Supercritical Fluid Extraction of Meat Lipids: An Alternative Approach to the Identification of Irradiated Meats

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ABSTRACT: Ionizing radiation is currently under study as an alternative method for extending the shelf life of meats and meat products. Accordingly, methods are needed to determine if a meat or meat product has been exposed to ionizing radiation. In this study, a method is described for the isolation and analysis of volatile hydrocarbons formed in meat lipids after exposure to ionizing radiation. The method is based on supercritical fluid extraction of the hydrocarbons from meat lipids and subsequent identification and quantitation of individual hydrocarbons by gas chromatography (GC) with a mass selection detector (MSD). Supercritical carbon dioxide at 175 bar and 40°C extracted the hydrocarbon fraction from total meat lipids within 20 min. The presence of radiolytic hydrocarbons, as determined by GC/MSD, was then correlated to the degree of irradiation of the meat from 0 to 10 kGy. Besides being faster, this method has the advantage of reduced solvent consumption when compared to current methods for determining if a meat or meat product has been irradiated.

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KEY WORDS: Beef lipids, gas chromatography, mass detection, radiolytic hydrocarbons, SFE.

In the United States, foodborne illnesses are still a problem. It is estimated that 9,000 deaths (1) occur each year as a result of food illness. The increased complexity of the modern food chain has provided new opportunities where pathogens can be introduced. Therefore, a modernized food safety process is needed. Food irradiation is a modern option to improve food safety.

Many scientific studies have been conducted over the years (2) that have shown food irradiation to be a safe process. Food irradiation reduces illness due to foodborne pathogens, extends product shelf life, and destroys insect pests. Irradiation of poultry (3 kGy) and pork (1 kGy) have already been approved by the U.S. government, and approval of low-level (<10 kGy) irradiation of most meats is expected in the near future. Because of this, methods for identifying irradiated meats must be available for regulatory purposes to be sure that irradiated foods are properly labelled in this country and in international trade (3). Currently, there are three main methods used for identifying irradiated foods. These methods are thermoluminescence, electron spin resonance, and analysis of volatile products from irradiated lipids (3). The analysis of volatile products would be the method of choice because most laboratories are equipped with gas-chromatographic equipment.

The literature, primarily of Nawar and co-workers (4–10), has shown that irradiation of meats produces radiolytic products from the lipids present, and that these lipid products can be used as irradiation markers. In particular, when lipids are irradiated, triglycerides undergo cleavage primarily at the ester carbonyl, which gives rise to specific hydrocarbons that are one and two (Cn_{-1} and Cn_{-2}) carbon atoms less than the parent fatty acid and have a terminal carbon–carbon double bond. Also, it has been reported that a linear relationship exists between radiation dose and the amount of each hydrocarbon formed (10). The two primary methods, cold-finger distillation and Florisil column chromatography, used to isolate these lipid degradation products are time-consuming and require extraction with organic solvents. Another approach is to use supercritical fluid extraction (SFE) to reduce the organic solvents used in the current methodology. SFE has been developed over the past ten years as an alternative extraction technique to conventional extractions, such as Soxhlet and liquid–liquid extraction (11). Lembke and co-workers (12) used SFE in the characterization of irradiated lipids from several foods but did not include beef in their study, nor did they study the effect of dose on the concentration of lipid radiolytic products.

The objectives of this research were to develop an SFE protocol to replace the currently used extraction methods to isolate radiolytic hydrocarbons from irradiated meat lipids, to correlate the amount of hydrocarbon products formed with irradiation dose, to characterize and identify the main radiolytic hydrocarbon products, and to extend the SFE method to include ground beef, which has not been studied previously.

EXPERIMENTAL PROCEDURES

Materials. Ground beef (20% fat), labeled as fresh ground beef, was obtained from a local supermarket. The extraction solvents, hexane and isopropanol, were of high-purity, high-performance liquid chromatography (HPLC) grade, obtained from Burdick and Jackson (Muskegon, MI). Deionized water was a laboratory preparation. Carbon dioxide, SFC-grade

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with dip tube, was obtained from Scott Specialty Chemical (Plumsteadville, PA). Glass beads (3 mm) were obtained from Thomas Scientific (Swedesboro, NJ). Florisil, reagent-grade, was obtained from Sigma Chemical Co. (St. Louis, MO). Hydromatrix (a special celite), chem-tube-grade, was obtained from Varian (Harbor City, CA). A hydrocarbon standard, UST Modified Diesel Range Organics, was obtained from Supelco, Inc. (Bellefonte, PA).

