Concentration and Identification of Volatile Compounds from Heated Beef Fat Using Supercritical CO_2 Extraction–Gas Liquid Chromatography/Mass Spectrometry

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Beef fat heated at 100 °C was fractionated with supercritical CO_2 into six fractions at 207 bar/50 °C and 345 bar/50 °C. The volatile compounds generated were analyzed by gas liquid chromatography and gas liquid chromatography/mass spectrometry. The concentration of volatiles from fractionated beef fat was higher in fractions extracted at 207 bar than in those extracted at 345 bar. The beefy odor intensity gradually decreased from fraction 1 to fraction 6, requiring from 1 to 6 kg of CO_2. Concentrations of volatiles in fraction 1 extracted at 207 bar were 5–20-fold higher than those in the nonextracted sample or residue. The compounds identified included 17 hydrocarbons, 4 terpenoids, 15 aldehydes, 3 ketones, 4 phenols, 10 carboxylic acids, 6 esters, and 7 lactones.

INTRODUCTION

The production of edible beef tallow exceeds 1.9 billon pounds/year in the United States (AMI, 1987); however, its utilization is decreasing. One reason is that consumers have been increasingly concerned about the nutritional and health characteristics of their foods. More than 45% of beef tallow is in the form of saturated fats, which are thought to be promoters of high blood cholesterol levels which can increase the risk of heart diseases (Haumann, 1987). Due to consumer demands, the fast-food industry has taken steps to switch from animal to vegetable fats in its frying operations. However, vegetable fats do not provide the characteristic desirable flavor of beef tallow, and the use of vegetable oil could lead to significant losses in consumer appeal for deep-fried foods.

Animal fat plays an important role in the formation of the characteristic flavor of cooked meat (Yamato et al., 1970). More than 100 compounds have been identified from heated beef fat, including aldehydes, *n*-alkanes, *n*-alkenes, free fatty acids, ketones, alcohols, lactones, and nitrogen-containing compounds (Watanabe and Sato, 1968; Yamato et al., 1970; Ohnishi and Shibamoto, 1984; Umano and Shibamoto, 1987; Ha and Lindsay, 1991b). Of these compounds, lactones, methyl ketones, and fatty acids are unique in their occurrence in beef and other animal fats (Watanabe and Sato, 1968; Hsu et al., 1982; Baines and Mlotkiewicz, 1984; Ha and Lindsay, 1991b). Also, carbonyl compounds from the fat may react with nitrogen-, oxygen-, and sulfur-containing heterocyclic compounds to produce major cooked flavor volatiles (Shibamoto, 1980; Bailey, 1983).

For many years, researchers have tried to isolate, concentrate, and purify the flavors produced by nature. Classical distillation and solvent extraction have two main disadvantages: First, the solvent must be removed, which is expensive in terms of energy and in some cases degrades the product because of heat treatment. Second, it is often not possible to remove the solvent completely, which results in adulteration of food products (Calame and Steiner, 1982). Current methods used for modifying edible beef tallow composition include drying and fractionation by solvents and detergents (Grompone, 1989). Unfortunately, these procedures involve high energy costs, damage the heat-sensitive oil products, and contaminate the product with undesirable residues.

Supercritical fluid extraction (SFE) is a separation procedure that exploits the use of the solvent properties of a simple compound such as CO_2 at the state beyond its critical point (Friedrich et al., 1982). A typical supercritical fluid has a diffusibility close to that of gas and density and viscosity similar to and lower than, respectively, those of liquid. SFE involves the phenomenon of simultaneous distillation and extraction (Zosel, 1978). The advantages of using CO_2 as a solvent for the SFE process applied to the food industry include low-temperature separations, near-zero solvent residue, varied solubility, fractionated extraction and separation, low energy consumption, and easy solvent recovery (Chao et al., 1991; Tiwari, 1989). Therefore, this process has recently received considerable attention from the food industry and has been successfully applied to reduce the cholesterol content of butter (Shishikura et al., 1986), milk fat (Arul et al., 1987), ground beef (Chao et al., 1991), and squid oil (Yeh et al., 1991).

The objective of this study was to investigate the feasibility of extracting flavor volatiles of beef fat using supercritical CO_2 (SC-CO_2) under different extraction conditions and to identify volatile compounds that may be related to beef flavor.

MATERIALS AND METHODS

Sample Preparation. Approximately 1 kg of subcutaneous beef fat from a corn-fed animal was obtained from the University of Missouri Meat Laboratory, minced into small pieces, and then heated in a 100 °C convection oven for 2 h. The melted fat was filtered through cheesecloth and transferred to glass jars. The jars were flushed with N_2 gas, capped tightly, and stored frozen (−18 °C) until separation by SC-CO_2 extraction.

