Technical

Identification of Radiolytic Compounds from Beef

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ABSTRACT

Several previous papers have dealt with the formation of radiolysis products in fats, triglycerides and fatty acids. Size excusion chromatography now provides a means of separating larger, less volatile compounds, and its application to irradiated beef has resulted in a nearly complete analysis of the radiolytic products formed. The components have been identified by gas chromatography/mass spectrometry (GC/MS).

INTRODUCTION

Much of the knowledge regarding the radiation chemistry of fats is based on the study of model systems. Such systems have included free fatty acids (1,2), methyl esters (3,4), and triglycerides (4-8) having alkyl chains ranging from C_3 to C_{18}. The radiolytic products derived from these systems have been shown to be similar, and a mechanism has been postulated to explain their formation (3).

Several of the radiolytic products observed in the studies of the model systems are low molecular weight volatile carbonyl compounds and series of saturated and unsaturated hydrocarbons (6,8). Prior studies of irradiated natural fats (9) and of irradiated beef (10) have also demonstrated the presence of similar radiolytic compounds. Some of the higher molecular weight compounds which include the glyceryl residues and various recombination products have been relatively easy to separate and identify in the model systems of relatively low molecular weight such as tricaproin and tributyrin (6-8). Isolation and identification of such compounds have not been possible from higher molecular weight triglycerides or from natural systems such as meat due to the limitations of the analytical techniques available.

Recently in the work on irradiated model systems (11), several higher molecular weight radiolytic compounds have been identified, and certain recombination products have been shown to be formed which have not been reported previously. This study was undertaken to ascertain the formation of such compounds in irradiated beef.

EXPERIMENTAL PROCEDURES

A choice top round beef was obtained from a commercial source (Kansas City Beef, Framingham, Massachusetts) and was kept frozen prior to irradiation treatment. Uniform samples of beef were divided into two portions representing the control and treatment. The samples were irradiated at 50 Mrads with gamma rays (Co^{60}-1.28 x 10^5rads/min) in air at 25 C and kept at 5 C until analyzed.

Extraction of the lipid was carried out with chloroform/methanol according to Bligh and Dyer (12). The methanol layer was discarded and the chloroform fraction was evaporated under nitrogen to a convenient volume. The lipid extract was passed through a silicic acid column to effect removal of phospholipids from the nonpolar lipids.

The analysis of free fatty acids was carried out on 10 g of extracted beef fat containing dodecanoic acid as an internal standard. The extract was passed through the KOH/Silicic acid column (13) to separate the free fatty acids. Subsequently, the free fatty acids eluate was analyzed by a Carbowax 20 M (FFAP) column.

Fractionation of the radiolysis products was carried out by means of size exclusion chromatography and analysis performed by gas chromatography/mass spectrometry (GC/MS) as described in a previous publication (11). In this work gas chromatographic analysis of the fractions was performed using the following five stationary phases: Carbowax 20 M, Dexil-300, Carbowax 20 M (FFAP), and SF-96.

Authentic compounds such as ketones, lactones, and triglycerides were purchased from several commercial sources. Long chain gamma lactones were obtained through the courtesy of Dr. J.S. Showell of Eastern Regional Research Lab and Dr. M.J. Diamond of Western Regional Research Lab, U.S. Dept. of Agriculture. Palmitone (16-hentriacontanone) and pentadecyl heptadecenyl ketone (16-tritriaconta-24-enone) were synthesized by the method of Gil-

TABLE I

Quantitative Analysis of the Major Free Fatty Acids Produced in Beef Irradiated at 50 Mrads at 25 C[a]

Free fatty acids	Beef mg/10 g fat	Irradiated beef mg/10 g fat	Increase due to irradiation mg/10 g fat
Myristic acid	14.5	27.1	12.5
Palmitic acid	69.9	134.3	64.3
Palmitoleic acid	14.1	22.9	8.8
Stearic acid	31.0	66.5	35.4
Oleic acid	83.6	146.6	63.0
Total	213.2[b]	397.4	184.0

[a]Average of 16 determinations.

[b]The background amount of free fatty acid in these samples is relatively high. The values have been corroborated by direct gc analysis of the fat extract on FFAP/Carbowax. Apparently some degree of lipolysis had occurred before irradiation.