Equipment. A rotary evaporator model RE111 (Büchi, Flawil, Switzerland) was used to evaporate solvents. A centrifuge, model HN-SII, International Equipment Co. (Needham Hts., MA) was used for centrifuging samples. A supercritical fluid extractor, model SPE-ED, Applied Separations (Allentown, PA), along with 24-mL stainless steel extraction vessels, rated at 10,000 psi (Keystone Scientific, Bellefonte, PA), was used for the SFE. Vials (25 mL) from Pierce Chemical Company (Rockford, IL) were used as traps. A capillary chromatograph, model Sigma 2000, Perkin Elmer Corporation (Norwalk, CT), was used for gas-chromatography analysis. Identification of radiolytic hydrocarbon products was made on a Hewlett-Packard gas chromatography/mass selection detector (GC/MSD), 5890 series II with a 5972 series mass selective detector (Palo Alto, Ca). The γ-radiation source was ^{137}Cs (134,000 Ci), obtained from Lockheed Corporation (Marietta, GA).

γ-Radiation. Fresh ground beef was packaged (100 g lots) into Cryovac E poultry bags (air transmission 4000 cm^3/m^2 per 24 h at 1 atm and 22.8°C) and hung from racks in the center of the radiation field for the irradiation process. Dose rate was 0.108 kGy min^{-1}. Dosimetry and dose distribution for the source have been described elsewhere (13). The temperature of the radiation chamber was 5 ± 0.5°C. The meat samples received average doses of 0, 1, 3, 5, and 10 kGy.

Solvent lipid extraction. Fifty grams of irradiated ground beef were mixed in a blender with 75 mL of hexane/isopropanol solution (3:2) for 2 min. The mix was then centrifuged at 5000 rpm for 10 min. The top layer was removed and evaporated on a rotary evaporator to dryness, about 10 mL. Hexane (20 mL) was added, along with 20 g of anhydrous sodium sulfate. The mixture was allowed to sit for 1 hr and filtered, and the solvent was evaporated on a rotary evaporator to obtain the meat lipids, which were stored in glass bottles at 4°C.

Heat lipid extraction. Fifty grams of irradiated ground beef in a centrifuge bottle were covered with a little water and heated in an oven at 60°C for 1.5 h with occasional stirring. The ground beef–water mix was then centrifuged at 2500 rpm for 10 min. After centrifuging, the mix was returned to the 60°C oven to melt the solidified lipid. After remelting the lipid, it was siphoned off with a pipette and stored in a glass bottle at 4°C.

SFE. Irradiated lipid (1 g) was weighed out on a balance, spiked with $C_{17:0}$ hydrocarbon, and mixed with 2 g Hydromatrix. [The Hydromatrix came from a blank sample that had previously been cleaned by SFE. The blank consisted of the extraction vessel with glass wool, Florisil (4 g), and Hydromatrix (2 g) arranged as in Figure 1. The blank was extracted at 9700 psi for 10 min at 80°C and a carbon dioxide flow rate of 2 L/min. Hydromatrix use with SFE has been previously described (14).] The fat–Hydromatrix sample mixture was then added to the extraction vessel, and the end nuts were tightened. The extraction vessel was placed in the supercritical extractor, where it was extracted with carbon dioxide gas at 2500 psi for 20 min at 40°C and a flow rate of 1 L/min. This results in 20 L of CO_2, measured as expanded gas, passing through the extraction vessel.

The compressed gas was passed through a high-pressure metering valve (heated to 80°C), which served as a restrictor to control the expanded gas flow into the trap. The trap consisted of a special vial with a glass tube insert and a lid that could be punctured. The vial, cooled in dry ice/acetone, contained 10 g of glass beads and 10 mL hexane, as shown in Figure 1. The vial provided for entrance of the CO_2 from the extractor and an exit to the flowmeter. An identical vial with glass beads and hexane and spiked with $C_{17:0}$ hydrocarbon was carried along as a control to evaluate the work-up procedure from the trap to the GC injection.