Extraction Procedures. The supercritical fluid extraction system (semipilot scale) used by Chao et al. (1991) was manufactured by the Superpressure Division of Newport Scientific Inc. (Jessup, MD). The apparatus extraction vessel (capacity, 1 kg) was rated for 689 bar at 100 °C, while the separation vessel was rated for 414 bar at 100 °C. For each test, approximately 100 g of heated fat was loaded into the extraction vessel. The SFE grade CO_2 (99%) was passed through a 5-μm particle filter before reaching the motor-driven diaphragm-type compressor. The desired pressure of CO_2 in the extractor was set by adjusting the back-pressure regulator. Either up-flow or down-flow of su-

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percritical fluid could be induced depending upon positions of two diagonal paired valves surrounding the extraction vessel. Both extraction and separation vessels (capacity, 250 g) were equipped with an electrical heater, temperature controller, and thermocouples to thermostabilize the system. Rupture disks were installed to provide overpressure protection for the vessels and pressure gauge. Also, back-pressure regulators were electrically heated and temperature controlled to prevent clogging of exit lines from a sudden pressure drop which could cause rapid cooling of the solute-laden CO_2 stream.

Fresh supercritical CO_2 was continuously in contact with the fat sample to extract soluble components. Precipitated solutes were fractionated by periodically collecting them from the separation vessel. The extraction vessel conditions were controlled by varying the pressure and temperature (P/T) at 207 bar/50 °C or 345 bar/50 °C, while the P/T condition for the separation vessel was maintained at 34.5 bar/40 °C. The mass flow rate of CO_2 at 20 °C was 1.7–2.1 kg/h, and extraction time ranged from 2 to 3 h.

Gas Chromatographic Analysis (GC). The volatile components of heated beef fat were analyzed using direct sampling (Suzuki and Bailey, 1985). A 0.32 mm × 50 m fused silica capillary column coated with SE-54 was used. The split ratio was adjusted to 1:24 with carrier gas (helium) at 25 psi. The linear flow rate was 41.7 cm/s, and the column flow was 2.0 mL/min. The injection port temperature was 200 °C. The column temperature was maintained at 35 °C for 5 min and programmed at 8 °C/min to 200 °C and then at 2 °C/min to 250 °C. The gas chromatograph used was a Perkin-Elmer Sigma 2 equipped with a FID detector heated at 265 °C.

Gas Chromatography/Mass Spectrometric (GC/MS) Analysis. A Carlo Erba 41-60 gas chromatograph coupled to a Kratos MS-25 mass spectrometer was used. The SE-54 capillary column (0.32 mm × 50 m) was connected directly to the source chamber of the mass spectrometer. The MS was equipped with a Kratos DS-55 data system with a NIH/EPA data base (NIH/EPA Chemical Information System, 1978) for library search. The ionization voltage was set at 70 eV, the ion source temperature at 250 °C, and the resolution at 600 (10% valley). Also, the gas chromatographic retention index (Kovats index) and MS fragmentation pattern of components were compared with those of the authentic compounds to identify the volatiles of the samples.

Quantitation of the Individual Components. Quantitative analysis of the individual constituents identified in the beef fat fractionated by SFE was carried out by spiking heated beef fat with 2-methyl-3-octanone (1.4 mg/10 mL in *n*-pentane) before volatiles were trapped.

From the peak areas of different known concentrations of 2-methyl-3-octanone, the amounts of individual constituents present in heated beef fat were calculated and expressed as parts per million.

Sensory Evaluation. One hundred milligrams of each fraction extracted with SC-CO_2 was placed into glass bottles and melted at 50 °C for 5 min. The fractions were then evaluated for beefy odor intensity by sniffing using 10 trained panelists. Sensory evaluation ballots consisted of 9-point structured scales with descriptive anchors at the 1 (no odor) and 9 (very strong beefy odor) points, respectively.

Statistical Analysis. Sensory and chemical data were analyzed by the analysis of variance and Fischer's least significant difference (SAS, 1988).

RESULTS AND DISCUSSION

To assess the capabilities of supercritical carbon dioxide for concentrating flavor fractions from heated beef fat, the effect of different pressure conditions on the degree of extraction was examined. Six fractions of heated beef fat were obtained by SC-CO_2 extraction as a function of the total kilograms of CO_2 passed through the extractor vessel, and their yields are presented in Figure 1. Higher pressure resulted in a higher density of the SC-CO_2 at isothermal SFE condition and gave higher solubility of fat (Bott, 1982). Data in Figure 1 clearly reveal that the higher pressure condition was more effective in obtaining

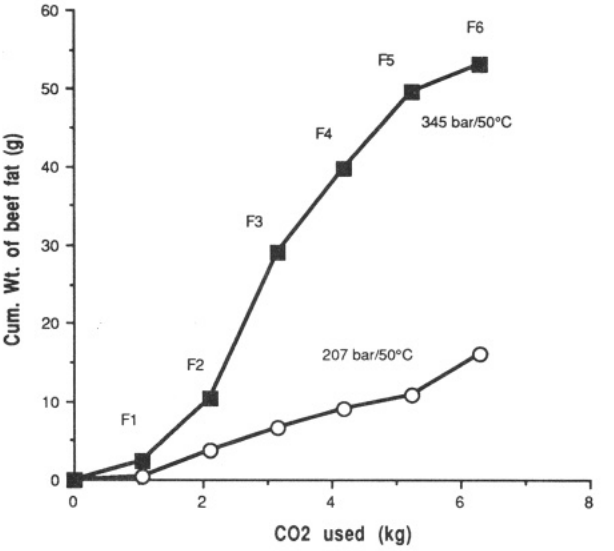

Figure 1. Effect of different pressure conditions on the cumulative yield of fractionated beef fat.

