

# Identification of Meat Treated with Ionizing Radiation by Capillary Gas Chromatographic Determination of Radiolytically Produced Hydrocarbons

Kim M. Morehouse,\* Marlene Kiesel, and Yuoh Ku

Division of Food Chemistry and Technology, Food and Drug Administration, Washington, D.C. 20204

When triglycerides or fatty acids are irradiated, some of the major stable products are hydrocarbons formed from the loss of CO<sub>2</sub> and CH<sub>3</sub>COOH in various free-radical reactions. A capillary gas chromatographic procedure has been developed to monitor the presence of these radiolytically generated hydrocarbons in meats treated with ionizing radiation. Several lipid extraction procedures for isolating the radiolytically generated hydrocarbons from the irradiated meat were compared. The radiolytically generated hydrocarbons were separated from the extracted lipids on a Florisil column and determined by capillary gas chromatography. The yield of these radiolytically generated hydrocarbons was linear with absorbed dose. Data indicating the utility of this methodology to identify meat products (poultry, beef, and pork) treated with ionizing radiation are presented.

## INTRODUCTION

Interest in the use of ionizing radiation for the treatment and preservation of food is 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. The U.S. Food and Drug Administration (FDA) has established regulations to allow the treatment of several foods with ionizing radiation (*Code of Federal Regulations*, 1992; *Federal Register*, 1986, 1988, 1990). 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 FDA's regulatory limitations on permissible food types and maximum allowable absorbed dose.

A series of saturated and unsaturated hydrocarbons arise from the termination of alkyl radicals formed during the radiolysis of lipids (Dubravcic and Nawar, 1968; Faucitano et al., 1972; Handel and Nawar, 1981; Howton and Wu, 1967; Merritt et al., 1978, 1985; Nawar, 1978; Nawar and Balboni, 1970). We recently reported a fairly simple procedure for the extraction and gas chromatographic (GC) determination of the specific hydrocarbons that are formed during the radiolysis of lipids (Morehouse and Ku, 1992; Morehouse et al., 1991). The procedure is based on an approach originally proposed by Nawar and Balboni (1970).

This paper describes the results of GC analyses for radiolytically generated hydrocarbons in several meat products containing 1-30% fat. Several lipid extraction techniques investigated during these experiments are compared.

Furthermore, the results obtained from the GC procedure are compared with those from a procedure proposed by Dodd et al. (1985, 1988), which involves the measurement by electron spin resonance (ESR) spectroscopy of radiation-induced free radicals trapped in the hard matrix of calcified tissues. ESR spectroscopy is currently one of

the most promising techniques to identify bone-containing foods that have been treated with ionizing radiation.

## EXPERIMENTAL PROCEDURES

**Test Materials.** The meat (whole chicken breast and thigh quarters, deboned chicken thighs, ground chicken, ground turkey, ground pork, and ground beef) was purchased from Washington, DC, area grocery stores. The deboned thighs were ground in a meat grinder and thoroughly mixed before treatment. The breast and thigh quarters were treated intact as purchased. All products were kept frozen.

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

**$\gamma$ -Radiolysis.** Nonirradiated meat was packed on dry ice and shipped to a commercial irradiation facility (Isomedix, Inc., Whippany, NJ) for treatment. The meat was irradiated at various doses (0.5-1 kg per absorbed dose) and shipped back to us on dry ice. Additionally, some of the frozen meat products were irradiated locally by using a Gammacell 220 (0.1 kGy/min in water, National Institute of Standards and Technology, Gaithersburg, MD). The absorbed dose was calculated with respect to water. No corrections were made for dose-depth distributions or the differences between the stopping power or absorption coefficient of the analytical samples and that of the dosimeter.

**Extraction.** Several different extraction procedures were investigated. These included the Folch (chloroform/methanol) extraction procedure (Bligh and Dyer, 1959; Folch et al., 1957); an automated Soxhlet extraction procedure (Soxtec System HT2, Tecator, Fisher Scientific, Pittsburgh, PA) with petroleum ether as the extraction solvent; a modification of the acetonitrile extraction procedure for determination of organochlorine pesticides in nonfatty foods [AOAC, 1990, Method 970.52, K, a, and e], described by Morehouse et al. (1991) and Morehouse and Ku (1992)]; and a modification of the extraction procedure for determination of organochlorine pesticides in fatty foods (AOAC, 1990, Method 290.52, L, e) (see below).

Approximately 10 g of meat was used for each analysis. When appropriate, the chicken was deboned and minced before the lipid and radiolytic hydrocarbons were extracted from the meat.

