Isolation and Identification of Volatiles and Condensable Material in Raw Beef with Supercritical Carbon Dioxide Extraction

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The purpose of this study was to identify the volatiles in raw beef that were isolated using supercritical carbon dioxide (SC-CO_2) extraction. The extraction produced two kinds of samples: a noncondensable volatile fraction that was concentrated from the CO_2 on an adsorbent (Tenax TA) and a condensed lipid fraction. The lipid fraction was heated subsequent to CO_2 extraction to produce additional volatiles. The noncondensable fraction produced beef-like aroma. The compounds in this fraction were desorbed from the Tenax directly into a GC/FID or GC/mass spectrometer. Lipid fractions were analyzed by direct headspace sampling, also using GC and GC/mass spectrometry. Eighty-six compounds were identified in noncondensable fractions and 59 compounds in lipid headspace samples. Twenty-six compounds were common to both fractions.

INTRODUCTION

Supercritical fluid extraction (SFE) is an appealing analytical tool in flavor and aroma applications for several reasons. First, the capacity and selectivity of a supercritical solvent can easily be manipulated by minor changes in temperature and pressure. By contrast, conventional solvents such as methanol and chloroform extract compounds on the basis of chemical affinity. There is little flexibility on the part of these solvents with regard to selectivity toward solutes. In addition, it is difficult or impossible to separate liquid solvent from the volatile extracts without losing the volatiles.

Supercritical carbon dioxide (SC-CO_2) will extract a wide variety of volatile and nonvolatile compounds according to the vapor pressure of the compounds and can then be completely separated from the extracted material simply by restoring atmospheric pressure. The critical state is at relatively mild temperatures and pressures, which facilitates its use in the laboratory. Additionally, CO_2 is nontoxic and nonflammable, thus allowing extracted compounds to be smelled and tasted. Thermally unstable compounds that decompose at high temperatures can be solubilized in dense CO_2. Because extraction takes place in an air-free environment at mild temperatures, oxidation can be eliminated. This is particularly important with beef, because oxidation is a mechanism in the production of both desirable and undesirable flavor and aroma compounds.

Rizvi et al. (1986) stated that carbon dioxide is an attractive solvent for use in the food industry because of its potential for extracting lipids and flavors. Rubin and Shahidi (1988) stated that SFE with carbon dioxide offers a new and exciting technique for identifying chemicals that control or result in beef flavor. For example, compounds unknown to us because of their degradation with conventional extraction will be recovered through supercritical carbon dioxide extraction. Some of these may be responsible for flavor in beef. The advantages of SC-CO_2 in the analysis of food have been discussed by other authors as well (Kerrola and Kallio, 1993; Calame and Steiner, 1982; Schultz and Randall, 1970).

The purpose of this study was to identify the volatiles obtained from raw ground beef using low-temperature SC-CO_2 extraction. Raw beef is the precursor to cooked products but has been little studied compared to cooked beef. The results from this study should help to elucidate the chemical changes that occur upon cooking. Future work will use the methodology described herein to study the chemistry of cooked beef. Comparison between studies of raw and cooked beef will give new insights into the chemistry and mechanisms forming beef aroma and flavor compounds.

Two main fractions were obtained from the supercritical extraction process. A lipid-like condensable fraction was condensed by reducing pressure from extraction conditions (6000 psi, or 41.4 MPa) to atmospheric pressure at the extraction temperature of 35 °C. The material that did not condense (the volatile fraction) was collected by adsorption onto Tenax TA. This fraction did produce a pleasant beefy aroma and therefore presumably is a fraction closely related to beef flavor. Because the lipid fraction is believed by some to play an important role in beef flavor chemistry, we subjected it to further heating to produce volatiles and analyzed these by headspace sampling. Comparisons were then made between compounds produced from the lipid and those in the noncondensable volatile fraction. Because the headspace samples from the lipid were subjected to heating they are not truly representative of raw beef but are more akin to the products produced in cooked fat.

MATERIALS AND METHODS

SC-CO_2 Equipment and Extraction. The supercritical carbon dioxide extraction (SC-CO_2) apparatus is shown in Figure 1. Carbon dioxide from a supply cylinder was transferred to a floating piston vessel and pressurized to the liquid state using nitrogen at a pressure of about 1500 psi. CO_2 was pumped through the extraction vessel using a liquid chromatography pump (Milton Roy VS minipump). A forced-air circulation oven housed CO_2 preheat tubing, the extraction vessel, and a metering valve (Autoclave Engineers). The extraction vessel had a volume of 50 cm^3 and held 10–40 g of sample. Approximately 15 g of raw ground beef was placed in the supercritical extraction vessel. Extraction was performed in duplicate at 35 °C and 6000 psia (41.4 MPa). Oxidative changes are minimal with this technique because the atmosphere about the sample was purged with CO_2. This allows study of the chemistry of volatile materials before cooking and oxidation.