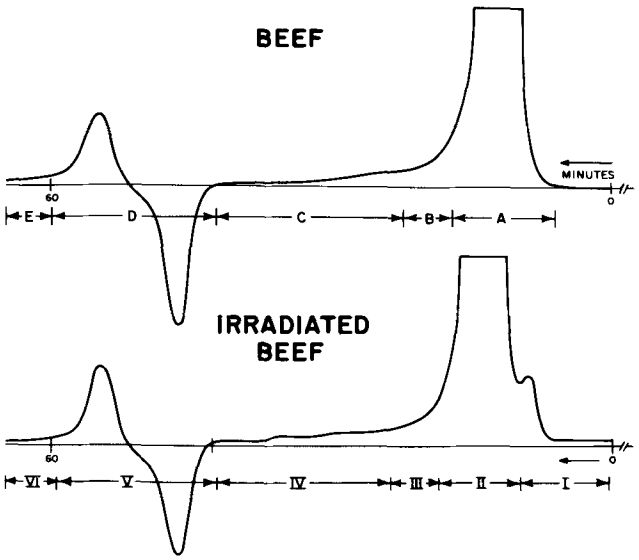

FIG. 1. Gel permeation chromatography of fat extracts. Column: 16' x 3/8" poragel 60°A + 8'x3/8" poragel 100°A. Solvent: Chloroform. Flow rate: 2 ml/min. at 800 psi. RI attenuation: 64X. Sample size; 2ml. Concentration: 50/50; wt/vol.

man and Nelson (14). Propanediol diesters were synthesized by using 1,2-propanediol and 1,3-propanediol and the appropriate acid chloride in solution of chloroform and pyridine following Baumann's procedure (15).

RESULTS AND DISCUSSION

In this study, the chromatographic analysis of free fatty acids in beef and irradiated beef showed an increase in all the free fatty acids in the irradiated samples. For comparative purposes, only the major free fatty acids were quantitized. Table I presents the quantitative analysis of five major free fatty acids in beef and irradiated beef.

The size exclusion chromatograms of the control and irradiated samples are presented in Figure 1. Five continuous fractions (A-E) were collected from each eluate of the control sample and pooled to obtain adequate concentration of lipid components within each designated fraction. Similarly, five fractions (II-VI) from the eluate of the irradiated sample were collected at intervals similar to those of the control sample. Due to the appearance of a shoulder on the triglyceride peak representing compounds of greater apparent size (Fig. 1), an additional fraction (Fraction I) was collected from the irradiated samples. The fractions collected were evaporated under nitrogen and analyzed by GC/MS.

Gas chromatographic analyses of these fractions were made on various columns. Two representative gas chromatograms (Figures 2 & 3) showing most of the radiolytic compounds are presented here. In most cases unequivocal identification of the radiolytic compounds was made by comparing their GC retention times and mass spectra with those of the corresponding authentic compounds. In a few cases where authentic compounds were not available, identification was based on the interpolation of retention time among the retention times of other members of the same homologous series and by comparison of the mass spectrum with the mass spectra of appropriate homologs. Table II presents a list of the radiolytic compounds identified in this study. No radiolytic compounds were identified from the corresponding fractions in the sample of unirradiated beef. These compounds include series of saturated and unsaturated hydrocarbons, aldehydes, alcohols, alkyl esters, fatty acids, lactones, ketones, and diol esters. The compounds without peak numbers correspond to those identified on gas chromatographic columns for which chromatograms are not shown here.

On the whole, the radiolytic compounds found in this study are consistent with those expected from consideration of the mechanisms and deduced from the prior studies of model systems. Many of the compounds found, however, have not actually been identified or reported previously in irradiated beef. The newly found compounds are distinguished by superscript[a] in Table II.

The compounds identified in Fraction IV (Fig. 2) are members of the series of classes corresponding to acyl and acyloxy cleavage of the aliphatic side chains of the triglycerides. The compounds in Fraction III (Fig. 3) represent the larger and less volatile radiolytic products such as the diol diesters and certain recombination products which constitute peaks 24-32 on this chromatogram. In this study and in previously reported work on model systems (11), several lactones were identified whose presence and possible route of formation will be published in a separate paper.

The corresponding fraction (B) from the control sample contained cholesterol and related compounds identified as cholestatriene, dehydrocholesterol, and cholestadiene-7-one. These compounds were also present in the irradiated sample. Since they are nonradiolytic in nature and were originally present in the sample, they are classified under miscellaneous in Table II.