Table I. Fatty Acid Composition of Various Meat Products<sup>a</sup>

product	fatty acid concn, <sup>b</sup> mg of fatty acid/g of meat extracted						
	myristic acid	palmitic acid	palmitoleic acid	stearic acid	oleic acid	linoleic acid	linolenic acid
whole chicken breast	0.05	1.68	0.53	0.44	2.44	1.11	0.04
whole chicken thigh	0.19	7.09	2.51	1.55	10.38	4.69	0.16
ground chicken thigh	0.74	21.94	6.74	5.20	32.32	15.07	0.58
ground chicken	0.83	29.39	10.68	6.66	47.31	22.65	0.94
ground turkey	1.19	21.39	3.97	8.45	37.33	30.25	1.78
ground pork	4.41	68.24	6.73	39.41	109.13	17.30	0.82
ground beef	9.23	77.50	9.58	49.68	109.86	4.07	1.26

<sup>a</sup> Determined by using the modified fatty foods extraction procedure. GC analysis performed by using a DB-23 capillary column. <sup>b</sup> The fatty acid concentration has an overall experimental error of  $\pm 10\%$ .

A reagent blank and a nonirradiated control were analyzed along with each set of radiation-treated replicates to determine and minimize possible experimental contamination. Minimum duplicate test portions of meat, at each radiation dose, were extracted. Each extract was analyzed in duplicate for radiolytic hydrocarbons by capillary GC.

For the modified fatty foods extraction procedure used for this investigation, approximately 10 g of meat was blended with 50 g of anhydrous sodium sulfate and 150 mL of petroleum ether. The supernatant was filtered through a Büchner funnel fitted with two sharkskin filter papers. The residue was re-extracted with two 100-mL portions of petroleum ether, and the extract was filtered through the same funnel. The residue was transferred to the funnel and pressed to force out remaining solvent. The combined extracts and rinses were passed through a column of anhydrous sodium sulfate to remove traces of water and collected in a Kuderna-Danish concentrator fitted with a graduated collection flask. The petroleum ether was evaporated to 20 mL by using the Kuderna-Danish concentrator and a steam bath. The extract was then brought to a final volume of 100 mL of petroleum ether in a graduated cylinder with glass stopper. A 1-mL aliquot of the petroleum ether extract was transferred to a Teflon-lined screw-cap vial containing 1 mg of the internal standard, tricosanoic acid methyl ester (C23:0), for fatty acid analysis (Morehouse and Ku, 1992; Morehouse et al., 1991).

**Florisil Column Cleanup.** The radiolytic hydrocarbons were separated from the extracted lipids by Florisil column chromatography (AOAC, 1990, Method 970.52, O), as described by Morehouse et al. (1991) and Morehouse and Ku (1992). The Florisil column was prepared by adding 10 cm of Florisil to a 22 mm i.d.  $\times$  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 extract containing the lipids and the radiolytic hydrocarbons dissolved in petroleum ether 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. The volume of petroleum ether extract applied to the Florisil column was dependent on the concentration of fat in the meat being analyzed and was adjusted so that less than 1 g of fat was used. The eluate was concentrated to 1 mL, after the addition of 1 mL of isooctane, by using a Kuderna-Danish concentrator equipped with a three-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 meat were determined according to a modification of the procedure of Eining and Ackman (1987) (Joseph and Ackman, 1992), as previously described (Morehouse and Ku, 1992). The concentration of each radiolytic hydrocarbon was then reported as nanograms per milligram of its precursor fatty acid. The fatty acid methyl esters were quantitated by using an internal standard method and a DB-23 capillary column as previously described (Morehouse and Ku, 1992).

**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- $\mu$ L aliquot of the concentrated Florisil column 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  $\times$  0.25 mm i.d., 0.25- $\mu$ m film thickness, J&W Scientific, Folsom, CA) and an Ultra-2 (5% phenyl, 95% methyl polysiloxane, 25 m  $\times$  0.2 mm i.d., 0.3- $\mu$ m 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 in the extracts were quantitated by using an external standard containing C<sub>14</sub>(1-ene), C<sub>15</sub>, C<sub>15</sub>(8-ene), C<sub>16</sub>(1-ene), C<sub>16</sub>(1,7-diene), C<sub>16</sub>(1,7,10-triene), C<sub>17</sub>, C<sub>17</sub>(8-ene), and C<sub>17</sub>(6,9-diene) at known concentrations.

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 [C<sub>15</sub>(8-ene), C<sub>16</sub>(1,7-diene), C<sub>16</sub>(1,7,10-triene), C<sub>17</sub>(8-ene), and C<sub>17</sub>(8,11-diene)] isolated from  $\gamma$ -irradiated triglycerides or fatty acids were also used as reference standards. The identities of the radiolytic hydrocarbons isolated from the irradiated triglycerides were confirmed by GC/MS and GC/Fourier transform infrared spectroscopy.

**Fortification Experiments.** Several hydrocarbons were added at various concentrations to nonirradiated meat. The hydrocarbons were extracted from these spiked controls, and recoveries were determined by the various extraction procedures described above.

**Electron Spin Resonance (ESR) Spectroscopy.** Chicken bones 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 broken into small pieces. A weighed portion (ca. 100 mg, but less than 2 cm long) was analyzed by ESR spectroscopy (Varian E109 X-band spectrometer equipped with a TE<sub>102</sub> cavity). The ESR signal intensity was normalized for the weight of bone used in the analysis (Morehouse et al., 1991).

