heated at 550°C overnight. Just before packing the column, it was heated again to 130°C for 5 h and cooled down at room temperature. It was then deactivated by the addition of 3% distilled water. A glass column (2.3 cm i.d.) with a Teflon stopcock was rinsed with hexane and filled with 20 g Florisil. One gram of fat sample, mixed with 1 mL of hexane that contained 2 µg/mL *n*-eicosane as internal standard, was applied to the column, followed by 60 mL hexane to elute at 3 mL/min. The eluate was concentrated to a volume of about 3 mL under a nitrogen stream. The concentrated sample was filtered through a Nylon membrane (13 mm, 0.2 µm; 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 0.6 mL under nitrogen into a GC vial. Hydrocarbons from the oleic and linoleic acids, unirradiated or irradiated, were also separated in the same way.

*GC analysis of hydrocarbons*. The isolated hydrocarbons were analyzed on a Hewlett-Packard 5890 series II gas chromatograph (Avondale, PA), equipped with a flame-ionization detector and a split injector. Helium was used as the carrier gas. The column was 0.25 mm i.d. × 30 m with 0.25 µm film thickness (DB-5; J&W Scientific, Folsom, CA). The initial column temperature was 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 for 5 min. The injector and detector temperatures were 200 and 250°C, respectively. One µL of sample was injected. All experiments were in duplicate unless otherwise stated.

## RESULTS AND DISCUSSION

*Irradiated oleic and linoleic acids.* Palmitic, stearic, oleic, and linoleic acids are the principal fatty acids in sesame oil. Oleic and linoleic acids comprise 33–47% and 33–48% of the total fatty acids in sesame oil, respectively (17). The predominant radiation-induced hydrocarbons could therefore be predicted as 8-heptadecene (17:1) and 1,7-hexadecadiene (16:2) from oleic acid and 6,9-heptadecadiene (17:2) and 1,7,10-hexadecatriene (16:3) from linoleic acid. Oleic and linoleic acids were irradiated at a fairly high dose (41 kGy) in the present study to induce large amounts of the expected hydrocarbons and to identify the GC retention times of the predominant hydrocarbons from sesame seeds because these unsaturated hydrocarbon standards were not commercially obtainable. The GC chromatogram of the hydrocarbon extract from the irradiated oleic acid showed two large peaks; one was just prior to the standard hydrocarbon 16:1 and the other prior to 17:0, suggesting they should be 16:2 and 17:1, respectively. The GC chromatogram from the irradiated linoleic acid also showed two large peaks; they should be 16:3 and 17:2. The hydrocarbons from the irradiated fatty acids were also confirmed by GC/mass spectrometry at Korea Ginseng and Tobacco Research Institute (Daejeon, Korea).

*Irradiated sesame seed*. Sesame seed that was irradiated even at 10 kGy was hardly distinguishable from unirradiated seed by appearance or flavor. The sesame seed in this study contained 48.6% fat and 4.5% moisture; therefore, less sample was needed than that for meats or fruits. Subsequently, less sodium sulfate was used to remove moisture.

No unsaturated hydrocarbons were detected in the oil extracted from unirradiated sesame seed (Fig. 1). It has been reported that hydrocarbons 16:1, 16:2, 16:3, 17:1, and 17:2 were detected in unirradiated oils from peanut, sunflower, and extra-virgin olive oils and that small amounts of 17 alkane and alkenes were naturally present in avocado–pear oil, which made quantitative analysis difficult (9). No predicted radiation-induced hydrocarbons were detected in sesame seed irradiated at 0.05 kGy (Table 1). Hydrocarbon 17:2 was detected in the sample at 0.1 kGy. The major irradiation-induced hydrocarbons, 16:2, 16:3, 17:1, and 17:2, were detected in the sample after 0.5 kGy. Hydrocarbons 16:2, 16:1 (from stearic acid), 16:3, 17:1, 17:2, and an unidentified peak (probably 17:3 from linolenic acid) were detected in the samples irradiated at 1.0 kGy or higher. The amount of hydrocarbons increased with the dose. The prominent hydrocarbons produced by λ-radiation of sesame seed were 16:2, 16:3, 17:1, and 17:2, among which 17:2 was the most abundant (Fig. 1). The amount of these radiation-induced hydrocarbons increased almost linearly with the irradiation dose; correlation coefficients were over 0.95 for the four hydrocarbons. The ratios of hydrocarbons n-2,1-ene (16:2 and 16:3) to n-1 (17:1 and 17:2) in sesame seed irradiated at 5 and 10 kGy were about 0.8.

