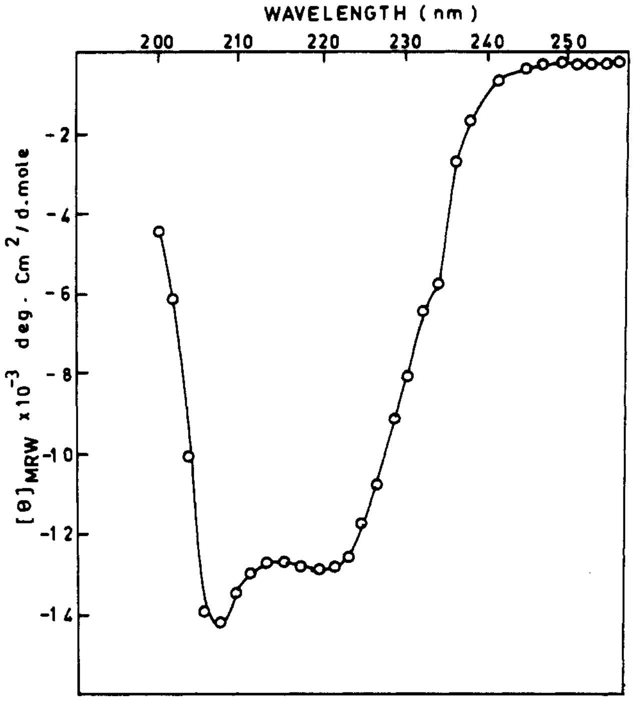

Figure 3. Far-UV-CD spectrum of poppy seed low molecular weight proteins in 0.5 M NaCl solution. Values are averages of two independent determinations.

linseed low molecular weight proteins but different from those of rapeseed proteins. This may be related to the composition and physicochemical properties of these proteins.

The data show that unlike the 10S protein that contains very little α-helix (Srinivas and Narasinga Rao, 1986), the low molecular weight protein fraction has a more ordered structure as evidenced by higher helical content.

The results indicated that the low molecular weight proteins consisted of three to five fractions. Apparently their molecular weights are very close to each other; SDS-PAGE indicated values ranging from 13000 to 18000. Because of this, it was difficult to resolve into homogeneous fractions in gel filtration. Attempts to isolate the homogeneous fractions by DEAE- or CM-Sephadex chromatography did not succeed. Since the proteins contained carbohydrate, attempts are being made to fractionate them to homogeneous protein by affinity chromatography.

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Received for review March 13, 1986. Accepted August 5, 1986.

Analysis of Headspace Volatiles from Overheated Beef Fat

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Headspace volatiles from overheated beef fat were purged into the water of the gas-washing bottle and simultaneously were continuously extracted with dichloromethane. Gas chromatography/mass spectrometry analysis of the dichloromethane extract resulted in identification of 87 compounds. The compounds identified included 7 alkanes, 31 alkenes, 18 aldehydes, and 6 ketones. When the water of the gas-washing bottle was replaced with an aqueous cysteamine solution, trace quantities of six branched aldehydes not previously found in the extract from the water were recovered and identified as thiazolidine derivatives. The apparatus prepared in the present study may be used to collect large quantities of headspace volatiles under mild temperatures unlikely to cause chemical alteration of the volatiles.

The volatile components of cooked meats have been investigated by many researchers. Several reviews on beef flavors have been appeared in the last decade (Dwivedi, 1975; Chang and Peterson, 1977; Shibamoto, 1980). Volatile chemicals formed from animal fat alone have, however, not been thoroughly studied. Buttery et al. (1977) reported basic volatile components of roasted lamb fat. Ohnishi and Shibamoto (1984) investigated volatile chemicals formed from heated beef fat. They used simulated cooking temperatures of 150 and 200 °C. Many studies of heated fats have been done with what were called "laboratory-heated fats" in order to determine

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0021-8561/87/1435-0014$01.50/0

chemicals formed from fat by high-temperature treatment. The fats have been overheated in the laboratory, with or without air or oxygen, at high temperatures of 200–300 °C for several days in most cases (Crampton et al., 1956; Firestone et al., 1961; Perkins, 1960; Schultz, 1962). The same circumstances can be seen in the cooking practice of some restaurants.