## RESULTS AND DISCUSSION

**Gas Chromatographic Method.** We have used a capillary GC-based procedure to monitor the hydrocarbons formed when meat is treated with ionizing radiation. This procedure is similar to that previously described for frog legs (Morehouse et al., 1991) and shrimp (Morehouse and Ku, 1992). The meat products used in this investigation contain appreciable amounts of palmitic, palmitoleic, stearic, oleic, and linoleic acids (Table I). When these lipids are treated with ionizing radiation, a series of radiolytically generated hydrocarbons is formed from the decarboxylation ( $n - 1$ ) and deacetylation ( $n - 2$ ) of these fatty acids (Table II) (Handel and Nawar, 1981; Merritt et al., 1985; Nawar, 1978).

The meat products used in this investigation varied in total fat content (Table III), and the ratios of the fatty acids in the various meat products were also drastically different (Table I). Therefore, the concentrations of radiolytically generated hydrocarbons present in irradiated meats would be expected to vary, depending on the product being analyzed.

Several extraction procedures were used during this investigation to separate the lipids and the radiolytic

**Table II. Radiolytic Hydrocarbons and Their Precursor Fatty Acids**

precursor fatty acid	radiolytic hydrocarbon	
	<i>n</i> - 1	<i>n</i> - 2
myristic acid	C <sub>13</sub>	C <sub>12</sub> (1-ene)
palmitic acid	C <sub>15</sub>	C <sub>14</sub> (1-ene)
palmitoleic acid	C <sub>15</sub> (8-ene)	C <sub>14</sub> (1,7-diene)
stearic acid	C <sub>17</sub>	C <sub>16</sub> (1-ene)
oleic acid	C <sub>17</sub> (8-ene)	C <sub>16</sub> (1,7-diene)
linoleic acid	C <sub>17</sub> (6,9-diene)	C <sub>16</sub> (1,7,10-triene)
linolenic acid	C <sub>17</sub> (6,9,12-triene)	

**Table III. Total Fat Content of Various Meats<sup>a</sup>**

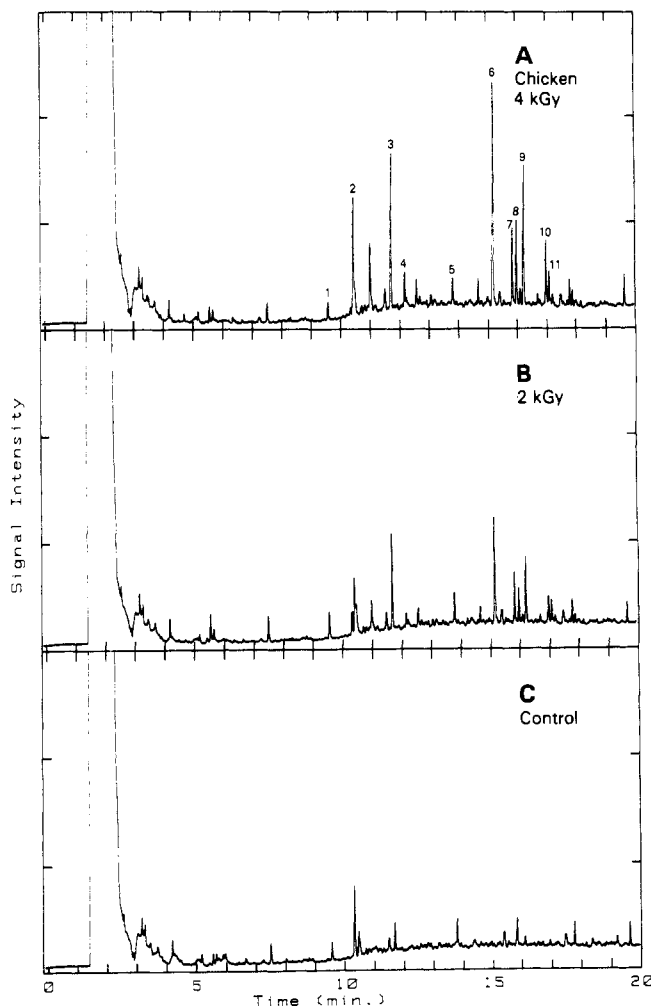
meat	% fat	meat	% fat
whole chicken breast	1.3	ground turkey	11.5
whole chicken thigh	6.4	ground pork	25.9
ground chicken thigh	10.6	ground beef	25.7
ground chicken	11.0		

<sup>a</sup> Determined by using the modified fatty foods extraction procedure.

hydrocarbons from the meat. These included a modification of the nonfatty foods procedure for pesticides that we have previously applied to shrimp (Morehouse and Ku, 1992) and frog legs (Morehouse et al., 1991), a modification of the fatty foods procedure for pesticides (described under Experimental Procedures), the classical Folch (chloroform/methanol) extraction, and an automated Soxhlet procedure using petroleum ether as the extraction solvent. The extraction, regardless of the procedure employed, was then followed by Florisil column cleanup to separate the radiolytic hydrocarbons from the fat, using petroleum ether as the eluting solvent.