Phenylheptane (10 ppm by weight) was added to raw beef samples as an internal standard before extraction. The internal

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standard was used in subsequent calculations to quantify the amount of extractable material relative to the standard. Extraction continued until approximately 150 g of CO_2 had been passed over the beef. This gave a "treatment ratio" of 10 g of CO_2/g of beef.

The extract at high pressure flowed through the metering valve, heated to 50 °C, where the pressure was reduced to 1 atm. The contact time in this heated valve is small, and we assume that any cooking in this region is small. Two sample phases were collected after pressure reduction—a condensable liquid/solid from the collector at 35 °C and a noncondensable volatile fraction on Tenax TA adsorbent. The volatile sample in the gas-phase CO_2 downstream of point e (Figure 1) was smelled to confirm that a beef-like odor was being produced. The small size of beef extracted precluded any tasting of the residual beef. Sample collection and analysis procedures are described in subsequent sections.

Collecting and Handling Volatile and Condensable Samples. Prior to an extraction, Tenax TA (60–80 mesh, Alltech Associates, Inc.) was conditioned at 275 °C for 4 h with a helium carrier flow of 50 mL/min. To collect volatile compounds, 400 mg of loose-packed Tenax TA 60–80 mesh polymer adsorbent was placed in a tube (40 cm long × 6 mm wide) at point f. CO_2 and volatile compounds flowed through the adsorption tube, where volatiles were retained. After each run, the Tenax was stored in a sealed vial with a Teflon-lined cap.

Analysis of adsorbed compounds was based on the procedure of Singleton and Pattee (1980). Thirty milligrams of Tenax plus adsorbate were packed into a glass liner (80 mm long × 4 mm wide) and placed into the injector port of a gas chromatograph. Volatile compounds were thus desorbed directly into the capillary GC column using the heat from the injection port. The Tenax tube was heated to 200 °C in the injector port for 1 min. During this time, the carrier gas transferred the heat-desorbed volatiles to the column, and the oven temperature program was then started. To ensure that a clean background chromatogram was obtained, a portion of clean, conditioned Tenax would routinely be analyzed.

Standard analyses have shown that the condensable liquid is primarily fat. Beef fat (mostly solid) collected from supercritical CO_2 extraction of raw beef was dissolved in chloroform and transferred into 10-mL headspace vials (Hewlett-Packard, Avondale, PA). We used chloroform to completely dissolve the lipid so it could be quantitatively transferred to the headspace vials. Chloroform appeared to be a better solvent for this purpose than hexane or methylene chloride. The chloroform was evaporated under a stream of nitrogen followed by equilibrating vials in air for 10 min. Because the supercritical fractionation procedure selectively extracted the volatiles from the lipid, the lipid fraction contained essentially no remaining volatile compounds to be lost during the chloroform evaporation step. This is an advantage of the supercritical fractionation process compared to conventional extraction with organic solvents. In conventional extraction, volatiles in the lipid fraction are likely lost during the solvent evaporation step. Following this step, the vials were sealed with Teflon-lined silica septa and then heated at 150 °C in an oven for 2 h. Using a heated gastight syringe, 5 mL of headspace volatile was injected into a GC. This headspace sample was subsequently analyzed by GC/MS and GC/FID, as described in the following section.

Capillary Gas Chromatography/FID/Mass Spectral Detection. Volatile and headspace samples were analyzed by two methods, either capillary GC/FID or capillary GC/mass spectrometry. The GC/FID instrument was a Hewlett-Packard 5790 capillary GC with FID detector. A 50 m × 0.32 mm i.d. fused silica capillary column (Ultra-2, Hewlett-Packard) with a 0.52-μm film thickness was used for separation. The column inlet pressure was 10 psi. Helium was used as the carrier gas with a split ratio of 8:1 with injector and detector temperatures of 200 and 270 °C, respectively. The GC was temperature programmed from 40 °C (3-min hold) at 4 °C/min to a final temperature of 250 °C and held for 30 min.

Tentative identification of volatiles was accomplished on two mass selective instruments with different ion fragmentation properties. A Hewlett-Packard 5890 GC coupled to a quadrupole mass spectrometer (Hewlett-Packard 5970) and a Perkin-Elmer

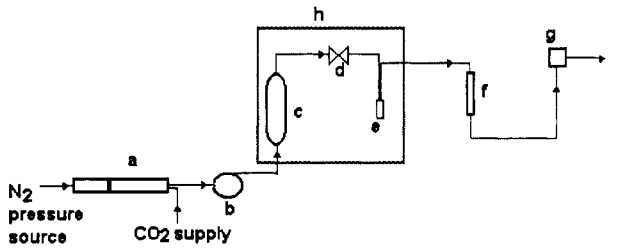

Figure 1. Supercritical extraction apparatus: (a) floating piston CO_2 transfer cylinder; (b) high-pressure pump; (c) extraction vessel; (d) pressure-reducing valve; (e) lipid collection trap; (f) Tenax adsorption trap; (g) gas flow totalizer; (h) constant-temperature air bath.