Isolation of volatile chemicals from a fatty sample is one of the most difficult of analytic procedures. Direct extraction with an organic solvent is practically impossible because most organic solvents dissolve fatty materials. Thus, steam distillation is the most common method of separating organic compounds from fatty materials. A simultaneous steam distillation–solvent extraction apparatus advanced by Likens and Nickerson (1964) was later applied to analysis of volatiles from fat by several researchers (Buttery et al., 1977; Ohnishi and Shibamoto, 1984). When steam distillation is used in a separation procedure, however, the volatiles still must undergo temperatures of 100 °C or higher, which may cause further alteration of chemicals after separation from the fat.

In the present study, a new apparatus was designed for recovering headspace volatiles formed from overheated beef fat using mild temperatures. This apparatus uses a gas-washing bottle and a liquid–liquid continuous extractor in tandem.

EXPERIMENTAL SECTION

Materials. Cysteamine hydrochloride, formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, and decanal were purchased from Aldrich Chemical Co., Milwaukee, WI. All other authentic chemicals were obtained from commercial sources. The fat used in the experiments was obtained from the renal periphery of beef carcasses. Frozen fatty tissue along with a little dry ice was ground to a powder in a blender and then melted in a flask in a hot water bath at 70–80 °C. All of the nonfatty tissue, including blood, muscle, and connective tissue, was removed from the liquid fat by filtration. The pure beef fat was then stored in a freezer for future experiments.

Collection of Headspace Volatiles from Heated Beef Fat. Pure beef fat (140 g) was placed in a 500-mL, two-neck, round-bottom flask. The flask was connected to the apparatus shown in Figure 1. The beef fat was heated at 300 °C. The headspace volatiles were purged into 250 mL of deionized water by a steam of purified nitrogen (flow rate 7.2 mL/min). The volatiles dissolved by the water were simultaneously and continuously extracted with dichloromethane (70 mL) for 6 h. The water temperature was maintained at 10 °C by a Brinkman RM6 constant-temperature water circulator. The dichloromethane extract was dried over anhydrous sodium sulfate for 12 h, and the solvent was removed by a rotary flash evaporator. The concentrated sample (0.3 mL) was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Analysis of Trace Aldehydes in Beef Fat Volatiles. Aldehydes formed in beef fat were trapped, by a method developed previously (Hayashi and Shibamoto, 1985). An aqueous solution of cysteamine (1.5 g in 245 mL of deionized water) was put in the apparatus. The solution was adjusted to pH 6.2 with 2 N sodium hydroxide solution. Purified beef fat (92 g) was placed in a 500-mL round-bottom flask and was heated to 300 °C. The headspace volatiles were purged into the cysteamine solution by a stream of purified nitrogen (7.2 mL/min). Thiazolidines derived from cysteamine from the solution and aldehydes from the volatiles were simultaneously and

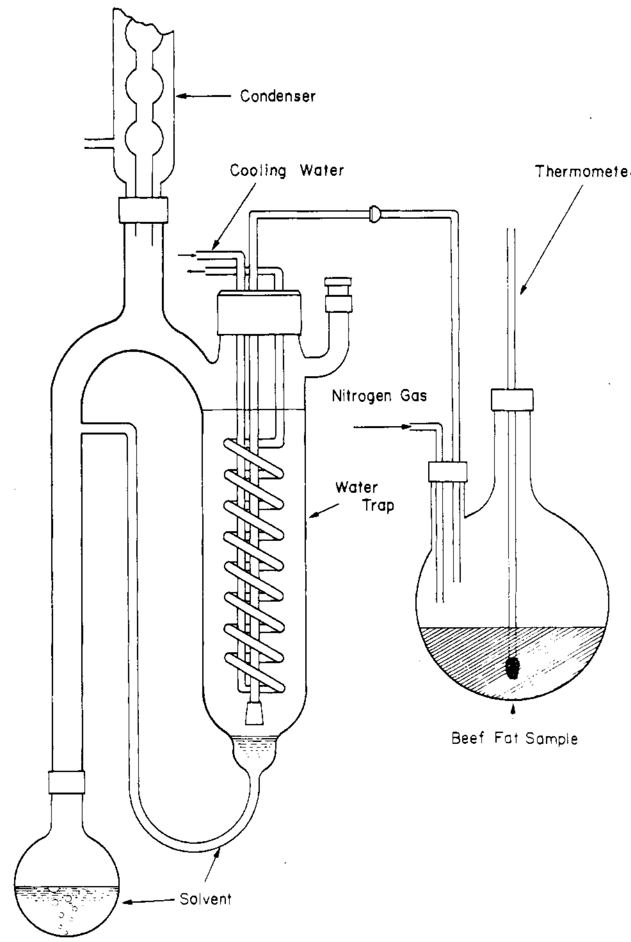

Figure 1. Apparatus used to collect headspace volatiles from heated beef fat.

continuously extracted with 70 mL of dichloromethane or chloroform for 6 h. Chloroform was used only for formaldehyde analysis because the dichloromethane contained formaldehyde as an impurity (Hayashi et al., 1986). The extract was dried over anhydrous sodium sulfate for 12 h, and then 2 L of extract was injected into a GC equipped with a thermionic nitrogen–phosphorus-specific detector (TSD).