Radiation-treated and fortified controls were analyzed to investigate the extraction yields and applicability of the various extraction procedures. The Soxhlet procedure exhibited a recovery for the radiolytic hydrocarbons of about half that of the other procedures, although good yields of total fat were obtained. The high temperature used to facilitate refluxing of the solvent may have caused volatilization losses of the hydrocarbons. The other three extraction procedures gave good recoveries (ca. 90%) for controls fortified with the radiolytically produced hydrocarbons. The nonfatty foods extraction procedure, however, did not lead to quantitative extraction of the fat. Therefore, this extraction procedure would lead to inaccurate approximation of the radiation dose, because the concentrations of the original fatty acids are used to estimate the absorbed dose. The modified fatty foods and Folch extraction procedures gave comparable results, and both adequately extracted the radiolytically produced hydrocarbons from the fortified controls with greater than 90% efficiency. Furthermore, because both of these procedures extracted the same proportion of the fat present in the meat, they would yield, within the limits of experimental error, the same value for the absorbed dose for an unknown. However, because the Folch extraction uses chloroform, we favored the use of the modified fatty foods extraction as the procedure of choice. The work described below used this latter extraction procedure.

Figures 1 and 2 display the gas chromatograms of the hydrocarbon fraction obtained from the extracts of radiation-treated and control ground chicken thighs by using two different capillary columns. Figures 1C and 2C demonstrate that most of the radiolytic hydrocarbons are absent, or present at very low concentrations, in the controls. Upon treatment of the chicken meat with ionizing radiation, the radiolytically generated hydrocarbons appear (Figures 1A,B and 2A,B) and increase in

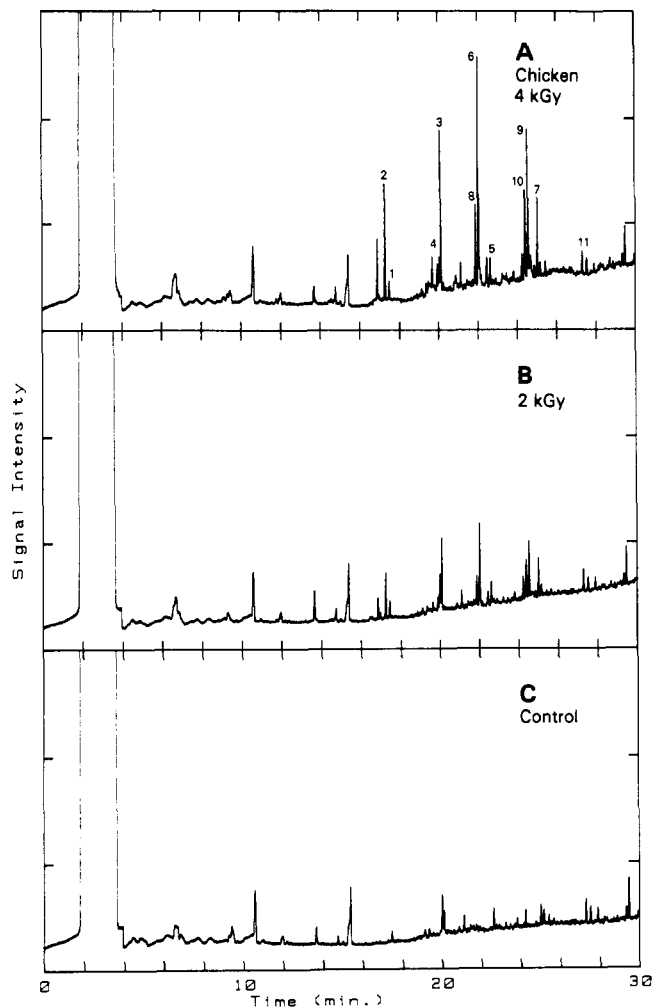


**Figure 1.** Gas chromatograms of the hydrocarbon fraction from  $\gamma$ -irradiated ground chicken thigh meat analyzed by using a DB-23 capillary column. Oven temperature program was as described under Experimental Procedures. (A) 4.1-kGy absorbed dose; (B) 2.1-kGy absorbed dose; (C) control. Peaks: 1, C<sub>14</sub>; 2, C<sub>14</sub>(1-ene); 3, C<sub>15</sub>; 4, C<sub>15</sub>(8-ene); 5, C<sub>16</sub>; 6, C<sub>16</sub>(1,7-diene); 7, C<sub>17</sub>; 8, C<sub>16</sub>(1,7,10-triene); 9, C<sub>17</sub>(8-ene); 10, C<sub>17</sub>(6,9-diene); 11, C<sub>18</sub>.

