Gas Chromatographic and Electron Spin Resonance Investigations of γ-Irradiated Shrimp

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When fats are irradiated, some of the major stable products formed are hydrocarbons. The most abundant radiolytic hydrocarbons are formed during various free-radical reactions as the result of the loss of CO_2. An extraction procedure followed by capillary gas chromatography (GC) to monitor the formation of these radiolytic hydrocarbons in γ-irradiated shrimp has been developed. Shrimp contain appreciable amounts of palmitic, palmitoleic, stearic, oleic, and linoleic acids. When irradiated, these fatty acids form the hydrocarbons pentadecane, 8-pentadecene, heptadecane, 8-heptadecene, and 6,9-heptadecadiene, respectively. The yield of these radiolytic hydrocarbons was found to be linear with absorbed dose. Data indicating the utility of the capillary GC technique for identifying radiation-treated shrimp are presented. The potential use of electron spin resonance (ESR) spectroscopy, another irradiation detection technique, to monitor the formation of free radicals trapped in irradiated shrimp shell and problems associated with using ESR spectroscopy are also described.

INTRODUCTION

The interest in and use of ionizing radiation for the treatment and preservation of food are increasing throughout the world. Foods are treated with ionizing radiation to decrease microbial and insect infestations, inhibit maturation, and extend shelf life (IAEA, 1978; Josephson and Peterson, 1982). Ionizing radiation can be used in place of, or in conjunction with, chemical treatment and other processes currently used to preserve foods. The treatment of food by ionizing radiation is accepted for specific purposes in several countries, although in other countries the sale of irradiated food for human consumption is prohibited. Recently, the U.S. Food and Drug Administration (FDA) established regulations to allow the treatment of several foods with ionizing radiation (*Code of Federal Regulations*, 1990; *Federal Register*, 1986, 1988). It would be advantageous to have a postirradiation dose-measuring method to determine whether a commercial food has been treated with ionizing radiation and is within the FDA's regulatory limitations on permissible food types and maximum allowable absorbed dose.

Many research groups have investigated the possibility of identifying a radiolytic product, or marker compound, that is formed when a food is treated with ionizing radiation (Bogle, 1990; Delincee et al., 1988; Desrosiers, 1989; Desrosiers and Simic, 1988; Dodd et al., 1985; Farkas et al., 1990; Goodman et al., 1989; Hayashi et al., 1982; Karam and Simic, 1988; Meier et al., 1988, 1990; Morehouse and Ku, 1990; Moriarty et al., 1988; Nawar and Balboni, 1970; Pfeilsticker and Lucas, 1987; Swallow, 1990). The marker compound could then be monitored to determine whether a particular food item has been treated with ionizing radiation. An ideal choice for such a marker would be a radiolytic product that changes in quantity predictably with absorbed dose and that is stable during storage of the irradiated commodity. Because of the variety of foods that could potentially be treated with ionizing radiation, one technique will not apply to all foods and the development of several techniques will be required.

This paper describes an analytical procedure for the determination of radiation treatment in shrimp and estimation of the absorbed dose. The procedure, which is based on an approach originally proposed by Nawar and Balboni (1970), involves the determination by capillary gas chromatography (GC) of stable hydrocarbons formed during the radiolysis of lipids after their extraction and isolation from shrimp.

When lipids (i.e., triglycerides or fatty acids) are irradiated, the absorption of the applied energy causes formation of several free radicals. The primary free radicals can undergo many further reactions to form secondary radicals and various stable products (Dubravcic and Nawar, 1968; Faucitano et al., 1972; Handel and Nawar, 1981; Howton and Wu, 1967; Merritt et al., 1978, 1985; Nawar, 1978; Nawar et al., 1969; Sevilla et al., 1983).

A series of saturated and unsaturated hydrocarbons arise from termination of alkyl radicals (Dubravicic and Nawar, 1968, 1969; Faucitano et al., 1972; Handel and Nawar, 1981; Howton and Wu, 1967; Merritt et al., 1978, 1985; Nawar 1978; Nawar and Balboni, 1970). One of the hydrocarbons produced by the radiolysis of each fatty acid or triglyceride contains one less carbon atom than its corresponding precursor fatty acid. This hydrocarbon product is formed as a result of the loss of the carboxylic acid group by several free-radical reactions. Furthermore, radiolytic decarboxylation is influenced by temperature, physical state, and chain length of the fatty acid. Another hydrocarbon, formed by the loss of CH_3COOH, contains two fewer carbon atoms than its parent fatty acid and a double bond at the C_1 position. In addition to these radiolytic hydrocarbons, many other products are formed by several other free-radical reactions.

We recently reported a simple extraction procedure for the isolation and identification of specific hydrocarbons that are formed during the radiolysis of certain lipids (Morehouse and Ku, 1990; Morehouse et al., 1991). This extraction technique is based on procedures normally employed for organochlorine pesticide residue analysis (AOAC, 1990). It was applied to the identification of frog legs that had been treated with ionizing radiation (Morehouse et al., 1991). The procedure gave equally good results for the extraction of the fat and radiolytic hydrocarbons present in radiation-treated shrimp. Our investigation of the relationship between the radiolytically generated hydrocarbons and the absorbed radiation dose in shrimp is described, as are the differences we observed among various species of shrimp.

Measurement of stable radiation-induced free radicals

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within the matrix of calcified tissue by electron spin resonance (ESR) spectroscopy is well established (Ikeya and Miki, 1980). An extension of this technique to food provides one of the most promising methods for the identification of foods treated with ionizing radiation (Dodd et al., 1985, 1988). Radiation-induced free radicals produced in hard matrices of foods (bone, shell, seeds) become trapped and may be monitored by ESR spectroscopy because the ESR signals persist for several months (Desrosiers and Simic, 1988; Dodd et al., 1988). The yield of the radiation-induced free radicals can be quantitated by ESR spectroscopy and, therefore, can be correlated with the absorbed dose.

Several research groups have recently reported the formation of a stable free radical in shrimp shell when it is irradiated (Desrosiers, 1989; Dodd et al., 1985, 1989; Goodman et al., 1989; Raffi and Agnel, 1989, 1990). However, there are some discrepancies in the reported spectra, in the reported stability of the trapped radical, and in the relationship of the ESR signal to the absorbed dose. The present study examined some of the problems associated with applying ESR to monitor radiaton-induced free radicals trapped in the shrimp shell.