A trap containing 250 mL of aqueous cysteamine solution was connected to the other end of the condenser to catch any highly volatile aldehydes such as formaldehyde that might escape from the apparatus. This cysteamine solution was then treated and extracted just as the experimental solution had been.

Preparation of Calibration Curves for Aldehydes. Cysteamine (1.5 g) was reacted with various amounts of an aldehyde (0.1–1.5 mg) in 250 mL of deionized water at pH 6.2. Each reaction solution was extracted with 70 mL of dichloromethane or chloroform (formaldehyde reaction solution only), with a liquid–liquid continuous extractor for 6 h. After removal of water from the extract, 50 μL of standard tripropylamine solution (10 μg/mL of dichloromethane) was added to each extract before GC analysis. The GC peak area ratio of a thiazolidine derivative to the internal standard was plotted against the quantity of the aldehyde in the original solution. Aldehydes used were formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, and decanal. A dichloromethane solution (10 μg/mL) was used with all aldehydes except formaldehyde. An aqueous solution (10 μg/mL) was used with formaldehyde.

Instrumental Analysis. A Hewlett-Packard Model 5880A gas chromatograph (GC) equipped with a 60 m ×

Table I. Compounds Identified in Headspace Extract of Heated Beef Fat

compound	peak area, %	I^a	ID MS	ID I	compound	peak area, %	I^a	ID MS	ID I
heptane	0.42	700	+	+	(*Z*)-2-undecene	2.64	1173	+	+
cyclohexane	0.23	722	+	+	2-heptanone	0.38	1180	+	+
(*E*)-3-heptene, cyclopentadiene	0.52	735	+	+	heptanal	3.06	1183	+	+
1-heptene	1.02	741	+	+	limonene	1.23	1192	+	+
(*E*)-2-heptene	*b*	778	+	+	dodecane, propylbenzene	0.98	1200	+	+
(*Z*)-2-heptene, propanal	*b*	784	+	+	(*E*)-2-hexenal	0.55	1212	+	+
octane	5.09	800	+	+	1-hexylcyclopentene	0.32	1217	+	+
acrolein	5.18	828	+	+	4-octanone	*b*	1224	+	+
1-octene	2.85	842	+	+	2-pentylfuran	0.79	1231	+	+
1,3-cyclohexadiene	*b*	849	+	+	(*E*)-3-dodecene	0.23	1237	+	+
(*E*)-2-octene	0.82	860	+	+	cyclododecane	0.55	1242	+	+
butanal	0.23	867	+	+	1-dodecene	0.60	1246	+	+
(*Z*)-2-octene	0.46	872	+	+	(*Z*)-3-dodecene	0.31	1254	+	
2-butanone	*b*	893	+	+	cycloundecene	0.6	1257	+	+
nonane	2.48	900	+	+	(*E*)-2-dodecene	0.27	1261	+	+
benzene	0.56	924	+	+	cyclohexanone	0.35	1282	+	+
1-nonene	3.86	929	+	+	2-octanone	0.31	1283	+	+
cyclooctene	0.38	944	+	+	octanal	2.57	1287	+	+
(*Z*)-3-nonene	0.83	948	+	+	(*Z*)-3-heptenal	*b*	1295	+	+
(*E*)-2-nonene	0.41	961	+	+	(*E*,*Z*)-2,4-undecadiene	*b*	1297	+	+
(*Z*)-2-nonene	1.66	972	+	+	tridecane	0.62	1300	+	+
pentanal	*b*	974	+	+	butylbenzene	1.28	1306	+	+
decane	1.23	1000	+	+	(*E*)-2-heptenal	2.07	1318	+	+
2-methylbicyclo[3.3.0]octane	*b*	1031	+		2-methyl-3-octanone	0.23	1323	+	+
bicyclo[5.1.0]octane	1.96	1035	+		(*E*)-5-undecen-3-yne	*b*	1327	+	+
toluene	1.19	1038	+	+	2-hexylfuran	0.45	1329	+	+
4-nonyne	0.48	1043	+		1-tridecene	*b*	1332	+	+
1-decene	1.79	1047	+	+	cyclododecene	0.46	1357	+	+
(*Z*)-3-decene	0.23	1049	+		2-nonanone	*b*	1386	+	+
3-methylbicyclo[3.3.0]octane	0.21	1056	+		nonanal	7.01	1392	+	+
cyclononene	0.93	1058	+	+	(*Z*)-3-octenal	0.26	1397	+	+
(*E*)-2-decene	0.70	1062	+	+	(*E*,*Z*)-2,4-dodecadiene	*b*	1402	+	+
(*Z*)-2-decene	0.28	1072	+	+	pentylbenzene	0.34	1409	+	+
butylcyclohexane	*b*	1075	+	+	(*E*)-2-octenal	1.28	1425	+	+
hexanal	2.53	1081	+	+	2-heptylfuran	*b*	1429	+	+
(*E*)-cyclodecene	*b*	1086	+		iso-β-terpineol	8.44	1437	+	
(*E*,*E*)-2,4-nonadiene	*b*	1092	+	+	1-octen-3-ol	0.51	1445	+	+
(*E*,*E*)-1,3,6-octatriene	*b*	1098	+	+	heptanol	0.39	1457	+	+
undecane, (*E*,*Z*)-1,3,6-octatriene	1.72	1100	+	+	2-butenylbenzene, (*E*,*E*)-2,4-heptadienal	0.37	1487	+	+
1-pentylcyclopentene	*b*	1110	+	+					
4,6-decadiene	*b*	1113	+	+	2-decanone	*b*	1493	+	+
ethylbenzene	0.4	1122	+	+	decanal	0.34	1495	+	+
(*E*)-2-pentenal	0.32	1124	+	+	benzaldehyde	0.51	1515	+	+
camphane	0.78	1131	+	+	(*E*)-2-nonenal	1.32	1532	+	+
(*E*)-5-undecene	0.35	1134	+	+	(*Z*)-2-nonenal	0.42	1543	+	+
(*E*)-3-undecene	*b*	1137	+		octanol	*b*	1565	+	+
1-undecene	3.59	1147	+	+	(*E*)-2-decenal	1.45	1642	+	+
(*Z*)-cyclodecene	0.31	1157	+	+	(*E*)-2-undecenal	*b*	1750	+	+
(*E*)-2-undecene	5.30	1163	+	+					