*Roasted and ground sesame seed*. The hydrocarbons detected in irradiated sesame seed should not be produced by other treatments if they are to be used as irradiation markers.

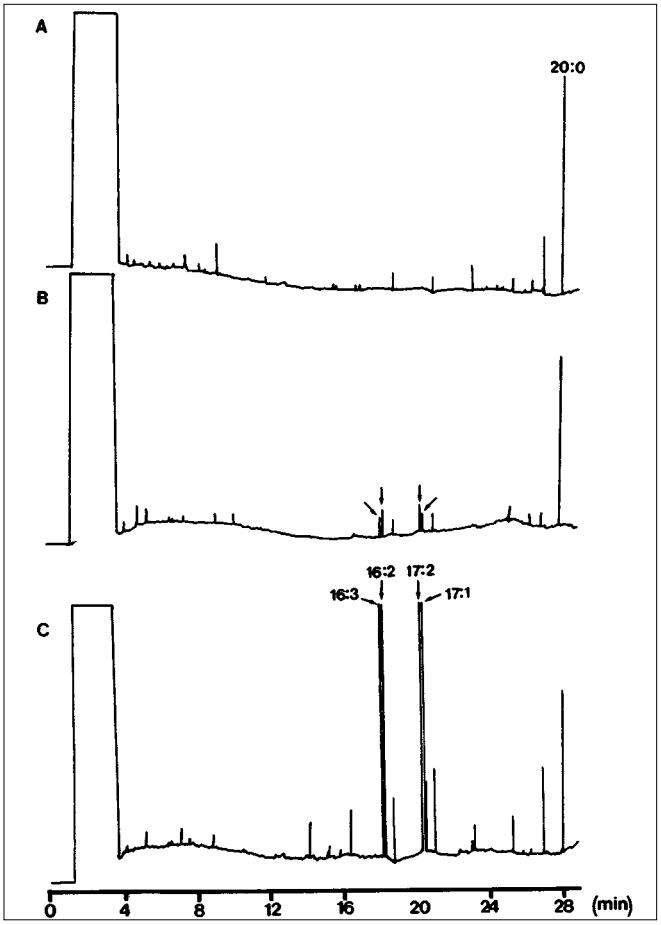


**FIG. 1.** Chromatograms of the hydrocarbons isolated from unirradiated and irradiated sesame seeds; (A) unirradiated; (B) 0.5 kGy; (C) 10 kGy.

Because sesame seed might be exposed to severe heat during shipment, the hydrocarbons were analyzed in sesame seed after storage at 37°C for 3 mon. Some sesame seed was roasted at an extreme condition, and part was ground and stored for 3 mon in air. It was reported that peanut oil characteristically developed 13:1 and 14:0 by heating, as well as 14:2 exclusively by heating in the presence of oxygen (9). The untreated sample stored for 3 mon did not develop any hydrocarbons inducible by irradiation; some hydrocarbons, such as 14:0, 16:0, and 17:0, which were present in the initial sample, were not detected in the stored sample (Table 2). Roasted sesame seed showed few differences in the types of hydrocarbons from the control, with an increase in some saturated hydrocarbons. After storage of the roasted samples, the seeds lost some hydrocarbons. The saturated hydrocarbons in roasted and ground sesame seed increased significantly during the 3-mon storage. However, no unirradiated sesame seed, regardless of the treatment, contained any irradiation-inducible hydrocarbons.

In conclusion, detection of the prominent radiolytic hydrocarbons, such as 16:2, 16:3, 17:1, and 17:2, in irradiated sesame seed may make it possible to identify whether sesame seed was previously irradiated at 0.5 kGy or higher. Irradiation dose for sesame seed would typically range from 1 to 5 kGy because the purpose of irradiation is mainly to kill insects. Therefore, detection of hydrocarbons can be an applicable method to identify post-irradiation of sesame seed.