8500 GC with a Perkin-Elmer ITD ion trap detector were used. The former system contained an HP-5 capillary column (30 m × 0.32 mm i.d., Hewlett-Packard). The ionization energy was 70 eV, and scan range was 35–510 m/z. The injector port was held at 200 °C with a carrier flow of 30 mL/min with a splitless mode. The GC was temperature programmed from −30 °C (1-min hold) at 10 °C/min to 250 °C with a 10-min final hold. The Perkin-Elmer 8500 GC with Perkin-Elmer ITD ion trap detector used an ionization energy of 70 eV and a scan range of 35–550 m/z in a 2-s cycle. The injector port was held at 250 °C with a split ratio of 8:1. Column and other relevant GC conditions were the same as described for capillary GC/FID.

Volatile compounds were tentatively identified using one or both of the following procedures: comparison of GC retention times to those of standard compounds and comparison of mass spectra with mass spectra of standard compounds. For mass spectra, the NBS (National Bureau of Standards) library was used. Duplicate analyses were performed on each SC-CO_2 extract.

RESULTS AND DISCUSSION

The small size of sample used, and the fact that the samples were raw, precluded taste-testing of the beef samples or the lipid extract. Therefore, we relied on smell to detect the presence of beef aroma and operated on the assumption that the compounds associated with aroma will be closely related to flavor compounds. The noncondensable fraction did produce a pleasant beef-like aroma in the gas phase (upstream of point f in Figure 1). In contrast, the condensed lipid fraction gave the aroma of beef fat, distinguishable from the aroma of the volatile fraction. This indicates that the supercritical CO_2 extraction procedure successfully and selectively isolates volatile aroma compounds into the gas phase.

The ability to smell (and potentially to taste) the extracted fractions is a distinct advantage of CO_2 extraction compared to conventional organic solvents. The latter have their own strong aroma which masks beef flavor, and tasting samples extracted with liquid solvents is not feasible. To remove the solvent from the extract requires vacuum, elevated temperatures, or both, and would cause loss of volatile compounds or thermal degradation of the sample.

Figure 2 shows a typical GC/MS profile of beef volatiles from condensed, heated lipid headspace samples (top) and from the noncondensable volatile fraction (bottom). The ordinate shows relative abundance, while the abscissa shows both the retention time (minutes) and the cumulative number of scans (one scan every 2 s). The internal standard peak (IS) is clearly visible in both chromatograms. Qualitatively, the noncondensible fraction shows a greater number of compounds overall and a larger number of compounds in the retention time window greater than 35 min. The noncondensable fraction still shows a region of poorly resolved compounds and a baseline shift in the region from 50 min onward. Part of the baseline shift is due to the high column temperature during this portion

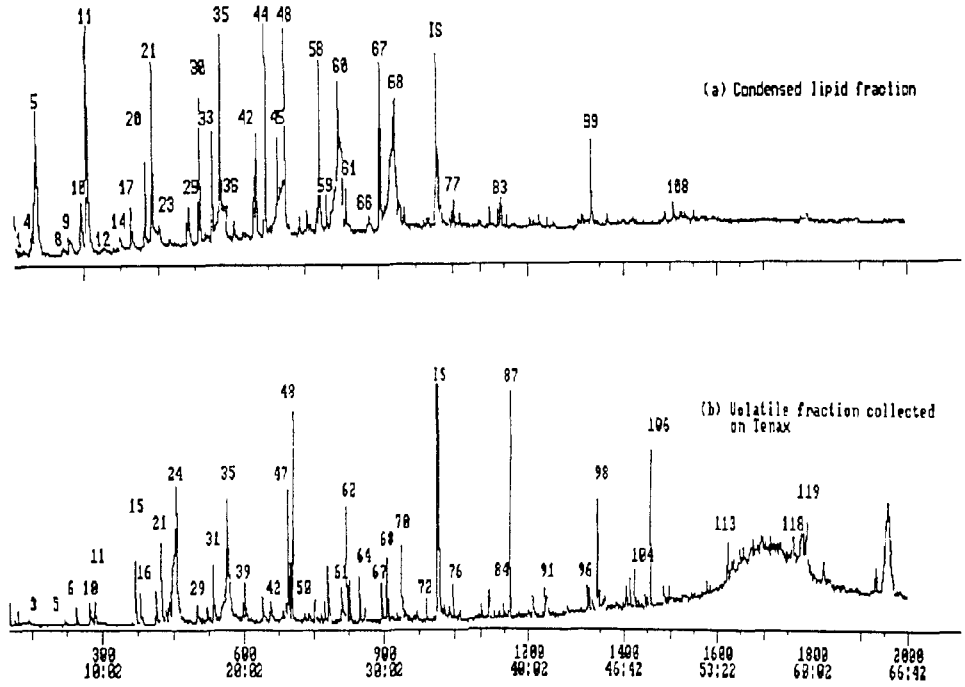

Figure 2. GC/MS chromatograms of volatile compounds obtained from (a) condensable beef fat from supercritical CO_2 extraction of raw beef using headspace sampling techniques and (b) Tenax traps from supercritical CO2 extraction of raw beef.

of the analysis. The heated lipid headspace samples, by contrast, show very little of the heavier compounds after about a 40-min retention time.