[a] Kovats index on DBWAX column. [b] Peak area percent less than 0.02.

0.25 mm i.d. DBWAX fused silica capillary column and a flame ionization detector (FID) was used for comprehensive analysis and with a 30 m × 0.25 mm i.d. DB-1 fused silica capillary column and a TSD was used for trace aldehyde analysis. The oven temperature was held at 40 °C for 10 min and then programmed to 200 °C at 2 °C/min. The GC peak areas were integrated with an HP 5880A series GC terminal. The injector and detector temperatures were 250 °C. The column was operated with helium carrier gas at an average linear velocity of 30 cm/s on the basis of a methane peak at 120 °C. The injector split ratio was 1:30. For the FID the gas flow rates were as follows (mL/min): hydrogen, 20; air, 250; makeup nitrogen, 20. For the TSD these rates were as follows (mL/min): hydrogen, 3; air, 60; makeup nitrogen, 20. A Hewlett-Packard Model 5792A GC interfaced to a VG ZAB-HS-2F high-resolution magnetic sector mass spectrometer (MS) with VG 11-250 computer data system was used for MS identification of the GC components at MS ionization voltage 70 eV. The GC column and oven conditions were as described for the Hewlett-Packard Model 5880A. The GC retention index (Kovats index) and MS fragmentation pattern of each component of the headspace volatiles were compared with those of the authentic compound for identification. An additional computer search of standard mass spectra to confirm the identity of unknowns was conducted as described in the *NIH/EPA Chemical Information System* (1978). Identities of compounds deduced from mass spectra, but for which no authentic samples are available, are considered as only tentatively identified.

RESULTS AND DISCUSSION

The compounds identified in the headspace volatiles of heated beef fat are listed in Table I in order of elution from the GC column DBWAX. Table I was prepared in the format used by Flath et al. (1983).