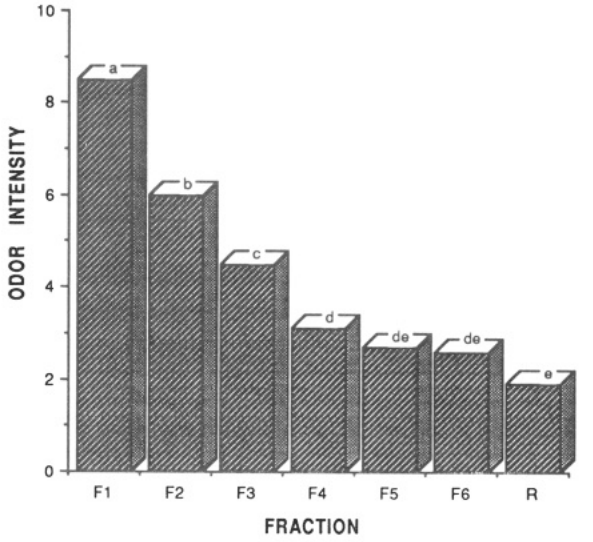

Figure 2. Odor intensity of heated beef fat samples fractionated by SC-CO_2 (extraction pressure, 207 bar/50 °C; separation pressure, 34.5 bar/40 °C). Means with different letters are significantly different ($p < 0.05$).

higher fraction yields. The cumulative weight of the extracted beef fat increased linearly from 1 to 6 kg of CO_2 at 207 and 345 bar at 50 °C. At 6 kg of CO_2 passed, the fat recovery was almost 3-fold at 345 compared to that at 207 bar. This observation is supported by the data of Shishikura et al. (1986), who reported that the solubility of butter oil increased linearly with an increase in pressure during CO_2 extraction.

Sensory data of beef fat odor are presented in Figure 2. The beefy odor intensity of each fraction gradually decreased from fraction 1 to fraction 6. The residue, which was almost odorless, had the lowest mean value for beefy odor. The physical state of fraction 1 was liquid at room temperature, but other fractions were solid at room temperature.

The fractions of heated beef fat extracted with SC-CO_2 at 207 bar/50 °C and 345 bar/50 °C were analyzed by gas liquid chromatography (GLC) and GLC/mass spectrometry (GLC/MS). Figure 3 illustrates the gas chromatogram of volatile compounds from heated beef fat. Fraction 1 was chosen from fractions extracted with SC-CO_2 because it contained the highest mean value for flavor intensity determined by descriptive analysis. The identified and

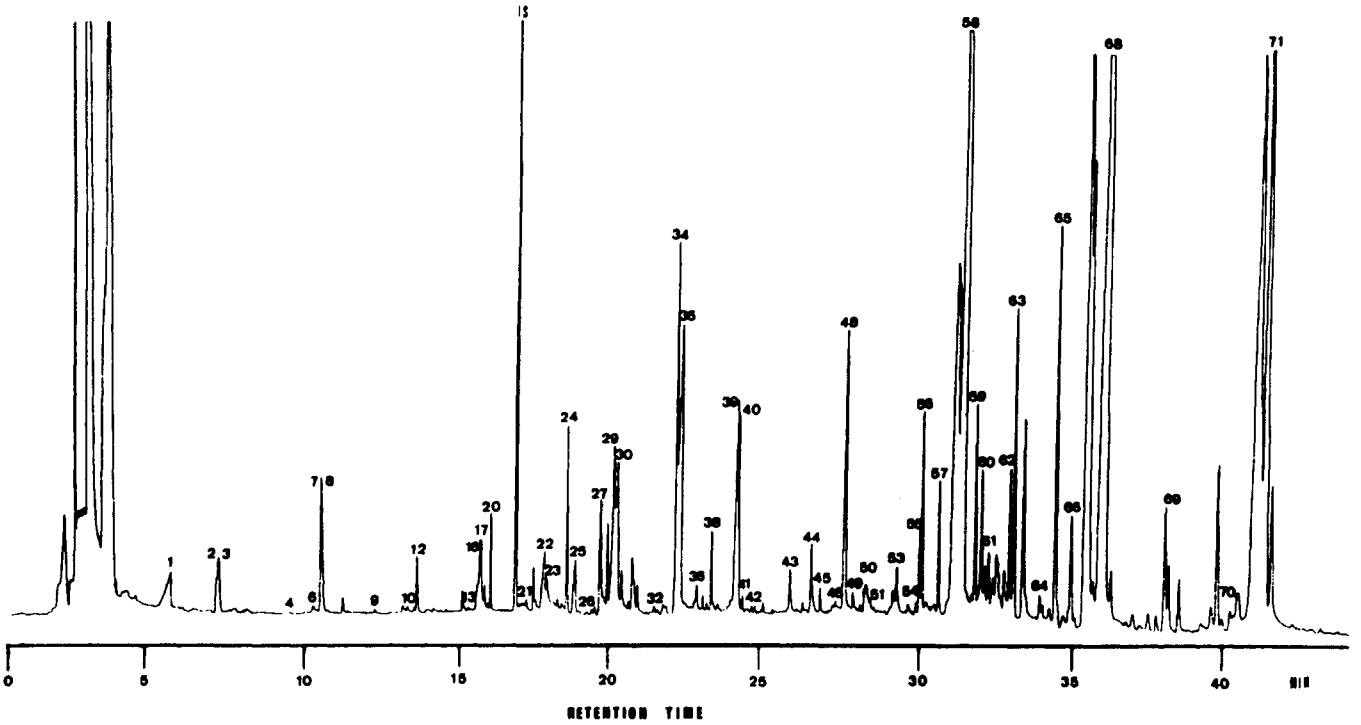

Figure 3. Gas chromatogram of volatile compounds from heated beef fat.