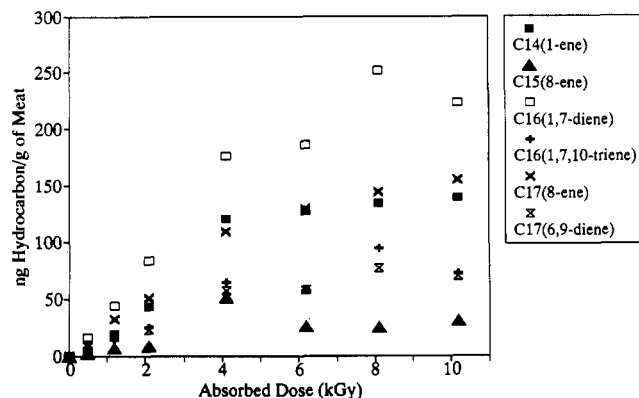
concentration with absorbed dose. Although both capillary columns gave acceptable results, the DB-23 capillary column was generally used for routine analysis because it gave better separation of the radiolytic hydrocarbons.

When the concentration of the radiolytically generated hydrocarbons from the radiation-treated ground chicken thighs is expressed as nanograms of radiolytic hydrocarbon per gram of meat extracted, or per gram of lipid, each of the radiolytic hydrocarbons exhibits a linear relationship to the absorbed dose up to 6 kGy (Figure 3). Table IV and Figure 4 display the radiolytic hydrocarbon data for the irradiated ground chicken thighs, expressed as nanograms of radiolytic hydrocarbon per milligram of originating fatty acid. If the results are calculated in this way, all of the radiolytic hydrocarbons that were monitored can be correlated and, within experimental error, they can be fitted to the same linear expression. The results obtained for chicken are similar to those previously published for radiation-treated shrimp (Morehouse and Ku, 1992).

The line in Figure 4 represents the regression equation for all of the data points displayed. This regression line differs from those constructed from shrimp (Morehouse and Ku, 1992) and frog leg (Morehouse et al., 1991) data representing separate regression equations for *n* - 1 and *n* - 2 hydrocarbons. The concentration of the *n* - 1 radiolytic hydrocarbons produced, expressed as nanograms



**Figure 2.** Gas chromatograms of the hydrocarbon fraction from  $\gamma$ -irradiated ground chicken thigh meat analyzed by using an Ultra-2 capillary column. Oven temperature was as described under Experimental Procedures. (A) 4.1-kGy absorbed dose; (B) 2.1-kGy absorbed dose; (C) control. Peaks are numbered as in Figure 1.



**Figure 3.** Plot of the GC data from the analysis of radiation-treated ground chicken thighs, expressed as nanograms of hydrocarbon per gram of meat extracted vs absorbed dose.

of radiolytic hydrocarbon per gram of meat, was an order of magnitude greater in the chicken than in the shrimp. This result was not surprising because the percentage of fat, the precursor of the radiolytic hydrocarbons, was much lower in the shrimp than in the chicken. When the data were expressed as nanograms of hydrocarbon per milligram of fatty acid, the values for chicken were an order of magnitude lower than those for shrimp treated in a similar manner. The differences in the concentrations of the

radiolytic hydrocarbons may be due to different amounts of fat, and possibly different types of fat, present in these fatty foods, compared with those in the previously studied commodities. The shrimp and frog legs were found to contain much less than 1% fat, whereas the meat products used in this investigation contained 1–30% fat (Table III). Apparently, the amount of fat present, or its form, alters the free-radical reactions in a way that changes the radiolytic yields for these products. Therefore, the radiolytic yields are matrix dependent, and standard dose-response curves will have to be established for different food items. From other work we know that when triglycerides are irradiated, the ratio of the  $n-1$  to  $n-2$  radiolytic hydrocarbons is about 1 to 1, whereas when the free fatty acids are irradiated, the ratio is about 100 to 1 (Morehouse, unpublished data).

In our previous papers on shrimp (Morehouse and Ku, 1992) and frog legs (Morehouse et al., 1991), the concentrations of fat in these commodities were similar and their dose-response curves overlapped. However, some problems in comparing different species of shrimp were noted, and it is possible that the differences in the fat content or type of lipid in the food may account for these discrepancies. Further work investigating this matrix effect is in progress.

For this investigation, whole chicken thighs and breasts, deboned chicken thighs, ground chicken, ground turkey, ground pork, and ground beef were analyzed. Although the chicken breasts contained about 1% fat and the ground beef and ground pork contained about 30% fat (Table III), when the data were expressed as nanograms of radiolytic hydrocarbon per milligram of precursor fatty acid, the data for all of the commodities investigated in this study were within the limits of the typical overall variability (Figures 4–6).

The deboned chicken thighs were analyzed by three analysts to evaluate interanalyst variability. The data in Table IV and Figure 4 are averages of the three analyses. The data from three analysts are in good agreement (exhibiting a slope of  $0.8 \pm 0.2$  and an experimental error of  $\pm 20\%$ ), although the experience level of the three analysts ranged from novice to expert for the procedure. Although the experimental error for this method is large, it is possible to construct a standard additions plot (similar to Figure 4) to approximate the unknown, original absorbed dose ( $\pm 0.5$  kGy) for irradiated chicken meat.