Direct SFE of meat. In one set of experiments, there was direct SFE of the meat. Four grams of irradiated ground beef were mixed with 3 g of Hydromatrix in a beaker. The mixture was then loaded into the extraction vessel and subjected to SFE without prior extraction of the lipid.

Gas-chromatographic analysis. The capillary chromatograph was fitted with an on-column injector (Perkin-Elmer) and a nonpolar DB5 column, 30 m × 0.25 mm i.d. × 25 μm film thickness (J&W Scientific, Folsom, CA). The parameters were true on-column injection (1.4 μL), with an 11-cm fused-silica needle (0.17 mm i.d.) to deposit the sample inside the top of column, and flame-ionization detection (FID). The column temperature program was 100°C for 4 min, 30°C min^{-1} to 115°C, 2°C min^{-1} to 190°C, 30°C min^{-1} to 300°C, and isothermal at 300°C for 5 min. The sample from the SFE trap was washed out three times, and the combined solvent was evaporated to about 1 mL with nitrogen gas flow. Hexane (200 μL), containing 2.96 μg eicosane ($C_{20:0}$), was added to the sample as an internal standard to calculate the amount of hydrocarbons produced from the irradiation.

GC/MSD. Another capillary chromatograph was fitted with a DB-5 column as described above. The parameters were 1 μL splitless injection, vacuum compensation on, 1.5 mL min^{-1} helium flow, and MSD. The column temperature program was 100°C for 2 min, 30°C min^{-1} to 115°C, 2°C min^{-1} to 190°C, 4°C min^{-1} to 280°C, and isothermal at 280°C for 2 min.

RESULTS AND DISCUSSION

The procedure for SFE and trapping of radiolytic lipid products from meat was adopted after several experiments had determined the conditions necessary to extract the radiolytic hydrocarbons. It was found that 2500 psi, 40°C, 1-L expanded gas flow rate, and a 20-min extraction time was sufficient to recover the hydrocarbons. Most of the recovered hydrocar-