Table I lists all compounds tentatively identified from Figure 2 along with retention times and relative peak areas obtained from GC/FID analysis. The chromatographic peak area percentages give a relative indication of the concentrations in the gas samples. The use of an internal standard allowed an approximate quantification of compound concentrations relative to the solid samples. In the volatile fraction, an area percentage of 1% based on FID response corresponds to a concentration of 30 ppb in the beef. For the lipid fraction, an FID area percentage of 1% corresponds roughly to a level of 4 ppm in the lipid fraction.

Table I also gives the identification method used for each compound. The notation RT means that peak retention time from the beef sample matched the retention time of a standard compound, analyzed under identical chromatographic conditions. The designation MS means that the compound was identified by matching sample spectrum with the spectrum in the library of standard compounds. The ability of the mass spectrum library search procedure to correctly identify known compounds was confirmed by analyzing known compounds on the mass spectrometer. When both designations (MS and RT) appear, both the mass spectrum and retention time of the sample were consistent with those of standard compounds. For most compounds, the Hewlett-Packard and Perkin-Elmer mass spectrometers both gave the same peak identification. However, in some cases the identification was made only by a single instrument. In these cases, MS1 indicates that the tentative identification was made only by the Hewlett-Packard, and MS2 means tentative identification by the Perkin-Elmer only. MS (1, 2) indicates that the tentative identification was made by both instruments.

Table I shows 86 compounds, including 26 hydrocarbons, 6 terpenoids, 18 aldehydes, 8 alcohols, 4 phenols, 4 ketones, 1 furan, 1 lactone, 11 acids or their esters, 1 S-containing compound, and 6 miscellaneous compounds, identified in the noncondensable volatile fraction. Fifty-nine compounds, including 10 hydrocarbons, 16 aldehydes, 9 alcohols, 11 ketones, 3 furans, 3 lactones, and 7 acids or their esters, were identified in condensed lipid headspace samples. Twenty-six compounds were in common in both fractions.

Careful inspection of Table I reveals the different chemical nature of the noncondensable sample and the heated lipid headspace sample. The non-condensable sample had higher concentrations of hydrocarbons, especially the heavier (C_{17}–C_{22}) *n*-alkanes, than the lipid headspace sample. Unique to the noncondensable sample were the diterpenoids, alkylbenzenes, and alkylnaphthalenes. Among the fatty acids, the longer chain fatty acids were found only in the volatile sample. In the headspace sample derived from the condensable lipids, the dominant species were aldehydes, ketones, lactones, and alcohols. These compounds are generally associated with thermal decomposition of fatty acids and other oxygenated species, consistent with the fact that the headspace sample was obtained after considerable heating. These observations confirm previous ideas about the role of oxidative degradation in the cooking process. More detailed discussion of the chemistry of the two samples follows.

Hydrocarbons were the dominant volatile compounds in the noncondensable volatile fraction, constituting over 30% of total volatiles. These hydrocarbons included 12 *n*-alkanes, 1 branched alkane, 5 alkenes, 1 cyclic alkane, 7 aromatic hydrocarbons, and 6 diterpenoids. The high molecular weight hydrocarbons such as hexadecane, heptadecane, octadecane, and diterpenoids are likely derived from grass feeds and have been related to "grassy" flavor (Urbach and Stark, 1975; Body, 1977; Larick et al., 1987; Um et al., 1992). Seven aromatic hydrocarbons, including alkylbenzenes and naphthalenes, were identified only in the noncondensable fraction adsorbed on Tenax. Occurrence of these compounds has been previously attributed to thermal decomposition of carbohydrates, fats, and proteins; degradation of carotenoids; decomposition of steroids; and environmental contaminations (Johnson et al., 1969; MacLeod and Ames, 1986; Berdague et al.,

1991; Cha et al., 1992). However, in this work these compounds were found in the volatile fraction in raw beef that had not been subjected to cooking or long-term heating. This casts some doubt on thermal degradation mechanisms as their source. The aromatic hydrocarbons may play only a minor role in beef flavor (Peterson and Chang, 1982).

Higher molecular weight n-alkanes (C_{17}–C_{22}) and diterpenoids were present only in the noncondensable volatile fraction. This confirms the observation of Um et al. (1992) that SC-CO_2 has high selectivity for extracting diterpenoids. The diterpenoids included pristane (2,6,10,14-tetramethylpentadecane), phytane (2,6,10,14-tetramethylhexadecane), phyt-1-ene (2,6,10,14-tetramethyl-1-hexadecene), phyt-2-ene (3,7,11,15-tetramethyl-2-hexadecene), farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol), and squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene). The present study is the first to identify squalene as a beef flavor volatile.