EXPERIMENTAL PROCEDURES

Sample Collection. Fresh shrimp, headless with shell on, were purchased from a local supplier in Washington, DC. Additional shrimp of different species [pink, white, brown (*Peneaus aztecus*), tiger prawns, china whites, and freshwater prawns] were obtained intact from the FDA New York District office and from the FDA Dauphin Island, AL, facility. The shrimp were kept and treated in the frozen state, unless otherwise indicated. Unless identified by species, the shrimp were classified by their observed color (i.e., pink, brown, white). For some of the experiments the shrimp were boiled in water for 5 min, before or after irradiation.

Chemicals. All solvents and chemicals used were of the highest purity available. Petroleum ether, distilled in glass, with a boiling range of 30–60 °C, and UV grade acetonitrile were obtained from Burdick & Jackson (Muskegon, MI). Granular anhydrous sodium sulfate, obtained from Mallinckrodt (Paris, KY), was heated at 700 °C for 12 h before use. Florisil was heated at 130 °C for 24 h the day before use. The *n*-alkane and 1-alkene standards were obtained from Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO). The fatty acid methyl ester standards were obtained from Sigma.

γ-Radiolysis. Nonirradiated shrimp were packed on dry ice and shipped to a commercial irradiation facility (Isomedix, Inc., Whippany, NJ) for treatment. Shrimp were irradiated at various doses (0.5–1 kg per absorbed dose) and returned to us on dry ice. Some of the shrimp were irradiated locally by a Gammacell 220 (0.12 kGy/m in water, National Institute of Standards and Technology, Gaithersburg, MD). The shrimp were generally irradiated whole, with meat and shell intact. However, for the ESR experiments some of the shrimp shell was irradiated after the meat had been removed and the clean shell had been dried under vacuum. The absorbed dose was calculated with respect to water. No corrections were made for dose-depth distributions or differences between the stopping power or absorption coefficient of the analytical sample and that of the dosimeter.

Extraction. The shrimp shell was removed, the shrimp meat was cut up, and the radiolytic hydrocarbons from the meat were extracted with the lipids. A modification of the acetonitrile extraction procedure of the Association of Official Analytical Chemists (AOAC) for determination of organochlorine pesticides in nonfatty foods (AOAC, 1990, Method 970.52, K, a and e) was used to extract duplicate 100-g test portions of shrimp meat from each irradiated batch. Each extract was analyzed in duplicate by GC for the radiolytic hydrocarbons. A reagent blank and a nonirradiated shrimp control were analyzed along with each set of duplicates.

Shrimp meat duplicates were blended in a Waring blender (fitted with Teflon gaskets) with 200 mL of acetonitrile. The supernatant was filtered through a Büchner funnel fitted with sharkskin filter paper. The residue was transferred to the funnel and pressed to force out remaining solvent. The acetonitrile extract was then partitioned with 100 mL of petroleum ether. A 5-mL aliquot of the petroleum ether extract was transferred to a Teflon-lined screw-cap vial containing 1 mg of tricosanoic acid methyl ester (C23:0), as the internal standard (ISTD), for fatty acid analysis.

Florisil Column Cleanup. The radiolytic hydrocarbons in the remaining petroleum ether extract were separated from the lipids by Florisil column chromatography (AOAC, 1990, Method 970.52, O). The column was prepared by adding 10 cm of Florisil to a 22 mm i.d. × 400 mm chromatographic tube fitted with a fritted glass disk and a Teflon stopcock. The Florisil was topped with 1 cm of anhydrous sodium sulfate and prewashed with 100 mL of petroleum ether. The petroleum ether extract containing the lipid and radiolytic hydrocarbons was placed on the Florisil column, and the column was eluted at about 5 mL/min with a total volume of 200 mL of petroleum ether. Because petroleum ether quantitatively elutes the hydrocarbons from the Florisil and yields a clean eluate, the Florisil column procedure was modified to use 200 mL of petroleum ether as the eluting solvent [modification of the AOAC procedure as reported by Yurawecz et al. (1976)]. The eluate was concentrated to 1 mL, after the addition of 1 mL of isooctane, by using a Kuderna-Danish concentrator equipped with a 3-ball Snyder column and heated with a steam bath.

Fatty Acid Methyl Ester Preparation. The lipid concentration and fatty acid composition of each test portion of shrimp were determined according to a modification of the procedure of Eining and Ackman (1987). The concentration of each radiolytic hydrocarbon was reported as nanograms per milligram of its precursor fatty acid. To determine the fatty acid content of the test portions of shrimp, the 5-mL aliquot of the petroleum ether solution containing the lipids and radiolytic hydrocarbons, which was taken prior to Florisil column cleanup, was transferred to a tube containing 1 mg of tricosanoic acid methyl ester ISTD. The solvent was evaporated with a stream of dry nitrogen, and the residual fat was processed and analyzed as previously described (Morehouse et al., 1991).

Gas Chromatography. The radiolytic hydrocarbons were quantitated by using a capillary gas chromatograph (HP 5890A, Hewlett-Packard Co., Avondale, PA), equipped with an HP 5895A workstation, an HP 7673A autosampler using a split/splitless injector (200 °C), and a flame ionization detector (250 °C). A 1-μL aliquot of the concentrated Florisil eluate was injected into the gas chromatograph operating in the splitless mode. Two capillary columns were used: a DB-23 (50% cyanopropyl polysiloxane, 30 m × 0.25 mm i.d., 0.25-μm film thickness, J&W Scientific, Folsom, CA) and an Ultra-2 (5% phenyl, 95% methyl polysiloxane, 25 m × 0.2 mm i.d., 0.3-μm film thickness, Hewlett-Packard). The capillary columns were used with the following temperature programs: DB-23, 50 °C, 5 °C/min to 200 °C, hold for 10 min; Ultra-2, 80 °C for 1 min, 5 °C/min to 200 °C, hold for 10 min. The concentrations of the radiolytic hydrocarbons present in the extracts were quantitated by using external standards of known concentrations. The concentrations of the radiolytic hydrocarbons in each original test portion were calculated as nanograms per gram in the meat, by using the equations in the AOAC method for nonfatty foods (AOAC, 1990, Method 970.52, K).