A typical GC of a headspace sample from the water trap showed over 120 peaks. This result suggests that water can be used as a trapping material for organic compounds. Solubility of the headspace volatiles in water was not important because they were rapidly removed from the water

Table II. Thiazolidine Derivatives Identified in the Cysteamine Trap

peak no. in Figure 2	thiazolidine	I^a	orig aldehyde	peak area, %	amt[b] recd, g
1	2-methylthiazolidine	890	acetaldehyde	11.84	161
2	tripropylamine (int std)	927		7.38	
3	2-ethylthiazolidine	991	propanal	17.61	835
4	2-isopropylthiazolidine	1055	isobutanal	0.37	c
5	unknown	1079		0.05	
6	2-propylthiazolidine	1086	butanal	7.69	104
7	unknown	1100		0.46	
8	2-isobutylthiazolidine	1146	isopentanal	0.50	c
9	2-(1-methylpropyl)thiazolidine	1160	2-methylbutanal	0.21	c
10	2-((Z)-2-butenyl)thiazolidine	1176	(Z)-3-pentenal	1.18	c
11	2-butylthiazolidine	1190	pentanal	9.99	422
12	unknown	1202		0.11	
13	2-(1-methylbutyl)thiazolidine	1254	2-methylpentanal	0.08	c
14	2-((Z)-2-pentenyl)thiazolidine	1289	(Z)-3-hexenal	0.46	c
15	2-pentylthiazolidine	1296	hexanal	11.47	715
16	unknown	1315		0.07	
17	unknown	1327		0.03	
18	2-((Z)-2-hexenyl)thiazolidine	1381	(Z)-3-heptenal	1.43	c
19	unknown	1390		0.59	
20	2-hexylthiazolidine	1402	heptanal	10.91	604
21	unknown	1436		0.29	
22	unknown	1471		0.27	
23	2-((Z)-2-heptenyl)thiazolidine	1481	(Z)-3-octenal	0.69	c
24	unknown	1492		0.42	
25	2-heptylthiazolidine	1509	octanal	7.41	474
26	2-((Z)-2-octenyl)thiazolidine	1589	(Z)-3-nonenal	0.43	c
27	unknown	1595		0.15	
28	2-octylthiazolidine	1616	nonanal	7.29	620
29	unknown	1700		0.12	
30	2-nonylthiazolidine	1722	decanal	0.35	20

[a] Kovats index on DB-1 column. [b] Amount of aldehydes found in headspace extract from the cysteamine trap. [c] Not calculated.

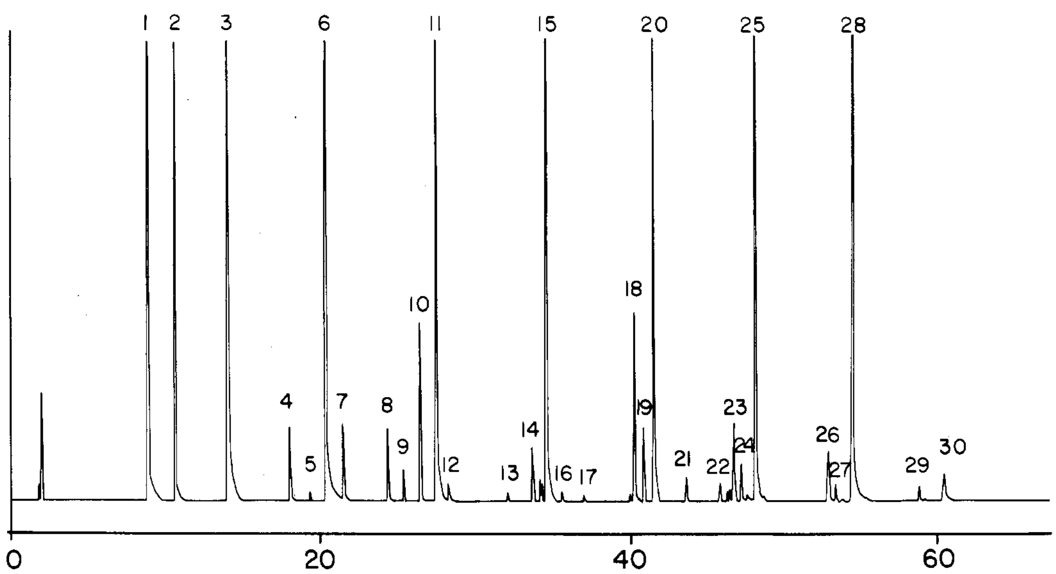

Figure 2. Typical gas chromatograph of the extract from a cysteamine trap. See Table II for peak identification, and see the Experimental Section for GC conditions.

by the organic solvent and accumulated in the solvent flask.