Fatty aldehydes were the major volatiles of the heated lipid fraction, constituting over 40% of the total volatiles. In the noncondensable volatile fraction, aldehydes constituted only about 20% of total volatiles. In general, aldehydes are oxidative products of unsaturated fatty acids, which can be generated at temperatures as low as 60 °C (Manley, 1988). Hexanal, the major compound produced from the thermal oxidation of linoleic acid, was the most abundant of the alkanal series in heated lipid fractions. On the contrary, nonanal was the most abundant of the alkanal series produced from the noncondensable volatile fraction. Nonanal is the decomposition product of the 10-hydroperoxide, produced from the oxidation of oleic acid (Forss, 1972); oleic acid (18:1) is the main fatty acid in beef fat. Alkanal and 2-alkenal compounds contributed to the oily and tallowy flavor of beef (MacLeod and Ames, 1986). Benzaldehyde was present only in the noncondensable fractions. This compound results from the thermal decomposition of amino acids and sugars and imparts a strong, sweet, and almond odor in beef (Peterson and Chang, 1982). It has been previously identified in heated beef fat (Maruri and Larick, 1992; Um et al., 1992). 2-Ethyl-2-hexenal tentatively identified in the present study appeared only in the noncondensible fraction. It has not been reported previously in the flavor literature.

A series of methyl ketones (C_5–C_{17}) and alcohols (C_4–C_9) were detected in the condensed lipid headspace samples. Ketones and alcohols constituted about 29% of the total volatiles in the heated lipid headspace; whereas fewer ketones and alcohols were present in the noncondensable volatile fractions. Manley (1988) reported that high temperatures of thermal oxidation were responsible for the formation of ketones and alcohols, and this is consistent with the higher level of such compounds we found in our lipid headspace samples. Methyl ketones have been known to cause off-flavors in foods. Three ketones, 2-heptanone, 2,3-octanedione, and 6,10-dimethyl-5,9-undecadien-2-one, were found only in the noncondensable fraction. 2-Heptanone produces a fruity, spicy, and cinnamon flavor, whereas 2,3-octanedione imparts a caramel-like sweet aroma in cooked beef volatiles (Winter et al., 1976). 2,3-Octanedione was related to grassy flavor in beef (Larick et al., 1987). The presence of 6,10-dimethyl-5,9-undecadien-2-one in beef has not been previously reported. Among alcohols, 2-butoxyethanol, 2-ethyl-1-hexanol, 1-octen-3-ol, 1-tetradecanol, and 1-octadecanol were identified only in the noncondensable fraction. 1-Octen-3-ol has a mushroom-like odor and was considered to play a significant part in the beef stew flavor (Peterson and Chang, 1982). 2-Ethyl-1-hexanol and 2-butoxyethanol were present in appreciable amounts (2.1% and 1.4% of the total volatiles, respectively) in the noncondensable fraction. 2-Ethylhexanol has been found in fried bacon (Ho et al., 1983), and 2-butoxyethanol was identified in roast beef volatiles by others (Liebich et al., 1972; Peterson and Chang, 1982). Of the phenolic compounds, 2,6-di-*tert*-butyl-4-methylphenol (BHT) and *tert*-butyl-4-hydroxyanisole (BHA) might be derived from the feed. These antioxidants could have a significant effect on beef flavor because their presence could control the amount of oxidation products formed during and after heating.

Eleven fatty acids or their esters were identified in the noncondensable fraction, and seven were identified in the condensed lipid headspace volatiles. The fatty acids were derived from hydrolysis of triglycerides and phospholipids (Selke et al., 1975; Berdague et al., 1991). Noncondensed fractions contained longer chain fatty acids (C_{10}, C_{12}, C_{16}, C_{18}) as compared to the volatiles produced from the heated condensed fraction. Long-chain fatty acids possessed a more fatty flavor character (Bailey and Einig, 1989). The concentrations of fatty acids may reflect the fatty acid composition of diet (Maruri and Larick, 1992). Ethyl hexanoate has been previously found in beef stew by Peterson and Chang (1982).

Three furans [dihyro-4,5-dimethyl-2(3*H*)-furanone, tetrahydro-2,5-dimethylfuran, and dihydro-5-propyl-2(3*H*)-furanone] were identified only in the lipid headspace samples. Ching (1979) reported that 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) and 4-hydroxy-5-methyl-3(2*H*)-furanone (HMF) were major flavor precursors in meat. When the dihydrofuranone derivatives were heated in the presence of hydrogen sulfide, they formed mercapto-substituted furan and thiophene derivatives and produced significant meat-like aromas (Van den Ouweland and Peer, 1975). The only furan compound identified in the noncondensable fraction was 2-(hydroxymethyl)furan. Watanabe and Sato (1971) also found 2-(hydroxymethyl)-furan to be the only furan derivative in heated beef fat. 2-(hydroxymethyl)furan was responsible for the cooked sugar taste (Maga, 1979). The lack of furan compounds in this study may have resulted from the use of low temperatures during extraction. Also, certain of the furan derivatives may not have been obtained because of ineffective gas chromatographic peak separation (Maga, 1979).