The radiolytic hydrocarbons were identified by GC/mass spectrometry (MS) or by comparison of their retention times with those of authentic hydrocarbon standards on both capillary GC columns. The radiolytic hydrocarbons isolated from γ-irradiated triglycerides or fatty acids were also used as reference standards.

The fatty acid methyl esters were quantitated by using an ISTD method and the DB-23 capillary column with the following temperature program: 170 °C for 1 min, 1 °C/min to 200 °C, hold for 5 min. A 1-μL aliquot of the solution containing the fatty acid methyl esters was injected into the gas chromatograph operating in the split mode (50:1). Concentrations of the fatty acids were then expressed as milligrams per gram of shrimp extracted, correcting for the extraction procedure (AOAC, 1990, Method 970.52, K). The fatty acid methyl esters were identified

by comparing their retention times with those of fatty acid methyl ester standards.

Fortification Experiments. Several hydrocarbons were added to untreated shrimp at concentrations from 5 to 80 ng/g. The hydrocarbons were extracted from these spiked controls, and hydrocarbon recoveries were determined.

Electron Spin Resonance (ESR) Spectroscopy. For ESR analysis, shrimp shells were cleaned, freed of meat and fat, dried under vacuum at room temperature in a bulk tray dryer (FTS Systems, Inc., Stone Ridge, NY), and ground to a powder. A weighed portion (ca. 100 mg) was analyzed by ESR spectroscopy (Varian E109 X-band spectrometer equipped with a TE_{102} cavity). The ESR signal intensity was measured as the peak-to-peak height of the major component of the signal and is reported in arbitrary units. Recorded signal intensities were normalized for the weight of shrimp shell used in the analysis. The *g* factors and splitting constants were measured relative to Fremy's salt, as described by Mason et al. (1977).

RESULTS AND DISCUSSION

GC Analysis. A capillary GC-based procedure was used to monitor the radiolytic hydrocarbons formed when shrimp were treated with ionizing radiation.

Of the radiolytically generated hydrocarbons, the decarboxylation products predominate and constitute the major hydrocarbons formed by the radiolysis of the fatty acids or triglycerides present in the shrimp. Shrimp fat contains large amounts of palmitic, palmitoleic, stearic, oleic, and linoleic acids; therefore, one would expect to find the hydrocarbons formed by decarboxylation of these fatty acids [i.e., C_{15}, C_{15}(8-ene), C_{17}, C_{17}(8-ene), and C_{17}(6,9-diene)] when shrimp are irradiated. The C_{14}(1-ene) formed by the loss of CH_3COOH from palmitic acid, the C_{16}(1-ene) formed from stearic acid, and the C_{16}(1,7-diene) formed from oleic acid can also be monitored. Because the $n-2$ alkenes from the other fatty acid precursors are formed in very small quantities, they were not monitored.

After the radiolytic hydrocarbons were extracted with the lipids from several lots of shrimp and isolated, gas chromatograms of the hydrocarbon fractions were obtained. Two GC columns with stationary phases of different polarities (Figures 1 and 2) were used. The figures show that the radiolytic hydrocarbons are absent (or present at very low concentrations) in the nonirradiated control (Figures 1B and 2B) but that they appear after the shrimp are treated with ionizing radiation (Figures 1A and 2A). The chromatograms for the nonirradiated shrimp meat control (Figures 1B and 2B) show the presence of very small amounts of C_{15} (1 ng/g) and C_{17} (1 ng/g) and the absence of the other radiolysis products (or amounts too small to be measured). The detection limit (at a signal-to-noise ratio of 3:1) for the hydrocarbons was approximately 0.5 ng/g based on the extraction of 100 g of shrimp. Any amount less than this was not detectable and is expressed as 0 ng/g. The quantities of the various radiolytically produced hydrocarbons are very low, and extreme care must be exercised during all of the steps of the analysis to minimize contamination. For an absorbed dose of 4 kGy, the concentrations of the individual radiolytic hydrocarbons ranged from 1 to 30 ng/g of meat. The amount of each radiolytic hydrocarbon produced is dependent on the concentration of its precursor fatty acid present in the shrimp (see below).

The identities of the various peaks in the chromatograms were confirmed by comparison of the retention times with those of known standards on both capillary GC columns or by GC/MS. Known quantities of C_{15} and C_{17} (5–80 ng/g) were added to minced shrimp meat controls, and the extracts were analyzed. Average recoveries of 80–90% were obtained for these two hydrocarbons. Long-time storage of shrimp after it was irradiated had no effect on the concentrations of the radiolytic hydrocarbons measured, which verifies an earlier study for irradiated beef (Merritt et al., 1978).

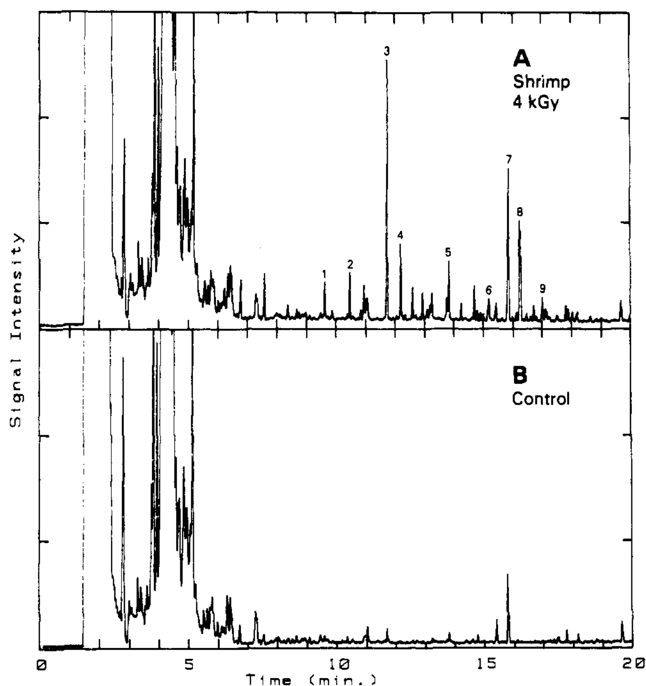

Figure 1. Gas chromatograms of the hydrocarbon fraction from γ-irradiated pink shrimp analyzed by using a DB-23 capillary column. (A) 4-kGy absorbed dose. Oven temperature program: 50 °C; 5 °C/min to 200 °C; hold. Peaks: 1, C_{14}; 2, C_{14}(1-ene); 3, C_{15}; 4, C_{15}(8-ene); 5, C_{16}; 6, C_{16}(1,7-diene); 7, C_{17}; 8, C_{17}(8-ene); 9, C_{17}(6,9-diene). (B) Control.