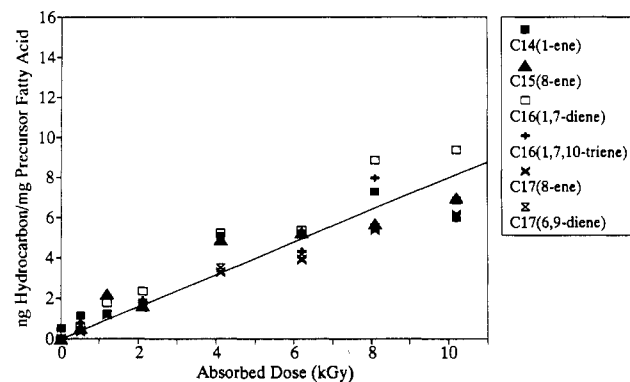
The effect of radiolysis temperature on the yield of the radiolytic hydrocarbons was also investigated. The whole chicken thigh and breast quarters and portions of the ground chicken thighs were irradiated at different temperatures, ranging from  $-80^\circ\text{C}$  for products frozen on dry ice to  $0-5^\circ\text{C}$  for products slightly thawed on ice. No effect of temperature, within the limits of experimental error, was found. This result is similar to that previously found for shrimp (Morehouse and Ku, 1992) or frog legs (Morehouse et al., 1991). The absence of a temperature effect is not surprising because the expected difference from the rise in radiolysis temperature is small, compared with the experimental error.

Various column chromatographic techniques were investigated for separating the radiolytic hydrocarbons from the extracted lipids. Silica gel was found to be an adequate substitute for Florisil. Two different sources of silica gel were used, Bio-Sil from Bio-Rad (Richmond, CA) (200–400 mesh) and Silica Gel 60 from EM Science (Gibbstown, NJ) (70–230 mesh). The silica with the larger particle size gave the faster elution rate, which was closer to that of Florisil. Large-capacity, solid-phase extraction tubes

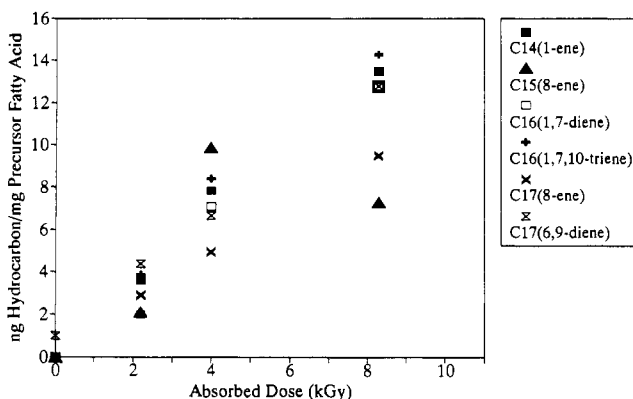
**Table IV. Levels of Radiolytic Hydrocarbons Found in Ground Chicken Thighs  $\gamma$ -Irradiated at Several Absorbed Doses<sup>a</sup>**

absorbed dose, kGy	ng of radiolytic hydrocarbon/mg of precursor fatty acid <sup>b</sup>								
	C <sub>14</sub> (1-ene)	C <sub>15</sub>	C <sub>15</sub> (8-ene)	C <sub>16</sub> (1-ene)	C <sub>16</sub> (1,7-diene)	C <sub>16</sub> (1,7,10-triene)	C <sub>17</sub>	C <sub>17</sub> (8-ene)	C <sub>17</sub> (6,9-diene)
0.0	0.5	0.7	0.0	0.2	0.0	0.0	3.0	0.0	0.0
0.5	1.1	1.6	0.5	0.7	0.6	0.5	4.3	0.3	0.9
1.2	1.2	2.8	2.2	1.8	1.8	1.3	7.2	1.2	1.2
2.1	1.6	2.9	1.7	3.0	2.3	1.9	7.5	1.6	1.6
4.1	5.1	4.7	4.9	4.7	5.2	5.0	12.1	3.3	3.5
6.2	5.2	6.1	5.3	4.9	5.3	4.3	14.7	3.9	4.2
8.1	7.3	7.1	5.7	6.5	8.8	7.9	16.2	5.4	5.5
10.2	6.8	8.5	7.0	7.8	9.4	5.9	22.2	6.1	6.0

<sup>a</sup> GC analysis performed by using a DB-23 capillary column. <sup>b</sup> The hydrocarbon concentration has an overall experimental error of approximately  $\pm 20\%$ .



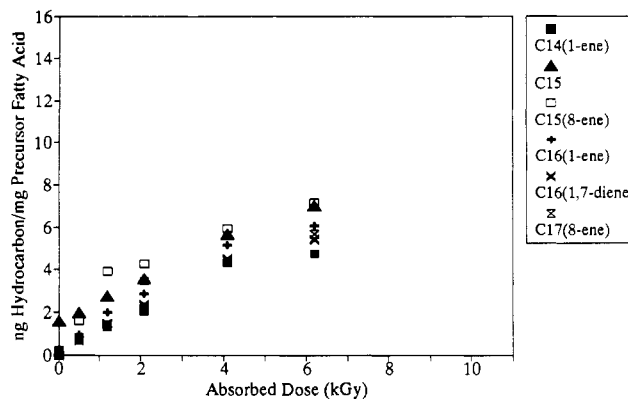
**Figure 4.** Plot of the GC data from the analysis of radiation-treated ground chicken thighs, expressed as nanograms of hydrocarbon per milligram of precursor fatty acid extracted vs absorbed dose (data from Table IV). The line was calculated from a linear regression of the data points for all of the radiolytic hydrocarbons displayed.