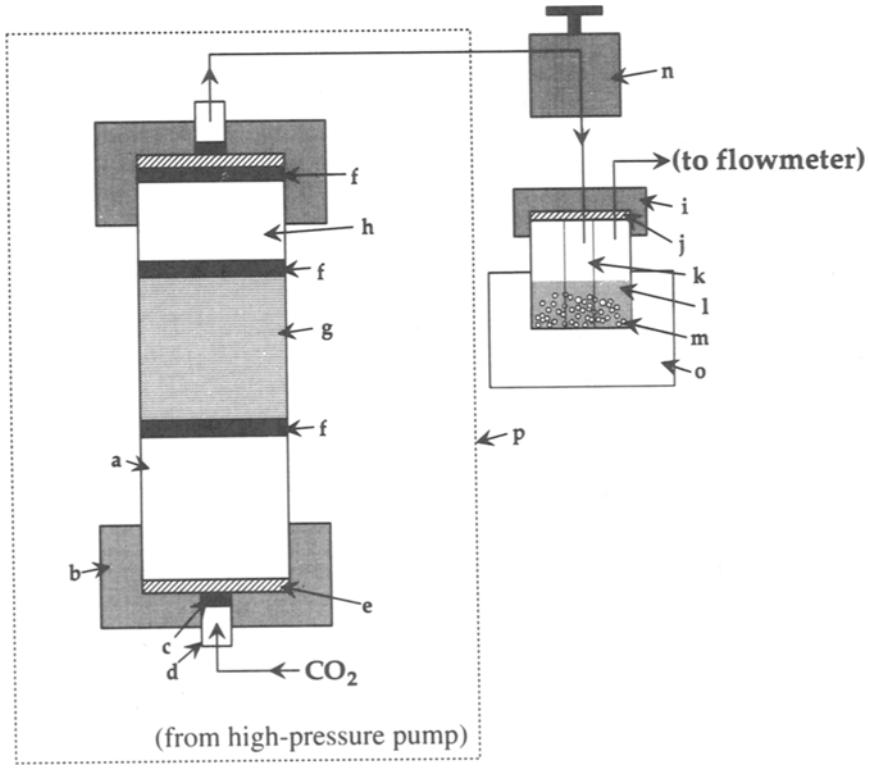

FIG. 1. Supercritical fluid extraction extraction vessel and trap: (a) SS extraction vessel—10 k psi, (b) threaded cap, (c) 2 μ frit, (d) threaded frit nut, (e) peek seal, (f) glass wool, (g) Hydromatrix (Varion, Harbor City, CA), (h) Florisil (Sigma, St. Louis, MO), (i) vial screw cap, (j) vial septum, (k) glass tube, (l) hexane, (m) glass beads (3 mm), (n) heated high-pressure metering valve, (o) acetone/dry ice bath, (p) constant temperature oven.

bons (90%) were extracted from the irradiated lipid in the first 10 min, with the remaining hydrocarbons extracted in the next 10 min. After 20 min, there was no further recovery of radiolytic hydrocarbons. In this procedure, the metering valve used as a restrictor was only heated to 80°C because preliminary experiments had shown that a temperature of 110°C caused breakdown of the hydrocarbons passing through it.

Examples of the type of results obtained by GC/FID analysis are seen in Figure 2. At zero kGy (Fig. 2), there was little indication of any major hydrocarbons attributable to radiolytic products. The two identified major peaks were heptadecane ($C_{17:0}$) at retention time 23.2 min, and eicosane ($C_{20:0}$) at retention time 37.5 min. Heptadecane was added to the samples to determine percentage recovery of hydrocarbons by SFE. Eicosane was used as the GC internal standard for quantitation of hydrocarbons. Some heptadecane has been reported to form as a radiolytic product but in low concentration at these dose levels. The peaks between heptadecane and eicosane were not identified but appear to be characteristic of beef. The peaks of interest, however, as indicated from the literature, were expected to elute before heptadecane because of the breakdown of triglycerides with C_{18}, C_{16}, C_{14} fatty acyl chains. At 5 kGy (Fig. 2), there was a definite amount of radiolytic hydrocarbon products. The peak with retention time of 17.3 min is of interest because its GC retention time corresponds to a C_{16} hydrocarbon with two double bonds, i.e., $C_{16:2}$. Another peak, retention time of 22.0 min, is also of interest because its GC retention time corresponds to a C_{17} hydrocarbon with one double bond. These two hydrocarbons, 16:2 and 17:1, have been reported (15) to be the radiolytic products of the highest concentration in irradiated beef lipids. Indeed, $C_{16:2}$ seems to be a marker for irradiated lipids in fresh ground beef, because this compound, $C_{16:2}$, was not detected in any nonirradiated ground beef samples. Figure 2 also shows a further increase in radiolytic hydrocarbon products at 10 kGy dose.

GC/MSD was performed on hydrocarbon standards and on the hydrocarbons isolated by SFE from irradiated meat samples. Positive identification was made for the major radiolytic products $C_{14:1}$, $C_{15:0}$, $C_{16:2}$, and $C_{17:1}$. Table 1 lists the standards used in this study and their retention times, along with the major radiolytic products identified by the GC/MSD instrument. The compound 1,7-hexadecadiene was not found in the instrument library. However, a known sample of 1,7-hexadecadiene was obtained, and its GC/MSD fragmentation pattern was added to the library. GC/MSD analysis for a beef lipid sample, irradiated to a dose of 5 kGy, is shown in Figure 3. Comparison of the fragmentation pattern for the peak at 15.95 min with the $C_{16:2}$ standard confirmed that this molecule was a $C_{16:2}$ hydrocarbon with a molecular weight of