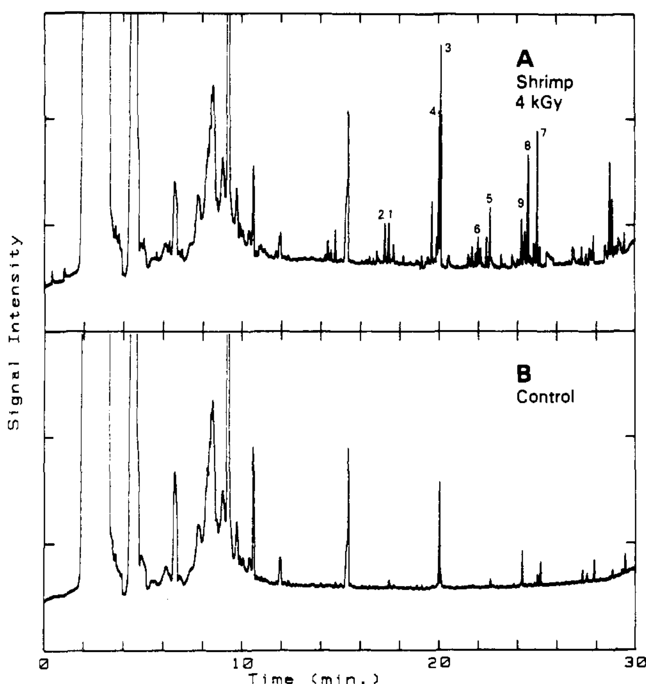

Figure 2. Gas chromatograms of the hydrocarbon fraction from γ-irradiated pink shrimp analyzed by using an Ultra-2 capillary column. (A) 4-kGy absorbed dose. Oven temperature program: 80 °C, 1 min; 5 °C/min to 200 °C; hold. Peaks: 1, C_{14}; 2, C_{14}(1-ene); 3, C_{15}; 4, C_{15}(8-ene); 5, C_{16}; 6, C_{16}(1,7-diene); 7, C_{17}; 8, C_{17}(8-ene); 9, C_{17}(6,9-diene). (B) Control.

The amount of radiolytic hydrocarbon formed is dependent on the amount of percursor fatty acid present in the shrimp being analyzed. Variation of the fatty acid content is demonstrated in Table I, which presents the

Table I. Fatty Acid Methyl Ester Analysis of Various Species of γ-Irradiated Shrimp[a]

batch description	fatty acid concn,[b] mg of fatty acid/g of meat extracted							
	palmitic acid	palmitoleic acid	stearic acid	oleic acid	linoleic acid	linolenic acid	EPA[c]	DHA[d]
pink shrimp								
batch 1	0.14	0.06	0.08	0.05	0.02	0.01	0.24	0.17
batch 2	0.20	0.08	0.11	0.10	0.03	0.01	0.44	0.43
tiger prawns								
batch 1	0.31	0.03	0.12	0.23	0.24	0.01	0.26	0.52
china whites								
batch 1	0.23	0.05	0.11	0.24	0.11	0.01	0.22	0.36
batch 2	0.36	0.08	0.11	0.32	0.19	0.01	0.43	0.50
prawns								
batch 3	0.20	0.10	0.07	0.20	0.09	0.04	0.15	0.08
white shrimp								
batch 1	0.21	0.07	0.13	0.11	0.04	0.02	0.27	0.19
grass shrimp								
Dauphin Island	0.3	0.12	0.11	0.14	0.04	0.03	0.34	0.37
popcorn shrimp								
batch 1	0.09	0.05	0.05	0.12	0.13	0.04	0.19	0.12

[a] GC analysis performed using a DB-23 capillary column. [b] The fatty acid concentration has an experimental error of ±0.01. [c] *cis*-5,8,11,14,17-Eicosapentaenoic acid. [d] *cis*-4,7,10,13,16,19-Docosahexaenoic acid.

Table II. Levels of Hydrocarbons Found in Various Species of γ-Irradiated (4 kGy) and Nonirradiated Control Shrimp[a]

batch description	ng of radiolytic hydrocarbon/g of meat[b]							
	C_{14}(1-ene)	C_{15}	C_{15}(8-ene)	C_{16}(1,7-diene)	C_{16}(1,7,10-triene)	C_{17}	C_{17}(8-ene)	C_{17}(6,9-diene)
pink shrimp								
batch 1, nonirradiated	1.0	1.6	0.0	0.0	0.0	0.0	1.2	0.0
batch 1, 4 kGy	2.3	9.1	1.9	1.5	0.7	1.6	5.7	2.4
batch 2, 4 kGy	2.4	13.7	4.3	2.2	0.0	1.4	8.6	9.2
tiger prawns								
batch 1, 4 kGy	3.0	24.6	2.8	2.0	0.7	14.6	10.8	18.0
china whites								
batch 1, 4 kGy[c]	2.6	29.5	7.0	1.9	0.0	10.2	13.3	26.1
batch 2, 4 kGy	3.3	19.3	4.1	3.0	0.6	7.5	5.5	17.1
prawns								
batch 3, 4 kGy	3.1	8.4	3.3	3.6	0.5	2.8	4.0	7.4
white shrimp								
batch 1, 4 kGy	2.7	16.0	4.6	1.9	0.3	2.4	9.4	8.2
grass shrimp								
Dauphin Island, 4 kGy	2.6	21.2	8.0	1.8	0.4	2.4	8.5	12.8
popcorn shrimp[c]								
batch 1, 4 kGy	2.1	24.4	8.4	1.7	0.6	12.4	11.8	17.6