GC analysis of Fraction II, corresponding to triglycerides, was made on a 2 ft SE-30 colum at high temperature and flow rate. The gas chromatogram of this fraction was similar to that of the corresponding Fraction A from unirradiated beef. Due to the high flow rate of the carrier gas, mass spectra of the components in these fractions could not be obtained.

It has been postulated from previous studies of model systems and natural products that the predominant free radicals produced as a result of radiolysis in beef fat are alkyl radicals such as pentadecanoyl and 9:10 heptadecanoyl. Consequently, long chain hydrocarbons, ketones and diketones are expected to be formed predominantly as recombination products of the above radicals. In this study two such recombination products were identified. These were palmitone and 16-tritriaconta-24-enone as expected from the relative abundance of the alkyl moities in beef fat. The amounts of the ketones are small compared to those of the primary radiolytic compounds.

The analysis of propanediol diesters revealed a greater abundance of 1,2-propanediol diesters than the corresponding 1,3-configurations. This is consistent with the higher probability of cleavage at the primary position.

The appearance of a peak before triglycerides on the size exclusion chromatogram (labeled as Fraction I in Fig. 1) suggests the presence of radiolytic compounds of high molecular weights. On the basis of the detector response, the components in Fraction I are estimated to be present in a greater quantity than other radiolytic compounds. This fraction was found to be very viscous after evaporation of the solvent. Because of its low volatility, this fraction could not be separated by gas chromatography. Further study of this fraction is needed to establish the nature of these components, and techniques such as thin layer chromatography, liquid-liquid chromatography, and high resolution mass spectrometry are currently in progress to augment the already existing data to establish the full scope of the radiolytic products found in irradiated beef.

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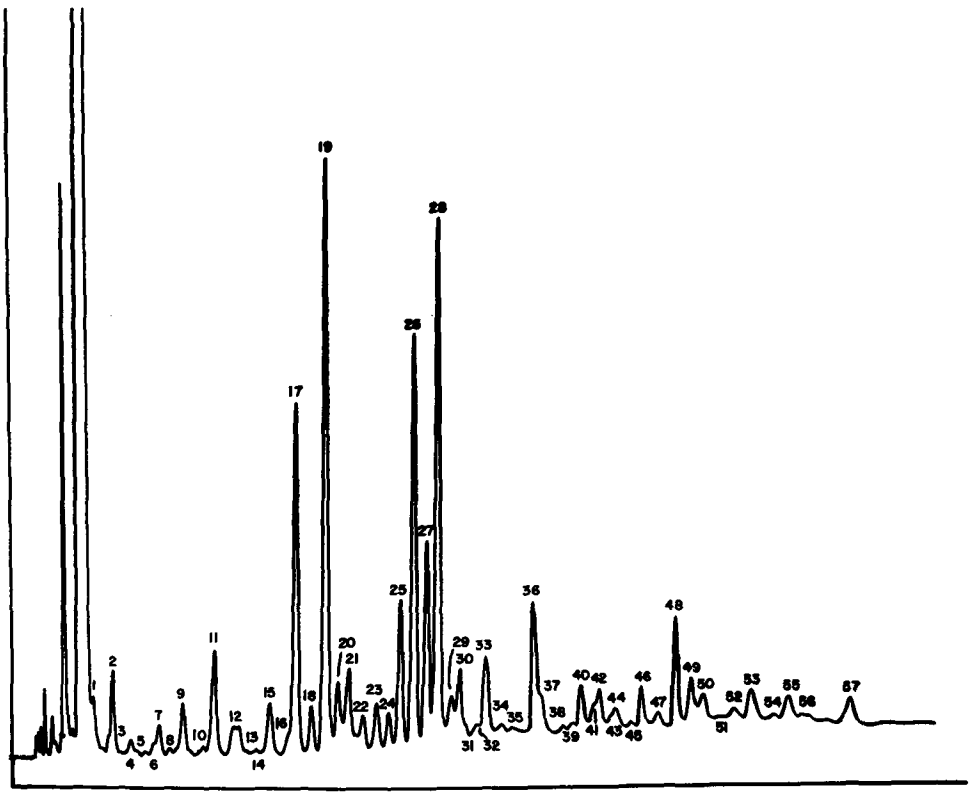

FIG. 2. Gas chromatogram of fraction IV. Column: 8'x1/8" – 15% CW20M (80/100 mesh chromosorb W). Temperature: 70-250 C (4 C/min). Flow rate: 20 ml/min. Detector: FID. Attenuation: 64x100.