Lactones are formed by heating the γ- or δ-hydroxy fatty acids that are known to be present in fats (Shahidi et al., 1986). Only three lactones (γ-butyrolactone, γ-hexalactone, and γ-decalactone) were identified in the heated condensable lipid headspace. Only γ-butyrolactone was present in high concentrations in the noncondensable fraction. High concentrations of γ-butyrolactone in roast beef were reported by Liebich et al. (1972). The absence of long-chain lactones in this study may have resulted from the low volatility of these compounds, and the low inlet temperature of the GC may have been insufficient to volatilize the compounds from the sample (Suzuki and Bailey, 1985).

Sulfur-containing compounds are extremely important contributors to roasted meat flavor profiles (Manley, 1988). In this study, only dimethyl sulfone was identified in the noncondensable fractions and was present in high concentrations. Dimethyl sulfone was identified as a roast beef volatile by Liebich et al. (1972).

Among the miscellaneous compounds, one nitrogenous compound and five esters were found in the noncondensable fraction. The only nitrogenous compound was

Table I. Volatile Compounds Analyzed by GC/MS in SC-CO_2 Noncondensable Volatile Fraction and in Condensable Lipid Headspace

peak no.	Kovats index[a]	compound	ID method[b]	volatile fraction area %[c]	lipid fraction area %[c]	refs[d]
		Aliphatic Hydrocarbons				
5	700	heptane	MS, RT	0.59	13.62	ABCDE
10	773	1-octene	MS, RT	0.18	2.70	C
20	887	1-nonene	MS, RT		6.08	CE
34	1000	decane	MS, RT	4.10	0.40	BCDE
47	1100	undecane	MS, RT	3.52		BC
60	1188	1-dodecane	MS, RT		0.34	ABC
62	1200	dodecane	MS, RT	2.14		ABC
64	1209	2,6-dimethylundecane	MS (1, 2)	0.45		
70	1300	tridecane	MS, RT	1.04	0.36	ABCD
72	1363	1-tetradecene	MS, RT	0.32	0.30	AC
76	1400	tetradecane	MS, RT	0.48		ACD
84	1500	pentadecane	MS, RT	0.48		ACDE
89	1571	1-hexadecene	MS, RT	0.11		ACE
91	1600	hexadecane	MS, RT	0.40	0.09	ACDE
94	1645	1-heptadecene	MS, RT	0.28		AC
96	1700	heptadecane	MS, RT	0.52		ACDE
103	1800	octadecane	MS, RT	0.57		ACDE
107	1900	nonadecane	MS, RT	0.63		CE
111	1969	1-eicosene	MS, RT	0.28		C
113	2000	eicosane	MS, RT	0.30		C
51	1141	cyclooctane	MS, RT		0.17	
53	1153	pentylcyclopentane	MS (2)		0.22	
80	1465	cyclododecane	MS (1)	0.13		
		Aromatic Hydrocarbons				
6	750	methylbenzene	MS, RT	0.90		ABCE
15	853	1,3-dimethylbenzene	MS, RT	2.59		AC
16	859	1,2-dimethylbenzene	MS, RT	0.86		C
19	886	ethylbenzene	MS, RT	0.75		ABC
40	1069	butylbenzene	MS, RT	0.74		C
57	1167	1,2,3,4-tetrahydronaphthalene	MS (1, 2)	0.45		
65	1229	1,2,3,4-tetrahydro-1-methylnaphthalene	MS (1, 2)	0.25		
74	1369	1-phenylheptane (IS)	MS, RT			
		Terpenoids				
97	1704	pristane	MS, RT	0.25		
102	1781	phyt-1-ene	MS (1, 2)	0.38		DE
104	1809	phytane	MS (1, 2)	0.72		ACD
106	1855	phyt-2-ene	MS (1, 2)	3.29		DE
118	2156	farnesol	MS	4.22		E
119	2179	squalene	MS, RT	2.64		
		Aldehydes				
1	678	2-butenal	MS (2)		0.78	BC
3	696	pentanal	MS, RT	0.17		ABCDE
7	753	3-methyl-2-butanal	MS, RT		0.36	B
11	778	hexanal	MS, RT	1.80	17.03	ABCDE
14	851	2-hexanal	MS, RT		0.67	ABC
21	900	heptanal	MS, RT	2.79	5.98	ABCDE
27	959	benzaldehyde	MS, RT	0.40		AE
29	975	2-heptenal	MS, RT	0.15	1.26	C
35	1004	octanal	MS, RT	2.43	4.73	ABCE
38	1031	2-ethyl-2-hexenal	MS (1, 2)	0.38		
42	1075	2-octenal	MS, RT	0.95	1.66	AC
48	1112	nonanal	MS, RT	8.43	3.01	BCDE
56	1163	2-nonenal	MS, RT	0.44	1.70	ACDE
63	1204	decanal	MS, RT	0.87		ABCD
67	1256	2-decenal	MS, RT	0.61	2.65	ADE
71	1306	undecanal	MS, RT	0.36	0.30	CE
73	1366	2-undecanal	MS (2)	0.10	0.89	ACDE
78	1408	dodecanal	MS, RT	0.19	0.17	CE
83	1499	2-dodecanal	MS (2)		0.22	CE
86	1519	tridecanal	MS, RT	0.11		CE
93	1614	tetradecanal	MS, RT		0.08	CE
105	1816	hexadecanal	MS (2)	0.11		C
112	1982	octadecanal	MS (2)	0.41		C
		Alcohols				
2	681	1-butanol	MS, RT		1.29	AC
8	759	1-pentanol	MS, RT		1.20	ABC
13	827	2,3-dimethyl-1-pentanol	MS (2)		0.08	
17	878	1-hexanol	MS, RT		1.72	ABC
22	904	2-butoxyethanol	MS, RT	1.42		AC
30	978	1-heptanol	MS, RT	0.37	3.39	BCE
31	984	1-octen-3-ol	MS, RT	0.82		BC