batch description	ng of radiolytic hydrocarbon/mg of precursor fatty acid[b]							
	C_{14}(1-ene)	C_{15}	C_{15}(8-ene)	C_{16}(1,7-diene)	C_{16}(1,7,10-triene)	C_{17}	C_{17}(8-ene)	C_{17}(6,9-diene)
pink shrimp								
batch 1, nonirradiated	7.3	11.8	0.0	0.0	0.0	0.0	16.4	0.0
batch 1, 4 kGy	15.4	61.1	28.4	28.3	13.2	84.2	68.7	45.3
batch 2, 4 kGy	12.0	68.5	54.4	21.8	0.0	50.0	75.4	91.1
tiger prawns								
batch 1, 4 kGy	22.4	78.3	96.6	8.8	2.9	60.6	89.3	79.6
china whites								
batch 1, 4 kGy[c]	11.3	127.7	134.6	7.8	0.0	93.6	126.7	107.8
batch 2, 4 kGy	9.1	53.3	52.6	9.4	3.2	40.3	52.4	54.1
prawns								
batch 3, 4 kGy	15.2	41.2	33.7	17.8	5.4	30.1	61.5	36.6
white shrimp								
batch 1, 4 kGy	12.9	76.6	65.7	17.4	6.8	54.5	75.2	75.2
grass shrimp								
Dauphin Island	8.7	70.9	69.6	12.7	9.1	54.5	78.0	90.1
popcorn shrimp[c]								
batch 1, 4 kGy	22.6	262.4	158.5	14.3	4.7	251.1	147.9	96.9

[a] GC analysis performed using a DB-23 capillary column. [b] The hydrocarbon concentration has an experimental error of approximately ±20%. [c] Possibly irradiated before collection.

results for the fatty acid determinations of several batches and species of shrimp. There is a difference not only in the total fatty acid content but also in the ratios of the various fatty acids present.

Several batches of shrimp of different species were analyzed for radiolytic hydrocarbons after being treated with ionizing radiation at an absorbed dose of 4 kGy. The results are presented in Table II. When the results are calculated per gram of meat, or per gram of fat, the different batches and species of shrimp do not give similar results. Furthermore, within the same batch of shrimp, each of the radiolytic hydrocarbons gives a different response (Table III and Figure 3) because of the variation in the concentrations of the precursors (Merritt et al., 1978, 1985; Nawar and Balboni, 1970).

When the results are calculated per milligram of precursor fatty acid present in the shrimp, the data from the different species of shrimp compare favorably (Table

Table III. Levels of Radiolytic Hydrocarbons Found in Pink Shrimp γ-Irradiated at Several Absorbed Doses[a]

	ng of radiolytic hydrocarbon/g of meat[b]							
absorbed dose, kGy	C_{14}(1-ene)	C_{15}	C_{15}(8-ene)	C_{16}(1-ene)	C_{16}(1,7-diene)	C_{17}	C_{17}(8-ene)	C_{17}(6,9-diene)
0.0	0.4	1.1	0.0	0.0	0.0	0.7	0.0	0.0
0.5	0.4	1.9	0.2	0.4	0.0	1.3	0.2	0.7
1.0	0.4	3.0	0.5	0.4	0.0	1.8	0.6	0.8
2.0	0.8	3.9	0.8	0.8	0.6	2.3	0.8	0.8
4.0	1.6	5.8	1.4	1.5	1.1	3.4	1.6	1.5
6.0	2.9	8.9	2.1	2.7	1.8	5.7	3.1	0.9
8.0	3.5	8.5	2.5	3.1	2.0	4.6	3.2	2.9
10.0	5.3	12.2	3.5	4.5	3.0	6.9	4.8	1.6
	ng of radiolytic hydrocarbon/mg of precursor fatty acid[b]							
absorbed dose, kGy	C_{14}(1-ene)	C_{15}	C_{15}(8-ene)	C_{16}(1-ene)	C_{16}(1,7-diene)	C_{17}	C_{17}(8-ene)	C_{17}(6,9-diene)
0.0	2.4	6.7	0.0	0.0	0.0	7.7	0.0	0.0
0.5	3.1	14.5	3.6	5.4	0.0	17.6	3.8	41.2
1.0	2.8	20.7	8.2	4.9	0.0	22.0	10.2	42.1
2.0	5.6	27.5	13.6	10.4	11.3	29.9	15.1	44.4
4.0	12.5	45.3	26.4	20.5	22.0	46.6	32.0	93.7
6.0	20.1	61.8	36.8	33.3	31.0	70.4	53.4	47.4
8.0	28.7	69.7	50.0	43.1	37.7	63.9	60.4	181.2
10.0	37.9	87.1	89.7	54.2	62.5	83.1	100.0	80.0

[a] GC analysis performed using a DB-23 capillary column. [b] The hydrocarbon concentration has an experimental error of approximately ±20%.

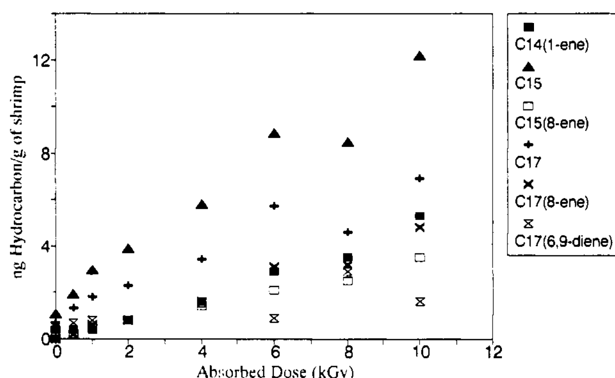

Figure 3. Plot of the gas chromatographic data from the analysis of radiation-treated pink shrimp, expressed as nanograms of radiolytic hydrocarbon per gram of meat vs absorbed dose (data from Table III).