quantitated volatile compounds in heated beef fat extracted with SC-CO_2 at 207 bar are listed in Table I. Use of gas chromatography/mass spectrometry analysis by the direct sampling method resulted in identification of 71 compounds. Compounds identified included 17 hydrocarbons, 4 terpenoids, 15 aldehydes, 3 ketones, 4 phenols, 10 carboxylic acids, 6 esters, and 7 lactones.

Among the hydrocarbons, eight *n*-alkanes, one branched alkane, four branched alkenes, and one cyclic alkene were detected. The identities of these hydrocarbons were similar to those reported by Urbach and Stark (1975), Alenca et al. (1983), and Ohnishi and Shibamoto (1984) for butter and beef fat.

A major difference in volatiles identified from these samples of heated beef fat was the presence of high amounts of diterpenoids. Also, concentrations of these diterpenoids were significantly increased in fraction 1. This means that SC-CO_2 has high selectivity for extracting diterpenoids. Urbach and Stark (1975) quantitated phyt-1-ene, phyt-2-ene, and neophytadiene in butter fat. The total concentration of the C_{20} hydrocarbons was 30 ppm, and phyt-1-ene was the most abundant. This is similar to the results of Table I. These diterpenoids, except farnesol, have been previously found in beef fat (Larick et al., 1987), beef stew (Peterson and Chang, 1982), butter fat (Urbach and Stark, 1975), and lamb fat (Suzuki and Bailey, 1985). The identification of farnesol in beef fat was the first. This compound could be derived from grass feed.

Among the 15 aldehydes identified, 2-nonenal, 2-decenal, and 2-undecenal were present in high concentrations. Also, benzeneacetaldehyde, 2-undecenal, and tridecanal sharply decreased from control to residue. The concentration of 2,4-decadienal was relatively low compared to that of other compounds present. All of these compounds have been found to contribute oily and tallowy flavor to beef (MacLeod and Ames, 1986). Yamato et al. (1970) reported that the concentration of long-chain aldehydes was greater and the oily odor in beef aroma was stronger when beef fat was heated at 200 °C in air. They also found a low concentration of 2,4-decadienal, which is a possible precursor of 2-pentylpyridine that has been identified in beef fat (Henderson and Nawar, 1981).

The ketones are represented by methyl ketones. Methyl ketones are the product of β-keto acids, which are derived from triglycerides by heat treatment (Selke et al., 1975). Ohnishi and Shibamoto (1984) identified 12 methyl ketones in beef fat heated at 200 °C, but only 3 methyl ketones were identified in this study. This may be due to the use of relatively low temperature.

Phenolic compounds have been known as mainly contributing to the aroma of smoked foods (Magna, 1978). Four phenols (phenol, *p*-cresol, 2,5- and 3,5-dimethylphenol) were found in heated beef fat. Of these phenols, *p*-cresol increased 7-fold in fraction 1 compared to the control, while other phenols increased only 1.5 times. These free phenols may have been derived from plant material such as lignin and diterpenoids. Recently, Ha and Lindsay (1991a) found 21 phenolic compounds from fats of six different species. They postulated that beefy flavors in bovine meats appeared to be influenced in part by cresol (*o*-, *m*-, *p*-), with *m*-cresol uniquely high in beef fat.

Ten fatty acids were identified in heated beef fat. Of the fatty acids, nonanoic acid and decanoic acid were most abundant. Ha and Lindsay (1991b) postulated that many of the volatile fatty acids present in beef tallow contributed to tallow-like flavor in deep-fried potatoes. Aliphatic acids appear to be the most ubiquitous of the volatiles and are derived from the hydrolysis of the triglycerides during heating (Selke et al., 1975; Stern et al., 1985).

Among the six esters identified, methyl tetradecanoate was most abundant. Esters arise from the esterification of the various alcohols and carboxylic acids in meat. Esters derived from the longer chain fatty acids possess a desirable fatty character (Forss, 1972).