Table I (Continued)

peak no.	Kovats index[a]	compound	ID method[b]	volatile fraction area %[c]	lipid fraction area %[c]	refs[d]
		Alcohols				
39	1033	2-ethyl-1-hexanol	MS, RT	2.09		
43	1091	4-methyl-2-propyl-1-pentanol	MS (2)		3.32	
44	1095	1-octanol	MS, RT	0.39	1.91	ABC
54	1157	3-nonen-1-ol	MS (2)		0.12	C
58	1178	nonanol	MS, RT		2.20	C
95	1697	1-tetradecanol	MS, RT	0.30		C
114	2007	1-octadecanol	MS, RT	0.28		C
117	2037	1,2-octadecanediol	MS (1)	0.32		
		Phenols				
82	1494	BHA	MS, RT	0.77		
87	1525	BHT	MS, RT	7.82		
90	1575	2,6-bis(1,1-dimethylethyl)-4-ethylphenol	MS (1, 2)	0.43		
101	1733	nonylphenol	MS (2)	0.10		
		Ketones				
4	682	2-pentanone	MS, RT		3.09	ABC
9	768	3-hexanone	MS, RT		0.71	B
18	884	2-heptanone	MS, RT	0.17		ABC
32	985	2,3-octanedione	MS (2)	0.14		CD
33	992	2-octanone	MS, RT		6.48	ABC
45	1097	2-nonanone	MS, RT		1.41	ABC
59	1180	2-decanone	MS, RT		1.02	ABC
66	1252	cyclononanone	MS, RT		0.11	
69	1284	2-undecanone	MS, RT		0.10	ABC
77	1402	2-dodecanone	MS, RT		0.08	C
79	1462	6,10-dimethyl-5,9-undecadien-2-one	MS (1, 2)	0.08		
85	1516	2-tridecanone	MS, RT		0.17	CDE
99	1723	2-pentadecanone	MS, RT		0.49	ACD
108	1915	2-heptadecanone	MS, RT	0.17	0.12	CDE
		Furans				
26	939	dihydro-4,5-dimethyl-2(3*H*)-furanone	MS (2)		0.15	
28	960	tetrahydro-2,5-dimethylfuran	MS (2)		0.27	E
49	1136	2-furanmethanol	MS, RT	1.22		AC
55	1159	dihydro-5-propyl-2(3*H*)-furanone	MS, RT		0.39	C
		Lactones				
23	908	γ-butyrolactone	MS, RT	0.99	0.35	BC
41	1072	γ-hexalactone	MS, RT		0.46	AC
81	1487	γ-decalactone	MS, RT		0.11	CDE
		Acids				
12	803	butanoic acid	MS, RT		0.25	BCE
25	934	pentanoic acid	MS, RT		0.21	ABCDE
36	1005	hexanoic acid	MS, RT	2.45	0.16	ACDE
37	1023	3-hexenoic acid	MS	0.35		
46	1098	heptanoic acid	MS, RT	0.33	0.43	ACDE
50	1139	2-ethylhexanoic acid	MS, RT	0.46	0.19	C
61	1189	octanoic acid	MS, RT	0.48	0.32	ACDE
68	1260	nonanoic acid	MS, RT	1.86	1.95	ACDE
75	1376	decanoic acid	MS, RT	0.10		CDE
88	1546	dodecanoic acid	MS, RT	0.06		CD
110	1969	hexadecanoic acid	MS, RT	0.43		C
115	2011	9-octadecenoic acid methyl ester	MS	0.19		C
116	2021	octadecanoic acid	MS	0.54		C
		Sulfur Compounds				
24	915	dimethyl sulfone	MS, RT	7.86		BC
		Miscellaneous Compounds				
52	1145	1-piperidinecarboxaldehyde	MS (1)	0.91		
92	1603	2-methyl-1-(1,1-dimethylethyl)-2-methylpropanoate 1,3-propanediyl ester	MS (1, 2)	0.55		
98	1711	1-butanol 3-methylbenzoate	MS (1, 2)	2.09		
100	1730	3-methoxybenzoate ethyl ester	MS (1, 2)	0.80		
109	1944	1,2-benzenedicarboxylic acid alkyl ester	MS (1, 2)	0.26		
120	2534	1,2-benzenedicarboxylic acid alkyl ester	MS (1, 2)	7.17		