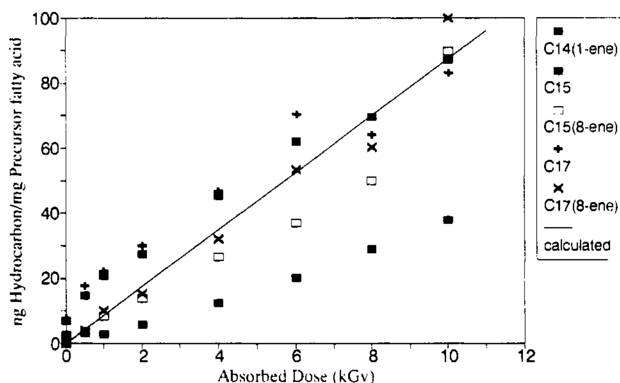

Figure 4. Plot of the gas chromatographic data from the analysis of radiation-treated pink shrimp, expressed as nanograms radiolytic hydrocarbon per milligram of precursor fatty acid vs absorbed dose (data from Table III). The line was calculated from a linear regression of the data points for the radiolytic hydrocarbons, except C_{14}(1-ene), found in the radiation-treated shrimp.

III and Figure 4). If the results are calculated in this way, the various yields of C_{n-1} radiolytic hydrocarbons can be compared with one another. It is not surprising that the scatter in the numbers for Tables II and III and for the data points in Figure 4 is quite large because there is significant variability in the determination of the trace levels of radiolytic hydrocarbons (ca. ±20%) as well as the fatty acid concentrations (ca. ±10%) that are used to obtain the tabulated or plotted ratios. These results are similar to our previously published data from the determination of these radiolytic hydrocarbons in frog legs (Morehouse and Ku, 1990; Morehouse et al., 1991) and to those reported by Merritt et al. (1985). Therefore, to overcome the differences in the concentrations of the various fatty acids present in different batches and types of shrimp and the differences in the total fat content, the final results are given as nanograms of radiolytic hydrocarbon per milligram of precursor fatty acid.

Table III displays the radiolytic hydrocarbon analysis data for a batch of pink shrimp treated at various irradiation doses (0.5–10 kGy) at −15 °C, expressed as nanograms of radiolytic hydrocarbon per milligram of precursor fatty acid. The quantity of the radiolytically generated hydrocarbons which determines the lowest absorbed radiation dose that can be measured depends on the amount of the precursor fatty acids present in the shrimp. The concentrations of the radiolytic hydrocarbons per milligram of precursor fatty acid exhibit a linear response with respect to the absorbed dose (Figure 4), and the yields of the C_{n-1} radiolytic hydrocarbons correlate well with one another, within the limits of experimental error. The line in Figure 4 represents a linear regression for the data points from the C_{15}, C_{15}(8-ene), C_{17}, and C_{17}-(8-ene) radiolytic hydrocarbons. The C_{n-2} alkenes, which are formed by a radiolytic mechanism different from that of the C_{n-1} alkanes, do not fit the same linear regression, but they can be fit to a regression line with a lower slope.

Although the major radiolytic hydrocarbons [i.e., C_{15}, C_{17}, C_{17}(8-ene), and C_{17}(6,9-diene)] deviate from linearity at higher absorbed doses, the C_{14}(1-ene) product, which is produced by a different mechanism, does not deviate but continues to increase in concentration with absorbed dose. Deviation from linearity is not as noticeable for the γ-irradiated shrimp as it was for frog legs (Morehouse et al., 1991). This may be due to the higher fat content in shrimp and to the resultant lower radiation saturation effect. Deviation from linearity is important because it is easier to underestimate high absorbed doses when only the major radiolytically generated hydrocarbons are determined. However, shrimp will probably not be treated with doses greater then 10 kGy because irradiation plants

Table IV. Levels of Radiolytic Hydrocarbons Found in γ-Irradiated Shrimp, before and after Cooking[a]

absorbed dose, kGy	ng of radiolytic hydrocarbon/mg of precursor fatty acid[b]							
	C_{14}(1-ene)	C_{15}	C_{15}(8-ene)	C_{16}(1-ene)	C_{16}(1,7-diene)	C_{17}	C_{17}(8-ene)	C_{17}(6,9-diene)
Irradiated and Analyzed Raw								
0.0	2.4	6.7	0.0	0.0	0.0	7.7	0.0	0.0
1.0	2.8	20.7	8.2	4.9	0.0	22.0	10.2	42.1
2.0	5.6	27.5	13.6	10.4	11.3	29.9	15.1	44.4
4.0	12.5	45.3	26.4	20.5	22.0	46.6	32.0	93.7
6.0	20.1	61.8	36.8	33.3	31.0	70.4	53.4	47.4
Irradiated Raw, Cooked, and Analyzed								
2.0	5.3	30.0	14.0	10.5	8.1	32.6	16.1	30.0
4.0	12.0	53.6	24.6	21.5	20.0	57.0	36.9	100.0
Irradiated Cooked and Analyzed								
0.0	3.3	7.7	0.0	6.0	0.0	7.0	0.0	34.6
2.0	5.0	28.7	12.2	11.2	8.7	27.6	18.8	26.1
4.0	10.6	37.9	19.3	19.1	19.2	35.5	28.2	14.8

[a] GC analysis performed using a DB-23 capillary column. [b] The hydrocarbon concentration has an experimental error of approximately ±20%.

will want to use the lowest, most economical doses possible to achieve the desired treatment.

During the course of these experiments, several different batches and species of shrimp were investigated. For most of the shrimp studied, the dose–response relationship for the C_{n-1} and C_{n-2} radiolytically generated hydrocarbons was similar to that displayed in Figure 4. However, for some species of shrimp the slope for the C_{n-1} radiolytic hydrocarbons deviated substantially from that displayed in Figure 4. Differences in the amount and type of fat present in the different batches of shrimp may account for the yield variations found for these C_{n-1} radiolytic hydrocarbons. Also, as expected, variations in the concentrations of the precursor fatty acids in the shrimp affected the determination of the radiolytically generated hydrocarbons. For shrimp with very low concentrations of linolenic acid, it was difficult to verify the presence of C_{17}(6,9-diene) in the lipid extract, and therefore, data error and scatter are expected to be larger than the patterns shown in Figure 4 (see Tables II and III).