Lactones are key compounds that contribute to beef aroma (Yamato et al., 1970). One γ- (C_9) and six δ-lactones (C_{10}, C_{11}, C_{12}, C_{14}, C_{15}, and C_{16}) were present in high concentrations in the heated beef fat. Among these six lactones, δ-C_{14} and δ-C_{16} were most abundant. These data agree with those in a paper by Watanabe and Sato (1968), who found 19 lactones in beef depot fat melted at 60 °C

Table I. Identification and Quantitation of Volatile Compounds in Heated Beef Fat Extracted with SC-CO_2 from Fraction 1 at 207 bar/50 °C

peak[a]	Kovats index[b]	compd	concentration,[c] ppm C	F1	R	ID[d]
		Hydrocarbons: *n*-Alkanes				
3[1]	700	*n*-heptane	1.71	1.28	2.03	A
7[2]	800	*n*-octane	2.94	2.78	2.74	A
19	1000	*n*-decane	0.30	2.01	0.33	A
47	1500	*n*-pentadecane	0.26	0.40	0.10	A
53	1600	*n*-hexadecane	0.90	2.36	0.63	A
55	1700	*n*-heptadecane	1.03	6.53	0.53	A
59	1800	*n*-octadecane[e]	2.58	11.86	0.52	A
64	1900	*n*-nonadecane	0.46	2.73	0.37	A
		Hydrocarbons: Branched, Cyclic, Alkenes				
4	763	toluene	0.17	0.08	0.23	A
5	773	2-methyleneheptane	0.43	0.15	0.18	A
11	889	1-nonene	0.12	0.27	t[f]	A
31	1228	naphthalene	0.10	0.40	0.10	A
33	1249	1-butylcyclohexene	0.09	0.09	0.12	A
42	1378	1-tridecene	0.27	0.91	0.25	A
45	1472	2-methyltridecene	0.56	2.08	0.23	A
51	1568	1-hexadecene	0.60	0.78	0.28	A
61	1812	2-methylhexadecane	1.09	4.73	0.69	A
		Terpenoids				
58	1776	phyt-1-ene[e]	23.89	86.46	12.42	B
62	1855	neophytadiene[e]	1.62	15.46	0.39	B
63	1859	phyt-2-ene[e]	3.73	26.20	1.50	B
67	1940	farnesol	0.33	2.05	0.20	A
		Aldehydes				
2[1]	700	pentanal	1.71	1.28	2.03	A
8[2]	800	hexanal	2.94	2.78	2.74	A
12	902	heptanal	0.76	1.49	1.08	A
14	976	benzaldehyde	0.07	t	0.10	A
20	1005	octanal	1.66	2.41	1.66	A
21	1054	benzene acetaldehyde	t	1.47	t	A
24	1107	nonanal	1.84	5.13	2.11	A
28	1168	2-nonenal	0.94	2.60	0.84	A
34	1280	2-decenal	3.77	6.51	2.70	A
36	1308	undecanal	0.73	1.93	0.23	A
38	1328	(*E*,*E*)-2,4-decadienal	0.75	2.78	0.48	A
39	1359	2-undecenal[e]	2.62	12.94	0.25	A
43	1425	dodecanal	0.65	1.58	0.56	A
46	1458	1-dodecanal	0.26	0.37	0.21	A
49	1534	tridecanal	0.48	0.65	0.17	A
		Ketones				
26	1131	3-hydroxy-4*H*-pyran-4-one	0.10	0.40	t	A
48	1520	2-tridecanone[e]	4.98	15.92	2.85	A
60	1807	2-hexadecanone	1.66	5.99	0.41	C
65	1910	2-heptadecanone[e]	5.27	27.92	3.41	A
		Alcohols				
15	980	heptanol	t	0.37	t	A
52	1599	tridecanol	0.25	2.07	0.23	A
		Phenols				
16[3]	982	phenol	2.09	4.71	0.78	A
23	1086	4-methylphenol	0.33	2.15	0.28	A
27	1165	2,5-dimethylphenol	2.25	4.12	2.05	A
30	1187	3,5-dimethylphenol	3.33	3.70	1.65	A
		Acids				
1	660	acetic acid	3.65	4.66	2.74	A
6	793	butanoic acid	0.56	0.25	0.34	A
9	858	3-methylbutanoic acid	0.31	0.08	0.14	A
10	885	pentanoic acid	0.15	0.15	t	A
17[3]	986	hexanoic acid	2.09	4.71	0.78	A
22	1080	heptanoic acid	2.18	3.38	2.17	A
25	1126	2-ethylheptanoic acid	1.43	1.71	0.69	A
29	1180	octanoic acid	3.85	5.67	2.47	A
35	1285	nonanoic acid	8.92	10.89	7.73	A
40	1364	decanoic acid	3.22	10.69	1.64	A
		Esters				
	1244	octanoic acid, Me ester	0.14	0.44	t	A
	1318	nonanoic acid, Me ester	0.22	0.44	0.14	A
	1458	heptanedioic acid, 4-Me-diMe ester	1.30	4.19	0.62	A
56	1706	tetradecanoic acid, Me ester[e]	2.24	14.76	0.99	A
66						
70	1935	hexadecanoic acid, Me ester	1.98	6.92	1.55	A
	2140	octadecanoic acid, Me ester	1.65	1.90	0.81	A
		Furans				
13	968	2,5-dimethyltetrahydrofuran	0.18	0.38	t	A
18	995	2-pentylfuran	1.10	4.27	1.07	A
		Lactones				
41	1370	γ-nonalactone	0.47	0.97	0.34	A
50	1550	δ-decalactone	0.79	3.54	0.47	A
54	1653	δ-undecalactone	0.30	1.13	0.28	A
57	1734	δ-dodecalactone	1.43	9.53	0.83	A
68	1970	δ-tetradecalactone[e]	23.30	185.59	12.29	A
69	2050	δ-pentadecalactone[e]	1.70	15.91	1.10	C
71	2190	δ-hexadecalactone[e]	17.60	176.03	13.28	C