[a] Kovats indices were determined by using a series of hydrocarbons on the fused silica column (Ultra-2) described under Materials and Methods. [b] MS, mass spectrum data were consistent with those of authentic compounds; RT, retention time data were consistent with those of authentic compounds; MS (1), tentatively identified from a Hewlett-Packard Model 5970 with NBS data; MS (2), tentatively identified from a Perkin-Elmer Model 8320 with NBS data; MS (1, 2), tentatively identified with both Perkin-Elmer and Hewlett-Packard mass spectrometers. [c] Area percents are based on FID response (solvent and internal standard excluded). Reported values are average of two analyses. [d] Reference where component was also identified: A, Peterson and Chang (1982); B, MacLeod and Ames (1986); C, Shahidi et al. (1986); D, Larick et al. (1987); E, Um et al. (1992).

1-piperidinecarboxaldehyde. The origin and role of this compound in the flavor of beef are not known. Five esters, 1-butanol 3-methylbenzoate, 3-methoxybenzoate, one branched-chain propanoate, and two alkyl benzenedicar-

boxylates, were tentatively identified in the noncondensable volatile fraction. Esters might have come from esterification of the various alcohols and carboxylic acids found in beef (Peterson and Chang, 1982). They are generally associated with fruit flavors (Nursten and Williams, 1967). 1,2-Benzenedicarboxylic acid alkyl esters were also found in ham (Berdague et al., 1991). Although the origin of these compounds is quite uncertain, compounds of this class are often used as polymeric plasticizers (Sears and Darby, 1982). This raises the possibility that SC-CO_2 extraction is powerful enough to extract and detect additives which may have migrated to the food from plastic wrapping. Use of supercritical chromatography to analyze plastics and polymers is now in practice.

Previous workers have identified many compounds associated with beef flavor and aroma using steam distillation or headspace sampling (Peterson and Chang, 1982; MacLeod and Ames, 1986; Shahidi et al., 1986; Larick et al., 1987). Both of these procedures expose the samples to high temperatures for significant periods of time. Sixty-five of the compounds from the noncondensable volatile fraction and 54 of the compounds from the condensable lipid headspace samples were previously identified by others. Um et al. (1992) recently used supercritical extraction to collect volatiles from beef fat that had been heated at 100 °C. We have confirmed 30 of the compounds from noncondensable volatile fraction and 25 of the compounds from the condensed lipid headspace samples identified by Um et al. (1992).

Compared to previous studies in which organic solvents were used in meat flavor analysis, we found approximately 25 new compounds that had not previously been identified. This may be due in part to the ability to remove the supercritical CO_2 completely from the sample before analysis, so it does not appear as background in the analytical instrument. Also, we were able to detect beef flavor by smell, which is not possible with strong-smelling liquid solvents. Using supercritical CO_2 extraction allows a coarse level of fractionation by reducing the pressure. With organic solvents, there is only one extract phase produced, and pressure is not a variable for fractionation.

CONCLUSIONS

SC-CO_2 is a very versatile extractant because of its efficiency and nontoxicity. Separation of the beef fat from volatile aroma fractions was successful. Raw beef was used in this study, and the extraction procedure did not involve high temperatures; thus, the volatile compounds present in beef before cooking were isolated in the noncondensable fraction. These data should be valuable in future studies aimed at supercritical extraction of whole cooked beef. Several new compounds not previously reported by others have been tentatively identified in this paper. This may be because raw, not cooked, beef was the sample extracted, or it may be due to the particular solvent selectivity of the supercritical CO_2. Direct on-column headspace injection involves high-temperature heating which may result in oxidation and cause the development of off-flavor. The chemical differences between the volatile (unheated) fraction and the lipid headspace (heated) fraction are indicative of oxidation and degradation caused by heating in air. The heated lipid samples may not be representative of the "true" cooked beef flavor because the heating times and temperatures were more extreme than those of ordinary cooking. The mechanism involved in extraction with SC-CO_2 and food is still not fully understood. Additional studies on flavor volatiles in cooked beef are continuing.

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