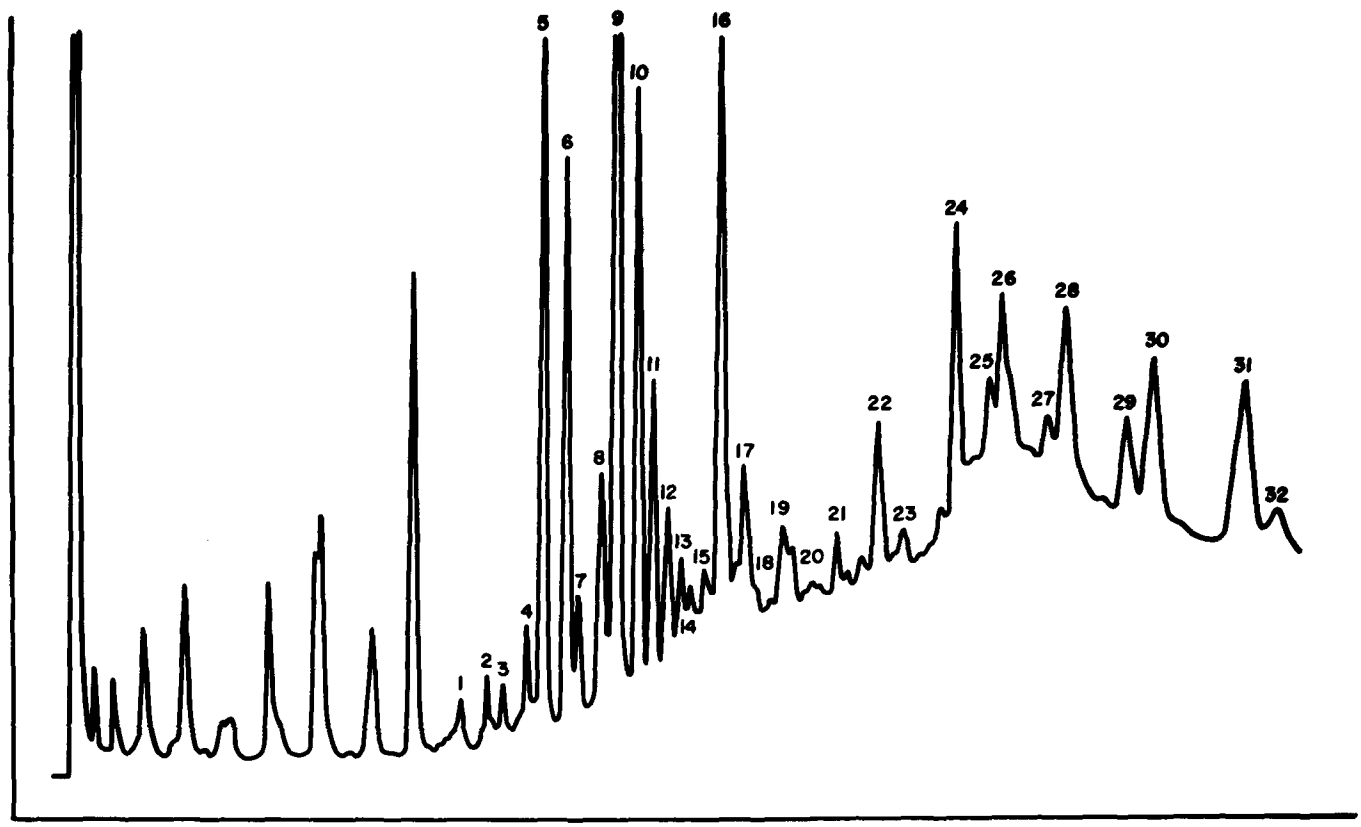

FIG. 3. Gas chromatogram of fraction III. Column: 5'x1/8"-3% OV-17 (80/100 mesh chromosorb W). Temperature: 100-340 C (8 C/min). Flow rate: 40 ml/min. Detector: FID. Attenuation: 64x100.