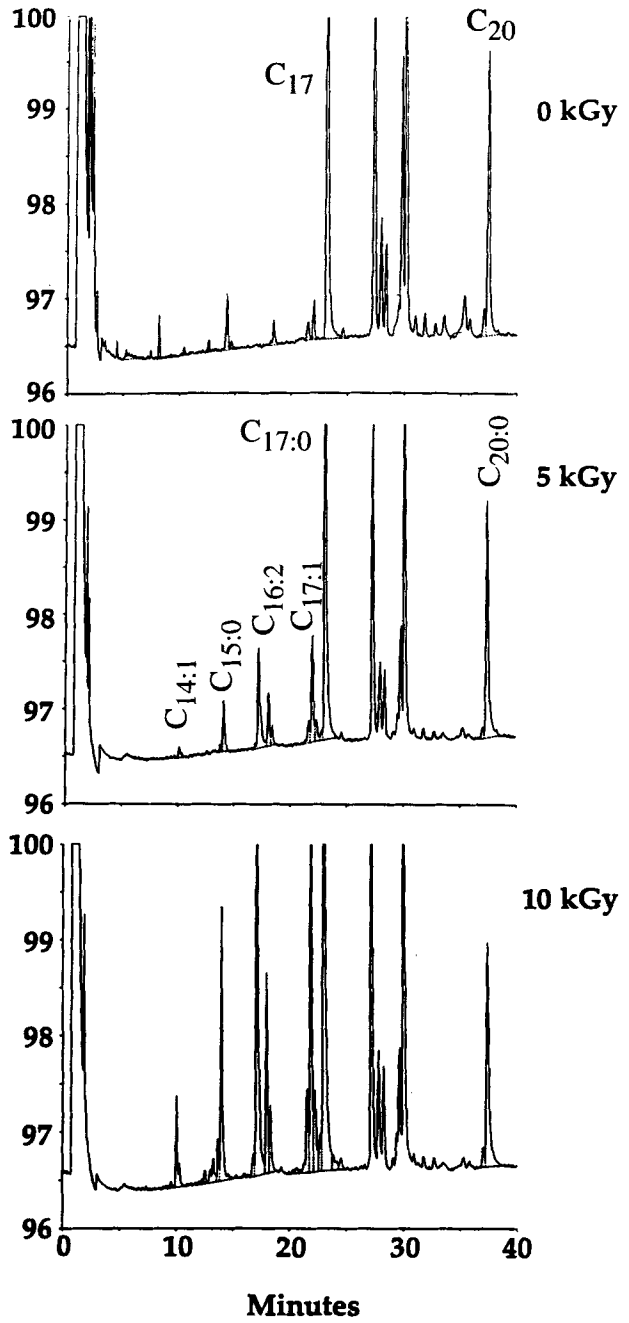

FIG. 2. Gas chromatography/flame-ionization detector analysis of extracted hydrocarbons from ground beef irradiated at 0, 5, and 10 kGy.

222. The hydrocarbons, $C_{16:2}$ and $C_{17:1}$, were also identified by comparing like GC data found in the literature with those from nonpolar DB5 capillary column.

The results of several replicate irradiation experiments are listed in Table 2. These values were obtained by comparing the GC/FID peak area of the internal standard, $C_{20:0}$, with the peak areas of the radiolytic hydrocarbon products. As the irradiation dose increases, so does the average amount of the major radiolytic hydrocarbon products, except $C_{14:1}$. Nonirradiated ground beef, when subjected to the SFE procedure,

TABLE 1
Identified Hydrocarbons by Gas Chromatography/Mass Selection Detector[a] (standards and those from irradiated lipids in fresh ground beef)

Hydrocarbon	MW[b]	Name	Retention time[c]
		Standards	
12:0	170	Dodecane	4.80
13:1	182	1-Tridecene	6.59
14:0	198	Tetradecane	9.55
15:0	212	Pentadecane	13.07
16:2[d]	222	1,7-Hexadecadiene	15.93
16:0	226	Hexadecane	17.19
17:1	238	8-Heptadecene	20.49
17:0	240	Heptadecane	21.68
18:0	254	Octadecane	26.35
20:0	282	Eicosane	35.55
		Major radiolytic products	
14:1	196	1-Tetradecene	9.24
15:0	212	Pentadecane	12.99
16:2	222	1,7-Hexadecadiene	15.95
17:1	238	8-Heptadecene	20.50