**Figure 5.** Plot of the GC data from the analysis of radiation-treated chicken breasts, expressed as nanograms of hydrocarbon per milligram of precursor fatty acid extracted vs absorbed dose.

from Analytichem (Harbor City, CA), containing Florisil or silica, were also used. The smaller amount of adsorbent in these tubes required that smaller quantities of fat be placed on them. This requirement could be a problem in analyses of meat products containing low concentrations of radiolytic hydrocarbons. With some adjustments, it may be possible to use these types of adsorbents instead of the standard Florisil column to separate the radiolytic hydrocarbons from the extracted lipids.

**Comparison with ESR Spectroscopy.** The application of ESR spectroscopy to the identification of foods which have been treated with ionizing radiation has been extensively studied. ESR spectroscopy exhibits great promise for the identification of bone-containing foods that have been treated with ionizing radiation. When bone is irradiated, a characteristic ESR signal develops and is easily monitored (Desrosiers and Simic, 1988; Dodd et al., 1985, 1989). The relative intensity of the ESR signal,



**Figure 6.** Plot of the GC data from the analysis of radiation-treated ground beef, expressed as nanograms of hydrocarbon per milligram of precursor fatty acid extracted vs absorbed dose.

corrected for test portion weight, is dose dependent and displays a linear relationship to absorbed dose. Unfortunately, few of the foods likely to be treated with ionizing radiation contain bone; therefore, another method for their monitoring and identification will be required. The FDA has recently approved the treatment of chicken and pork, including mechanically deboned and ground products, with ionizing radiation (*Code of Federal Regulations*, 1992). Although ESR spectroscopic analysis of these products may be possible if enough bone fragments are isolated from chicken and pork products treated in this way, it would be desirable to have an alternative method to identify irradiated meat products that do not contain appreciable amounts of bone.

The absorbed radiation dose calculated from the radiolytic hydrocarbon results from whole chicken thigh and breast quarters treated with ionizing radiation agrees, within the limits of experimental error ( $\pm 1$  kGy), with that determined by ESR spectroscopy. Although the GC procedure is more complex and takes longer to perform, it may be possible to develop one standard dose-response curve for chicken meat, or for the particular commodity of interest, which could be used to determine the original absorbed dose for the products being analyzed. To obtain an accurate estimate of the absorbed dose with ESR spectroscopy, ESR spectra should be obtained after the bone fragment has been reirradiated at several different absorbed doses. Then the original absorbed dose is determined by extrapolating back to zero ESR intensity. Although the GC procedure exhibits a greater error in the calculation of the absorbed dose, it is possible that the GC dose-response curve would have to be established only once and then checked periodically. This possibility might make the GC procedure more desirable.

**Conclusions.** The results presented here demonstrate that meats which have been treated with ionizing radiation can be monitored by GC to determine the major hydro-

carbons formed during the radiolysis of lipids. When bone is expected to be present in the meat, ESR spectroscopy can be used to measure the concentration of free radicals trapped in the bone.

Although ESR spectroscopy is one of the most accurate and reliable techniques to identify irradiated foods, one of the drawbacks in its application to meat products is the requirement that a bone fragment be present during radiolysis. The application of ionizing radiation to mechanically deboned chicken and ground pork limits the use of ESR spectroscopy as a monitoring technique, because bone would be absent or present only in very small quantities.

We have demonstrated previously that both the GC and ESR spectroscopic techniques can be used to estimate the unknown absorbed dose for irradiated food, with good agreement between the two techniques (Morehouse et al., 1991). The GC-based procedure for the determination of the radiolytically generated hydrocarbons is an alternative to other procedures under development to identify meat products which have been treated with ionizing radiation.

It is hoped that further work in this area will ultimately lead to methodology for monitoring a variety of irradiated foods and for determining the approximate absorbed radiation dose after the food has left the radiation treatment facility (postirradiation dosimetry). We intend to extract and analyze several different types of foods by this GC procedure to obtain a more complete quantitative and statistical picture of the data generated by the procedure and to determine its limitations and reliability as a radiation dose-measuring method.