[a] Peak numbers with superscripts contain more than one volatile. [b] Kovats indices were determined by using a series of hydrocarbons on the fused silica column (SE-54) described under Materials and Methods. [c] C, control (before extraction); F1, fraction 1 (extraction pressure, 207 bar/50 °C; separation pressure, 34.5 bar/40 °C); R, residue (after fractionation). The analysis of each sample was performed in triplicate. [d] The identification is indicated by the following symbols: A, mass spectrum and retention indices identical with those of an authentic sample; B, mass spectrum consistent with spectra found in the literature of Urback and Stark (1975); C, tentative identification by mass spectrum consistent with retention predicted from homologous compounds. [e] Means within the same row are significantly different ($p < 0.05$). [f] t, trace amount.

for 1 h. The concentrations of four δ-lactones (C_{12}, C_{14}, C_{15}, and C_{16}) were increased 9–10-fold in SFE fraction 1. A series of γ- and δ-lactones are produced during the thermal oxidation of saturated and unsaturated fatty acids. During autoxidation oxygen attacks γ- or δ-carbon atoms of fatty acids to produce hydroperoxides, which are converted to hydroxy acids (Watanabe and Sato, 1971). The interesterification of the hydroxy acid gives the corresponding lactone.

To study the influence of extraction pressures on extractability of beef fat flavor volatiles, 11 volatile compounds, which have odor profiles of roasty, tallowy, and buttery in heated beef fat and cooked beef, were selected on the basis of the literature reviews of beef flavor compounds (Yamato et al., 1970; MacLeod and Ames, 1986; Gasser and Grosch, 1988; Ha and Lindsay, 1991b). Data in Table II reveal that concentrations of selected volatile compounds of heated beef fat extracted with SC-CO_2 at 207 bar/50 °C are higher than those extracted at 345 bar/50 °C. δ-Tetradecalactone in fraction 1 was significantly different compared to the control and residue within each pressure ($p < 0.05$). The concentrations of volatile compounds of heated beef fat were approximately 2-fold higher in fraction 1 extracted at 207 bar/50 °C compared to those extracted at 345 bar/50 °C. This indicated that the flavor volatile compounds of heated beef fat were selectively extracted due to their high solubility with SC-CO_2 at lower pressure. The principle of extraction with SC-CO_2 lies in the difference in the molecular weight and enthalpic (intermolecular interaction) and entropic (mo-

Table II. Influence of Extraction Pressures on Extractability of Beef Fat Flavor Volatiles

		207 bar/50 °C		345 bar/50 °C	
compd	C[a]	F1	R	F1	R
2-nonenal	0.94[b]	2.60	0.84	1.22	0.11
2-decenal	3.77	6.51	2.70	2.87	1.83
(*E,E*)-2,4-decadienal	0.75	2.78	0.48	0.80	0.58
2-tridecanone	4.98	15.92	2.85	10.81	3.11
2-heptadecanone	5.27	27.92	3.42	16.51	3.05
2-ethylheptanoic acid	1.43	1.71	0.69	1.32	0.11
octanoic acid	3.85	5.67	2.47	3.51	0.25
decanoic acid	3.21	10.69	1.64	8.62	2.34
δ-dodecalactone	1.43	9.53	0.83	6.53	1.09
δ-tetradecalactone	23.30	185.59	12.30	97.29	4.35
neophytadiene	1.62	15.46	0.39	6.71	0.91

[a] C, control (before extraction); F1, fraction 1 (separation pressure, 34.5 bar/40 °C); R, residue (after extraction). [b] Concentration, ppm. $n = 3$.

lecular packing of crystals) characteristics of solute molecules as well as the solvent effect. This finding is in agreement with the results of Brogle (1982), who postulated that low molecular weight compounds such as essential oils and terpene esters can be extracted by SC-CO_2 at 60 bar/60 °C, while more extreme conditions (300 bar/60 °C) are needed to dissolve higher molecular weight compounds such as triglycerides, chlorophylls, pigments, and waxes. Fine tuning of SC-CO_2 extraction methodologies should result in more selective separation and identification of meat flavor.

The present investigation involved quantitation of SC-CO_2 concentrated volatile compounds, which are responsible for species difference and might contribute to cooked beef flavor. The concentrations of 2-undecenal, 2-tridecanone, 2-heptadecanone, methyl tetradecanoate, nonanoic acid, phyt-1-ene, phyt-2-ene, neophytadiene, δ-tetradecalactone, δ-pentadecalactone, and δ-hexadecalactone in fraction 1 extracted with SC-CO_2 significantly ($p < 0.05$) increased compared to that of nonextracted sample or residue. These compounds may contribute to characteristic beef tallow flavor since they are unique to the fat fraction and have not appeared in lists of compounds identified by investigation studying the flavor of cooked beef or pork roast. The concentrated volatile fraction extracted with SC-CO_2 of heated beef fat could be directly applied to the formulation of a flavor mixture to use with vegetable oil to impart beefy aroma. This mixture extracted with supercritical carbon dioxide would be inexpensive, nontoxic, and a liquid at room temperature.