In our initial experiments, several *n*-alkanes, including C_{15} and C_{17}, were found in reagent blanks and in nonirradiated control shrimp, as they were in frog leg analyses (Morehouse et al., 1991). Because the measured nanograms per gram levels are very low, it is important to take proper precautions to avoid or limit contamination, particularly by the C_{15} and C_{17} hydrocarbons. If *n*-alkanes are present as a result of contamination and only C_{15} and C_{17} are monitored, it is possible to misidentify untreated food as having been irradiated. Therefore, all radiolytically generated hydrocarbons expected to be present in a food must be monitored in blanks and controls to ensure accurate interpretation of the results for radiation-treated foods by this procedure. However, the presence of the C_{15}(8-ene), C_{17}(8-ene), and C_{17}(6,9-diene) radiolytic hydrocarbons is a good indication that a product was treated with ionizing radiation, because these hydrocarbons are generally not found as contaminants, and thus can be easily monitored. Therefore, it is recommended that these three hydrocarbon groups be used to determine the absorbed radiation doses for shrimp of unknown origin, by averaging the doses determined for the compounds measured. It is also desirable to use GC/MS to confirm the identity of these radiolytic hydrocarbons in selected test samples. Our results indicate that when shrimp with very low fat content are contaminated with petroleum byproducts, it is very difficult to measure the radiolytic hydrocarbons generated by low doses of absorbed radiation (1 kGy or less). Extreme care must be taken to confirm treatment of shrimp at low dose levels of ionizing radiation.

The absence of radiolytically generated hydrocarbons is a good indication that fresh (uncooked) shrimp have not been treated with ionizing radiation, whereas the presence of these hydrocarbons is a good indication that the shrimp have been irradiated.

Although the error in estimating the original absorbed dose from a single measurement is large because of the error inherent in determining the concentration of the radiolytic hydrocarbons, the final result can be improved by monitoring several radiolytic hydrocarbons and averaging the results expressed as absorbed dose. The experimental error for this procedure leads to a large uncertainty in the calculated absorbed dose (±1 kGy). The accuracy of the estimated absorbed dose can be improved by irradiating the shrimp in question at several doses and extrapolating the results to 0 ng/g (method of standard additions), as demonstrated by Desrosiers (1989), who estimated absorbed dose by ESR spectroscopy.

Various parameters in the radiolysis process can affect the yields of the radiolytic products. For simple fatty acids the yield of the radiolytic hydrocarbons increases with the radiolysis temperature (Faucitano et al., 1972), and similar effects were reported for irradiated meats and meat substances (Merritt, 1980; Merritt et al., 1978). However, other authors have stated that there is no temperature effect on the yields of the radiolytic hydrocarbons (Morehouse et al., 1991; Vajdi et al., 1982). Several nonirradiated shrimp controls were irradiated at three temperatures (−78, −15, and 0 °C) and at several absorbed doses to check whether radiolysis temperature affects the production of the radiolytic hydrocarbons in shrimp. Extracts from the shrimp meat were analyzed by capillary GC. Within experimental error, the concentrations of the radiolytic hydrocarbons did not differ for the shrimp irradiated at the same dose but at different temperatures. Thus, it appears that radiolysis temperature has a minimal effect on the estimated absorbed dose.

Another factor that could affect the yields of radiolytic products is whether the shrimp were fresh or cooked. We investigated the effect of cooking, before and after treating the shrimp with ionizing radiation (Table IV). Cooking the shrimp, before or after radiolysis, neither increased nor decreased the quantity of radiolytic hydrocarbons in control or irradiated shrimp. If control shrimp had shown an increase in radiolytic hydrocarbons after cooking, the result would have given a false positive. Because cooking the shrimp did not decrease the concentrations of radiolytic hydrocarbons, we were able to show that it would not be possible to disguise irradiated shrimp by cooking them before or after radiolysis. Therefore, either raw or cooked

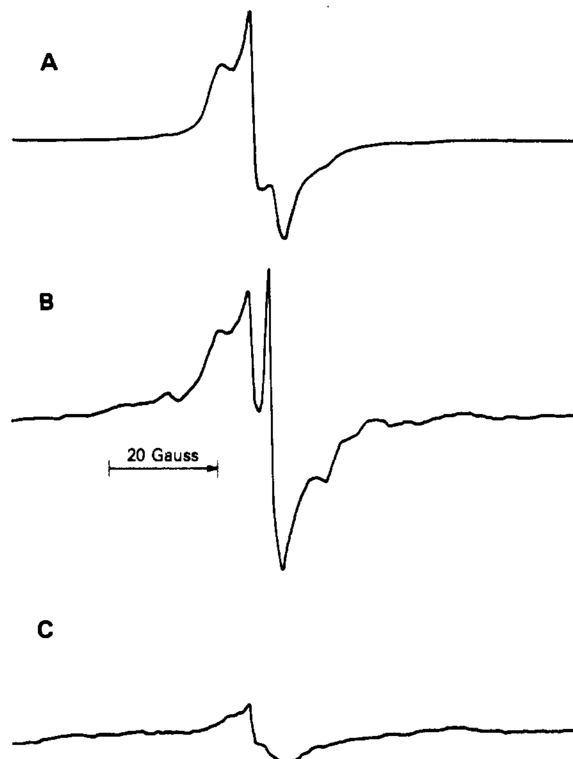

Figure 5. ESR spectra of γ-irradiated pink shrimp shell. (A) Cleaned, dried, and ground; then irradiated (2 kGy). (B) Irradiated (2 kGy) shrimp, whole; then cleaned, dried, and ground. (C) Control shrimp, cleaned, dried, and ground. (Raw data, not normalized for the weight of shrimp shell.) Spectrometer settings: 10-mW microwave power, 2-G modulation amplitude, 1-s time constant, and a 16-min scan time.

shrimp may be examined by this procedure for evidence of previous irradiation.

Several batches of shrimp suspected of having been treated with ionizing radiation were obtained from the FDA's New York and Chicago District offices. Extraction and GC analysis were applied to these shrimp to determine if they had been so treated. Three of the eight test samples analyzed exhibited the pattern of radiolytic hydrocarbons normally present in irradiated shrimp, indicating treatment with low doses (1–3 kGy) of ionizing radiation. An interlaboratory investigation is under way to validate this procedure for possible regulatory use.