#### ABBREVIATIONS USED

C<sub>12</sub>(1-ene), 1-dodecene; C<sub>13</sub>, *n*-tridecane; C<sub>14</sub>(1-ene), 1-tetradecene; C<sub>14</sub>(1,7-diene), 1,7-tetradecadiene; C<sub>15</sub>, *n*-pentadecane; C<sub>15</sub>(8-ene), 8-pentadecene; C<sub>16</sub>, *n*-hexadecane; C<sub>16</sub>(1-ene), 1-hexadecene; C<sub>16</sub>(1,7-diene), 1,7-hexadecadiene; C<sub>16</sub>(1,7,10-triene), 1,7,10-hexadecatriene; C<sub>17</sub>, *n*-heptadecane; C<sub>17</sub>(8-ene), 8-heptadecene; C<sub>17</sub>(6,9-diene), 6,9-heptadecadiene; C<sub>17</sub>(6,9,12-triene), 6,9,12-heptadecatriene; C<sub>18</sub>, *n*-octadecane; ESR, electron spin resonance; FDA, U.S. Food and Drug Administration; GC, gas chromatography; MS, mass spectrometry.

#### LITERATURE CITED

- AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990; Chapter 10, Method 970.52, K, L, and O, pp 274-275.
- Bligh, E. G.; Dyer, W. J. A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* 1959, 37, 911-917.
- Code of Federal Regulations Title 21; Part 179; U.S. Government Printing Office: Washington, DC, 1992.

- Desrosiers, M. F.; Simic, M. D. Postirradiation Dosimetry of Meat by Electron Spin Resonance Spectroscopy of Bones. *J. Agric. Food Chem.* 1988, 36, 601-603.
- Dodd, N. J. F.; Swallow, A. J.; Ley, F. J. Use of ESR to Identify Irradiated Food. *Radiat. Phys. Chem.* 1985, 26, 451-453.
- Dodd, N. J. F.; Lea, J. S.; Swallow, A. J. ESR Detection of Irradiated Food. *Nature* 1988, 334, 387.
- Dodd, N. J. F.; Lea, J. S.; Swallow, A. J. The ESR Detection of Irradiated Food. *Appl. Radiat. Isot.* 1989, 40, 1211-1214.
- Dubravcic, M. F.; Nawar, W. W. Radiolysis of Lipids: Mode of Cleavage in Simple Triglycerides. *J. Am. Oil Chem. Soc.* 1968, 45, 656-660.
- Eining, R. G.; Ackman, R. G. Omega-3 PUFA in Marine Oil Products. *J. Am. Oil Chem. Soc.* 1987, 64, 499-502.
- Faucitano, A.; Locatelli, P.; Perotti, A.; Faucitano-Martinotti, F. Gamma-Irradiation of Crystalline Oleic Acid. *J. Chem. Soc., Perkin Trans.* 1972, 2, 1786-1791.
- Federal Register* 1986, 51 (April 18), 13376-13399.
- Federal Register* 1988, 53 (Dec 30), 53176-53209.
- Federal Register* 1990, 55 (May 2), 18538-18544.
- Folch, J.; Lees, M.; Stanley, G. H. S. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J. Biol. Chem.* 1957, 226, 497-509.
- Handel, A. P.; Nawar, W. W. Radiolytic Compounds from Mono-, Di-, and Triacylglycerols. *Radiat. Res.* 1981, 86, 428-436.
- Howton, D. R.; Wu, G.-S. Gamma-Radiolysis of Oleic Acid. *J. Am. Chem. Soc.* 1967, 89, 516-525.
- IAEA. *Food Preservation by Irradiation*; International Atomic Energy Agency: Vienna, Austria, 1978; Vol. I, II.
- Joseph, D. J.; Ackman, R. G. Capillary Column Gas Chromatographic Method for Analysis of Encapsulated Fish Oils and Fish Oil Ethyl Esters: Collaborative Study. *J. AOAC Int.* 1992, 75, 488-506.
- Josephson, E. S., Peterson, M. S., Eds. *Preservation of Food by Ionizing Radiation*; CRC Press: Boca Raton, FL, 1982; Vol. I-III.
- Merritt, C., Jr.; Angelini, P.; Graham, R. A. Effect of Radiation Parameters on the Formation of Radiolysis Products in Meat and Meat Substances. *J. Agric. Food Chem.* 1978, 26, 29-35.
- Merritt, C., Jr.; Vajdi, M.; Angelini, P. A Quantitative Comparison of the Yields of Radiolysis Products in Various Meats and Their Relationship to Precursors. *J. Am. Oil Chem. Soc.* 1985, 62, 708-713.
- Morehouse, K. M.; Ku, Y. Gas Chromatographic and Electron Spin Resonance Investigations of Gamma-Irradiated Shrimp. *J. Agric. Food Chem.* 1992, 40, 1963-1971.
- Morehouse, K. M.; Ku, Y.; Albrecht, H. L.; Yang, G. C. Gas Chromatographic and Electron Spin Resonance Investigations of Gamma-Irradiated Frog Legs. *Radiat. Phys. Chem.* 1991, 38, 61-68.
- Nawar, W. W. Reaction Mechanisms in the Radiolysis of Fats: A Review. *J. Agric. Food Chem.* 1978, 26, 21-25.
- Nawar, W. W.; Balboni, J. J. Detection of Irradiation Treatment in Foods. *J. Assoc. Off. Anal. Chem.* 1970, 53, 726-729.

Received for review October 29, 1992. Revised manuscript received March 11, 1993. Accepted March 11, 1993.