Continued research is needed to identify low molecular weight characteristic flavor compounds of beef fat flavor with SFC/MS analyzed by multivariate analysis. A further challenge is to improve the extraction yield of the volatile fractions using multiextraction and other separation methods associated with SC-CO_2 extraction.

Certainly this method has a great future as a procedure for extracting and studying flavor compounds of foods and, coupled with supercritical fluid chromatography and mass spectrometry, will add a new dimension to the study of both volatile and nonvolatile flavor components.

LITERATURE CITED

Alenca, J. W.; Alves, P. B.; Craveiro, A. A. Pyrolysis of tropical vegetable oils. *J. Agric. Food Chem.* **1983**, *31*, 1268–1270.

AMI. *MEATFACTS*; American Meat Institute: Chicago, 1987.

Arul, J.; Boudereau, A.; Makhlouf, J.; Tardif, R.; Sahasrabudhe, M. R. Fractionation of anhydrous milk fat by supercritical carbon dioxide. *J. Food Sci.* **1987**, *52*, 1231–1236.

Bailey, M. E. The Maillard reaction and meat flavor. In *The Maillard reaction in food nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society:Washington, DC, 1983; pp 169–184.

Baines, D. A.; Mlotiewicz, J. A. The chemistry of meat flavor. In *Recent Advances in the Chemistry of Meat*; Bailey, A. J., Ed.; The Royal Society of Chemistry: London, 1984; Chapter 7.

Bott, T. R. Fundamentals of carbon dioxide in solvent extraction. *Chem. Ind.* **1982**, *7*, 394–396.

Brogle, H. CO_2 as a solvent: its properties and applications. *Chem. Ind.* **1982**, *7*, 385–390.

Calame, J. P.; Steiner, R. CO_2 extraction in the flavor and perfumery industries. *Chem. Ind.* **1982**, *7*, 399–402.

Chao, R. R.; Mulvaney, S. J.; Bailey, M. E.; Fernando, L. N. Supercritical CO_2 conditions affecting extraction of lipid and cholesterol from ground beef. *J. Food Sci.* **1991**, *56*, 183–187.

Forss, D. A. Odor and flavor compounds from lipids. *Prog. Chem. Fats Other Lipids* **1972**, *13*, 181–258.

Friedrich, J. P.; List, G. R.; Heakin, A. J. Petroleum-free extraction of oil from soybeans with supercritical CO_2. *J. Am. Oil Chem. Soc.* **1982**, *59*, 288–292.

Gasser, U.; Grosch, W. Identification of volatile flavor compounds with high aroma values from cooked beef. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 489–494.

Grompone, M. A. Physicochemical properties of fractionated beef tallows. *J. Am. Oil Chem. Soc.* **1989**, *66*, 253–255.

Ha, J.; Linday, R. C. Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. *J. Food Sci.* **1991a**, *56*, 1197–1202.

Ha, J.; Lindsay, R. C. Volatile fatty acids in flavors of potatoes deep-fried in a beef blend. *J. Am. Oil Chem. Soc.* **1991b**, *68*, 294–298.

Haumann, B. R. Trends in frying fat usage. *J. Am. Oil Chem. Soc.* **1987**, *64*, 789–799.

Henderson, S. K.; Nawar, W. W. Thermal interaction of linoleic acid and its esters with valine. *J. Am. Oil Chem. Soc.* **1981**, *58*, 632–635.

Hsu, C.; Peterson, R. J.; Jin, Q. Z.; Ho, C. T.; Chang, S. S. Characterization of new volatile compounds in the neutral function of roasted beef flavor. *J. Food Sci.* **1982**, *47*, 2068–2069.

Larick, D. K.; Hedrick, H. B.; Bailey, M. E.; Williams, J. E.; Hancock, D. L.; Garner, G. B.; Morrow, R. E. Flavor constituents of beef as influenced by forage- and grain-feeding. *J. Food Sci.* **1987**, *52*, 245–251.

MacLeod, G.; Ames, J. The effect of heat on beef aroma: Comparisons of chemical composition and sensory properties. *Flavour Fragrance J.* **1986**, *1*, 91–104.

Maga, J. A. Simple phenol and phenolic compounds in food flavor. *CRC Crit. Rev. Food Sci. Nutr.* **1978**, *10*, 338–372.

NIH/EPA Chemical Information System; U.S. Government Printing Office: Washington, DC, 1978.

Ohnishi, S.; Shibamoto, T. Volatile compounds from heated beef fat and beef fat with glycine. *J. Agric. Food Chem.* **1984**, *32*, 987–992.

Peterson, R. J.; Chang, S. S. Identification of volatile flavor compounds of fresh, frozen beef stew and a comparison of these with those of canned beef stew. *J. Food Sci.* **1982**, *47*, 1444–1453.

SAS. *SAS User's Guide: Statistics*, version 6.0; Statistical Analysis System Institute: Cary, NC, 1988.

Selke, E.; Rohwedder, W. K.; Dutton, H. J. Volatile components from tristearin heated in air. *J. Am. Oil Chem. Soc.* **1975**, *52*, 232–235.

Shibamoto, T. Heterocyclic compounds found in cooked meats. *J. Agric. Food Chem.* **1980**, *28*, 237–243.