TABLE II

Radiolytic Compounds Identified from Beef

Compound	Fr. IV/CW20M Peak No.	IDT	Compound	Fr. IV/CW20M Peak No.	IDT	Compound	Fr. IV/CW20M Peak No.	IDT	Compound	Fr.III/OV-17 Peak No.	IDT
Alkanes			Alkynes			Esters[a]			Lactones[a]		
Heptane			Decyne	8	A[b]	Et-tetradecanoate	42	A[b]	γ-palmitolactone	11	A
Octane			Undecyne	12	H[c]	Et-tetradecenoate			δ-palmitolactone	12	H
Nonane			Dodecyne	16	A	Et-pentadecanoate			γ-sterolactone	16	A
Decane						Et-hexadecanoate	49	A[b]	δ-sterolactone	16	H
Undecane			Aldehydes			Et-hexadecenoate			γ-oleolactone	17	H
Dodecane	7	A				Et-octadecanoate			δ-oleolactone	17	H
Tridecane	11	A	Hexenal	3	H[c]	Et-octadecenoate	56	H[c]			
Tetradecane	15	A	Nonanal[a]			Pr-hexadecanoate			Ketones[a]		
Pentadecane	19	A	Undecanal[a]	24	A[b]	Pr-octadecenoate					
Hexadecane	23	A	Dodecanal[a]	29	H[c]	Pr-octadecanoate			2-pentadecanone		A
Heptadecane	27	A	Tetradecanal[a]	37	A[b]				2-heptadecanone		H
			Tetradecenal[a]			Fatty acids[a]			Butyl tridecenyl ketone		H
Alkenes			Pentadecanal[a]	41	H[c]				Palmitone	24	A
			Hexadecanal	46	A[b]	Heptanoic acid			16-tritriaconta-24-enone	25	A
Nonene			Hexadecenal[a]	47	H[c]	Octanoic acid					
Decene	1	H	Octadecanal	52	A[b]	Nonanoic acid			Diol Esters[a]		
Undecene	4	H	Octadecenal	53	H[c]	Decanoic acid					
Dodecene	9	H				Tetradecanoic acid			2-hydroxy propyl hexadecanoate	13	A
Tridecene	12	H	Alcohols			Tetradecenoic acid			1,2-tetradecanoyl propanediol diesters	25	A
Tetradecene	17	A				Pentadecanoic acid			Hexadecanoyl, tetradecanoyl 1,2-propanediol diesters	24	A
Pentadecene	21	H	Hexanol	13	H[c]	Hexadecanoic acid			Tetradecanoyl, hexadecanoyl 1,3-propanediol diesters	27	A
Hexadecene	25	A	Decaonol[a]	31	H[c]	Hexadecenoic acid			1,2-hexadecanoyl propanediol diesters	28	A
Heptadecene	28	A	Undecanol[a]			Heptadecanoic acid			1,3-hexadecanoyl propanediol diesters	29	A
			Tridecanol[a]	43	H[c]	Octadecanoic acid			Tetradecanoyl, octadecenoyl 1,2-propanediol diesters	29	A
Alkadienes			Tetradecanol[a]			Octadecenoic acid			Hexadecanoyl, octadecenoyl 1,2-propanediol diesters	30	A
			Hexadecanol[a]						Hexadecanoyl, octadecanoyl 1,2-propanediol diesters	30	A
Decadiene			Octadecenol[a]						Glyceryl-1-tetradecanoate-2-octadecanoate or isomers	31	H
Dodecadiene	10	A							1,3-dipalmitin	31	A
Tridecadiene	14	H	Esters[a]						1,2-octadecenoyl propanediol diesters	32	A
Tetradecadiene	18	H									
Pentadecadiene	22	H	Me-dodecanoate	23	A[b]				Miscellaneous		
Hexadecadiene	26	H	Me-tetradecanoate	40	A[b]						
Heptadecadiene	30	H	Me-pentadecanoate	45	H[c]				Cholesterol	24	A
			Me-hexadecanoate	48	A[b]				Dehydrocholesterol	24	H
			Me-hexadecenoate						Cholestatriene	22	H
			Me-heptadecanoate	51	H[c]				Cholestadiene-7-one	25	A
			Me-octadecanoate	54	A[b]						
			Me-octadecenoate								

[a]Compounds not identified in previous work.
[b]GC/MS identification based on authentic compounds.
[c]GC/MS identification based on homologous series and literature.

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