ESR Analysis. When bone is irradiated, a characteristic ESR signal associated with the trapping of free radicals in the bone matrix develops and is easily measured. Recently, ESR spectroscopy has been applied to shrimp to identify those that have been treated with ionizing radiation (Desrosiers, 1989; Dodd et al., 1985, 1989; Goodman et al., 1989; Raffi and Agnel, 1989). An ESR signal associated with a stable free radical was observed in the irradiated shrimp shell (cuticle). Unfortunately, there are some discrepancies in the reported ESR spectra, in the reported stability of the trapped radical, and in the relationship of the ESR signal to the absorbed dose. Therefore, we have investigated the ESR signal formed in the shell of irradiated shrimp to determine the feasibility of using this signal as a marker of radiation-treated shrimp.

Three different ESR signals were obtained for pink shrimp, depending on how the shrimp shell was treated. Figure 5A displays the ESR spectra obtained when the shell from pink shrimp was irradiated after being cleaned free of meat and dried. The signal producing this spectrum was found to be stable for at least 2 weeks, to increase linearly with the absorbed dose, and to be similar to that reported by Dodd et al. (1985). However, when the shrimp shell was irradiated wet or with the meat intact and then dried, a different ESR spectrum was obtained (Figure 5B), one that is similar to the spectrum reported by Raffi and Agnel (1989). This signal was found to decay with time (at room temperature). The dried, nonirradiated control gave a different, and much-reduced, ESR signal (Figure 5C). Various species of shrimp were investigated by ESR spectroscopy. Shell that was dried and then irradiated exhibited similar ESR spectral results regardless of the variety of shrimp investigated. However, different batches of pink shrimp exhibited different ESR signals when the shrimp shell was irradiated while frozen and in contact with the meat (Figure 6). The ESR spectra also varied greatly from species to species, with the spectra of some species exhibiting a large singlet, which occasionally increased after the shrimp were irradiated. One of the batches of pink shrimp gave an ESR spectrum similar to that reported by Desrosiers (1989), and another batch exhibited a large Mn^{2+} ESR signal (Goodman et al., 1989; Raffi and Agnel, 1990). It was also observed that the ESR signal response was not dose-dependent for some of the shrimp investigated. As long as the shrimp and shells were kept frozen (−20 °C), the ESR signal was stable for several months.

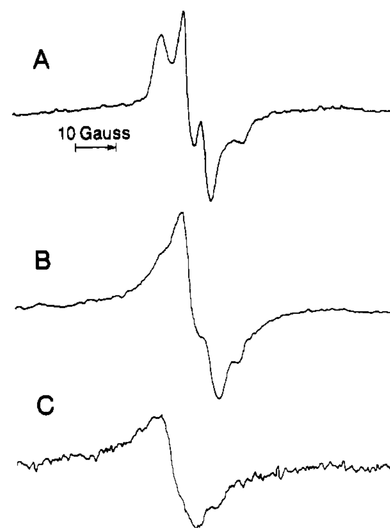

Figure 6. ESR spectra of γ-irradiated (4 kGy) shrimp shell from three different batches of pink shrimp. Spectrometer settings were as in Figure 5.

The shrimp shell was dried to remove the water before analysis in the ESR spectrometer to prevent microwave absorption by water. When a small amount of the wet shrimp shell was placed in the ESR cavity, the ESR spectrum obtained was the same as that for the dry shrimp shell, but the intensity of the ESR signal was much lower. Therefore, the removal of the water did not affect the spectral characteristics. Because the ESR spectral characteristics observed for the different species of shrimp varied during the course of this investigation, it is probable that the discrepancies in the ESR spectra reported previously in the literature are due to the use of different species of shrimp.

The shrimp suspected of being irradiated were analyzed by both the GC procedure and the ESR procedure if the shell was present with the shrimp. None of the shrimp that were found to be negative by the GC procedure exhibited a radiation-induced ESR signal. Of the three test samples that displayed the radiolytic hydrocarbon pattern, only one exhibited a radiation-dependent ESR signal. The ESR spectra for the other two could not be

determined because, in one case, the original shrimp was provided to us without the shell and the spectrum for the other lot exhibited a large manganese signal, which made it difficult to identify a radiation-dependent ESR signal in the shell. Further work in our laboratory on the ESR analysis of shrimp shell is reported elsewhere (Morehouse and Desrosiers, 1992).

Conclusions. The present study demonstrates that shrimp that have been treated with ionizing radiation can be identified by extracting and monitoring specific radiolytically generated hydrocarbons. The radiolytic hydrocarbons that will be formed can be predicted by first determining the fatty acid profile of the shrimp under investigation. If due care is taken to avoid hydrocarbon contamination during the analysis and if the shrimp being investigated are re-irradiated, a good estimation of the absorbed dose can be obtained. An interlaboratory study of this procedure is in progress.

This study also reports the use of ESR spectroscopy to identify radiation-processed shrimp by monitoring free radicals present in the shrimp shell. Many problems were encountered with this procedure, including ESR spectral differences from species to species and batch to batch. How the shrimp shell was processed before and after ionizing radiation treatment also drastically influenced the spectra. Further ESR spectral investigations of radiation-treated shrimp shell are in progress.

ABBREVIATIONS USED

C_{14}(1-ene), 1-tetradecene; C_{15}, *n*-pentadecane; C_{15}(8-ene), 8-pentadecene; C_{16}(1-ene), 1-hexadecene; C_{16}(1,7-diene), 1,7-hexadecadiene; C_{16}(1,7,10-triene), 1,7,10-hexadecatriene; C_{17}, *n*-heptadecane; C_{17}(8-ene), 8-heptadecene; C_{17}(6,9-diene), 6,9-heptadecadiene; DHA, *cis*-4,7,10,13,16,19-docosahexaenoic acid; EPA, *cis*-5,8,11,14,17-eicosapentaenoic acid; ESR, electron spin resonance; GC, gas chromatography; ISTD, internal standard(s); MS, mass spectrometry.

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Registry No. Palmitic acid, 57-10-3; palmitoleic acid, 373-49-9; stearic acid, 57-11-4; oleic acid, 112-80-1; linoleic acid, 60-33-3; pentadecane, 629-62-9; 8-pentadecene, 15430-98-5; heptadecane, 629-78-7; 8-heptadecene, 2579-04-6; 6,9-heptadecadiene, 81265-03-4.