Shishikura, A.; Fujimoto, K.; Kaneda, T.; Arai, K.; Saito, S. Modification of butter oil by extraction with supercritical carbon dioxide. *Agric. Biol. Chem.* **1986**, *50*, 1209–1215.

Stern, D. J.; Flath, R. A.; Mon, T. R.; Teranishi, R.; Lundin, R. E.; Benson, M. E. Crude oleic acid volatiles. *J. Agric. Food Chem.* **1985**, *33*, 180–184.

Suzuki, J.; Bailey, M. Direct sampling capillary GLC analysis of flavor volatiles from ovine fat. *J. Agric. Food Chem.* **1985**, *33*, 343–347.

Tiwari, K. K. Technological aspects of supercritical fluid extraction. In *Trends in Food Science and Technology: Proceedings of the Second International Food Convention*

(*IFCON-88*), held Feb 18–23, 1988, at Mysore, India; Rao, M. R., Chandrasekhara, N., Ranganath, K. A., Eds.; Association of Food Scientists and Technologists: India, 1989; pp 59–70.
Umano, K.; Shibamoto, T. Analysis of headspace volatiles from overheated beef fat. *J. Agric. Food Chem.* **1987**, *35*, 14–18.
Urbach, G.; Stark, W. The C-20 hydrocarbons of butter fat. *J. Agric. Food Chem.* **1975**, *23*, 20–24.
Watanabe, K.; Sato, Y. Studies on the changes of meat fats by various processings: Part II. Gas chromatographic identification of aliphatic γ- and δ-lactones obtained from beef fats. *Agric. Biol. Chem.* **1968**, *32*, 191–196.
Watanabe, K.; Sato, Y. Lactones produced through thermal oxidation of higher fatty acids. *Agric. Biol. Chem.* **1971**, *35*, 278–281.
Yamato, T.; Kurata, T.; Kato, H.; Fujimaki, M. Volatile carbonyl compounds from heated beef fat. *Agric. Biol. Chem.* **1970**, *34*, 88–94.
Yeh, A.; Liang, J. H.; Hwang, L. S. Separation of fatty acid esters from cholesterol in esterified natural and synthetic mixtures by supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* **1991**, *68*, 224–229.
Zosel, K. Separation with supercritical gases: Practical applications. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 702–709.

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Registry No. CO_2, 124-38-9; *n*-heptane, 142-82-5; *n*-octane, 111-65-9; *n*-decane, 124-18-5; *n*-pentadecane, 629-62-9; *n*-hexadecane, 544-76-3; *n*-heptadecane, 629-78-7; *n*-octadecane, 593-45-3; *n*-nonadecane, 629-92-5; toluene, 108-88-3; 2-methyleneheptane, 15870-10-7; 1-nonene, 124-11-8; naphthalene, 91-20-3; 1-butylcyclohexene, 3282-53-9; 1-tridecene, 2437-56-1; 2-methyltridecene, 18094-01-4; 1-hexadecene, 629-73-2; 2-methylhexadecane, 1560-92-5; phyt-1-ene, 30221-44-4; neophytadiene, 504-96-1; phyt-2-ene, 2437-93-6; farnesol, 4602-84-0; pentanal, 110-62-3; hexanal, 66-25-1; heptanal, 111-71-7; benzaldehyde, 100-52-7; octanal, 124-13-0; benzeneacetaldehyde, 122-78-1; nonanal, 124-19-6; 2-nonenal, 2463-53-8; 2-decenal, 3913-71-1; undecanal, 112-44-7; (*E,E*)-2,4-decadienal, 25152-84-5; 2-undecenal, 2463-77-6; dodecanal, 112-54-9; tridecanal, 10486-19-8; 3-hydroxy-3*H*-pyran-4-one, 496-63-9; 2-tridecanone, 593-08-8; 2-hexadecanone, 18787-63-8; 2-heptadecanone, 2922-51-2; heptanol, 111-70-6; tridecanol, 26248-42-0; phenol, 108-95-2; 4-methylphenol, 106-44-5; 2,5-dimethylphenol, 95-87-4; 3,5-dimethylphenol, 108-68-9; acetic acid, 64-19-7; butanoic acid, 107-92-6; 3-methylbutanoic acid, 503-74-2; pentanoic acid, 109-52-4; hexanoic acid, 142-62-1; heptanoic acid, 111-14-8; 2-ethylheptanoic acid, 3274-29-1; octanoic acid, 124-07-2; nonanoic acid, 112-05-0; decanoic acid, 334-48-5; methyl octanoate, 111-11-5; methyl nonanoate, 1731-84-6; dimethyl 4-methylheptanedioate, 4751-49-9; methyl tetradecanoate, 124-10-7; methyl hexadecanoate, 112-39-0; methyl octadecanoate, 112-61-8; 2,5-dimethyltetrahydrofuran, 1003-38-9; 2-pentylfuran, 3777-69-3; γ-nonalactone, 104-61-0; δ-decalactone, 705-86-2; δ-undecalactone, 710-04-3; δ-dodecalactone, 713-95-1; δ-tetradecalactone, 2721-22-4; δ-pentadecalactone, 7370-38-9; δ-hexadecalactone, 7370-44-7.