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**TABLE 1**
**Hydrocarbons in Irradiated Sesame Seed (μg/g oil)[a]**

| | Dose (kGy) | | | | | | |
|---|---|---|---|---|---|---|---|
| Hydrocarbon | 0 | 0.05 | 0.1 | 0.5 | 1.0 | 5.0 | 10.0 |
| 14:1 | — | — | — | — | — | — | 0.61 (0.12) |
| 14:0 | 0.07 (0.12) | 0.18 (0.10) | 0.19 (0.15) | — | — | — | 0.17 (0.10) |
| 15:0 | — | 0.41 (0.31) | 0.31 (0.11) | — | 0.13 (0.02) | 0.39 (0.00) | 0.98 (0.39) |
| 16:3 | — | — | — | 0.23 (0.07) | 0.53 (0.01) | 2.65 (0.00) | 6.55 (0.01) |
| 16:2 | — | — | — | 0.30 (0.08) | 0.62 (0.01) | 2.98 (0.02) | 7.07 (0.46) |
| 16:1 | — | — | — | — | 0.11 (0.00) | 0.35 (0.00) | 0.92 (0.29) |
| 16:0 | 0.14 (0.04) | 0.45 (0.14) | 0.29 (0.04) | 0.22(0.02) | 0.15 (0.00) | 0.17 (0.00) | 0.29 (0.07) |
| 17:3 (?) | — | — | — | — | 0.14 (0.08) | 0.53 (0.00) | 1.41 (0.07) |
| 17:2 | — | — | 0.16 (0.72) | 0.38 (0.06) | 0.55 (0.03) | 3.67 (0.00) | 8.25 (0.65) |
| 17:1 | — | — | — | 0.26 (0.05) | 0.42 (0.04) | 3.17 (0.01) | 7.59 (0.82) |
| 17:0 | 0.09 (0.00) | 0.26 (0.05) | 0.36 (0.06) | 0.23 (0.05) | 0.31 (0.00) | 0.44 (0.03) | 1.27 (0.26) |
| 18:0 | 0.18 (0.05) | 0.31 (0.12) | 0.60 (0.39) | 0.24 (0.06) | 0.13 (0.01) | 0.27 (0.01) | 0.28 (0.01) |
| 19:0 | 0.12 (0.05) | 0.22 (0.01) | 0.22 (0.04) | 0.53 (0.20) | 0.16 (0.02) | 0.31 (0.02) | 0.44 (0.02) |

[a]Mean (standard deviation) of duplicate samples.

**TABLE 2**
**Hydrocarbons in Raw, Roasted, and Ground Unirradiated Sesame Seed (μg/g oil)[a]**

| | Raw | | Roasted | | Roasted and ground | |
|---|---|---|---|---|---|---|
| Hydrocarbons | 0 d | 37°C; 3 mon | 0 d | 20°C; 3 mon | 0 d | 20°C; 3 mon |
| 14:1 | — | — | — | — | — | — |
| 14:0 | 0.07 (0.12) | — | — | — | — | — |
| 15:0 | — | — | — | — | — | — |
| 16:3 | — | — | — | — | — | — |
| 16:2 | — | — | — | — | — | — |
| 16:1 | — | — | — | — | — | — |
| 16:0 | 0.15 (0.00) | — | 0.29 (0.07) | — | 0.39 (0.00) | 0.62 (0.14) |
| 17:3 (?) | — | — | — | — | — | — |
| 17:2 | — | — | — | — | — | — |
| 17:1 | — | — | — | — | — | — |
| 17:0 | 0.09 (0.00) | — | 0.25 (0.07) | — | 0.15 (0.04) | 0.93 (0.00) |
| 18:0 | 0.18 (0.05) | 0.18 (0.15) | 0.39 (0.05) | 0.12 (0.02) | 0.27 (0.14) | 1.31 (0.29) |
| 19:0 | 0.12 (0.05) | 0.13 (0.32) | 0.24 (0.04) | 0.09 (0.03) | 0.21 (0.01) | 0.30 (0.05) |

[a]Mean (standard deviation) of duplicate samples.


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