[a]Probability-based matching >96 for instrument library compounds.
[b]Molecular weight (g/mol).
[c]Time in minutes.
[d]Not in instrument library, matched to a reference sample.

sometimes contained traces of $C_{14:1}$, $C_{15:0}$, and $C_{17:1}$, but no $C_{16:2}$ hydrocarbons. At 1 kGy dose, the average concentration was less than 1 μg/g fat for all four hydrocarbons. At 3 kGy, the concentration is about 2 μg/g fat. At 5 kGy, the concentration is about 3 μg/g fat for $C_{16:2}$ and $C_{17:1}$, and the highest hydrocarbon amounts were at 10 kGy. The literature (15) indicates that a concentration of 3 μg/g fat for $C_{16:2}$ was found for irradiated beef at 5 kGy at a temperature of 0°C. The standard deviation is rather large for the 1-kGy sample but improves at the 3- and 5-kGy levels and is comparable to that found by other investigators (15).

In conclusion, this research shows that an alternative method to cold-finger distillation and Florisil chromatographic solvent methods can be used to detect whether fresh ground beef has been irradiated over the range 1–10 kGy. This SFE procedure is fast and extracts a lipid sample in 20 min so that trapped hydrocarbons are ready for GC analysis in 1 h. This method may be applied directly to ground beef with known fat content, thus avoiding a fat extraction step. There was no obvious difference found between the direct SFE extraction of hydrocarbons from irradiated meat and the two solvent-extraction methods used in this study (data not presented).

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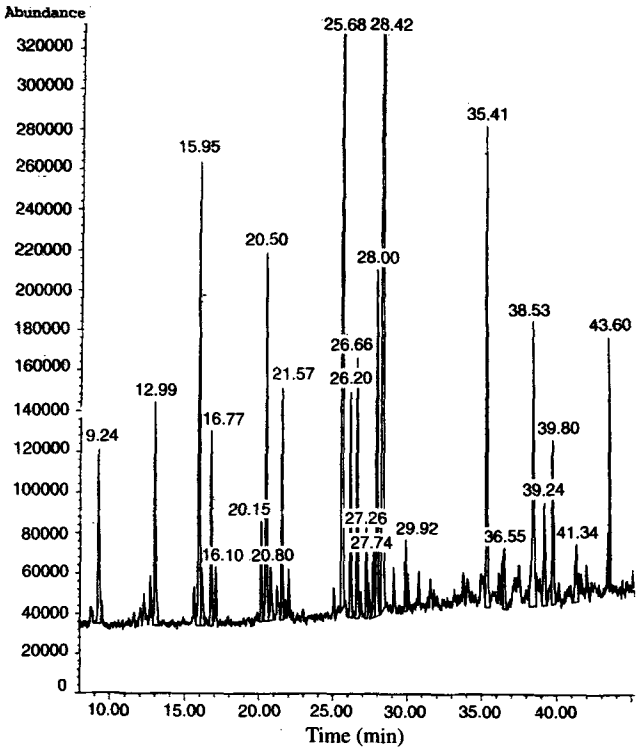

FIG. 3. The 5 kGy irradiation of ground beef, gas chromatography/mass selection detector analysis of extracted hydrocarbons.

TABLE 2
Yields of Major Hydrocarbons Produced by Irradiation of Lipids in Fresh Ground Beef[a]

Dose[b]	$C_{14:1}$	$C_{15:0}$	$C_{16:2}$	$C_{17:1}$
0	trace	trace	n.d.[c]	trace
1[d]	0.80 ± .40	0.92 ± .61	0.72 ± .41	0.77 ± .55
3[d]	1.58 ± .65	1.74 ± .40	2.33 ± .76	2.28 ± .22
5[e]	1.01 ± .65	1.52 ± .47	2.95 ± .53	3.22 ± .61
10[f]	1.11	2.96	6.13	4.92

[a]Data shown are amount of hydrocarbon formed (μg/g lipid) ± standard deviation. [b]Dose in kGy. [c]n.d. = Not detected. [d]n = 3 Replications. [e]n = 4 Replications. [f]n = 1 Sample.

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