The highest temperature of volatiles after separation from the heated fat was the boiling point of dichloromethane 39.75 °C. This is an advantage over other techniques because this relatively low is less likely to promote decomposition of any unstable chemicals obtained from the sample than procedure typically requiring temperature of 100 °C or more. For example, the most widely used techniques for concentrating headspace samples involve entraining headspace vapor on adsorbents such as silica gel, active alumina, charcoal, or porous polymers; but the trapped sample must then be heated above 100 °C to recover it from the adsorbents. In addition, the surface of adsorbents is somewhat active chemically and may alter components of a sample.

Usually, the amount of adsorbent used to trap headspace volatiles is limited and the quantity of headspace sample recovered is small (about 1–2 μL). In contrast, an almost unlimited amount of the headspace sample can be collected with the apparatus developed in the present study.

In the present study, the compounds of highest boiling point present in the headspace samples from heated beef fat may be compounds such as octanol, C_8 alcohol. By contrast, when heated beef fat was steam distilled, must less volatile octadecanol (C_{18} alcohol) was one of the compounds of highest boiling point recovered (Ohnishi and Shibamoto, 1984). Thus, the apparatus prepared for the present study may lead to the isolation of compounds too volatile for isolation by steam distillation and a more complete knowledge of the volatile components of food flavors. This is important because these components may be inhaled during cooking and eating.

The isolation of *n*-alkanes, *n*-alcohols, *n*-aldehydes, *n*-alkylcyclohexanes, and 2-ketones was consistent with previous reports (Alcencar et al., 1983; Ohnishi and Shibamoto, 1984). *E*,*Z* isomers of *n*-alkenes, however, have never before been found in fat samples. Aldehydes were the major constituents of headspace volatiles. The total GC peaks area percent of aldehydes was 23.41%, which included 7.67% unsaturated aldehydes. Further analysis of trace aldehyde, in the headspace sample, was done with a cysteamine trap.

Table II shows the aldehydes identified as thiazolidine derivatives. Figure 2 shows a typical GC of the extract from the cysteamine trap. The major advantage of this trap is that some trace chemicals such as aldehydes can be trapped in the form of a derivative. Because thiazolidines derived from aldehydes contained a nitrogen atom, the highly sensitive and selective TSD could be used for analysis. No acetaldehyde and just a trace of propanal were found in the sample from a water trap, yet propanal was the major component found in the sample from a cysteamine trap. This suggests that these highly volatile aldehydes may escape from the water trap during the experiment. But with the cysteamine trap no thiazolidines were found in the cysteamine trap connected to the condenser. This result suggests that with the cysteamine all of the volatile aldehydes were trapped as thiazolidines. Certain branched aldehydes that were not found in a water trap were detected in a cysteamine trap. But α,β-unsaturated aldehydes (acrolein, 2-pentenal, 2-hexenal, 2-heptenal, 2-octenal, 2-nonenal, 2-decenal) did not form thiazolidine derivatives with cysteamine. Standard α,β-unsaturated aldehydes were reacted with cysteamine in an aqueous solution at pH 6.2, but no thiazolidine derivatives were formed. The α,β-unsaturated aldehyde and cysteamine might undergo reactions other than thiazolidine formation because α,β-unsaturated aldehydes, (*Z*)-3-heptenal and (*Z*)-3-octenal, gave corresponding thiazolidine derivative 2-((*Z*)-2-hexenyl)thiazolidine and 2-((*Z*)-2-heptenyl)thiazolidine in the cysteamine trap, respectively.

The source of terpenes camphane, limonene, and iso-β-terpineol (tentative) in the heated beef fat was unknown, but they might have come from a cattle feed made from citrus peels.

The apparatus developed in the present study to collect headspace volatiles from heated beef fat using mild temperatures in isolation performed adequately. This apparatus may be used with other food samples to collect headspace volatiles, especially with those volatiles that might be lost by methods requiring higher temperatures of isolation.

ACKNOWLEDGMENT

We thank D. Sehnert, Animal Science Department, University of California, Davis, for supplies of beef fat. This investigation was supported by the National Cancer Institute (Grant 1R01CA38697-01).

Registry No. Heptane, 142-82-5; cyclohexane, 110-82-7; (*E*)-3-heptene, 14686-14-7; cyclopentadiene, 542-92-7; 1-heptene, 592-76-7; (*E*)-2-heptene, 14686-13-6; (*Z*)-2-heptene, 6443-92-1; propanal, 123-38-6; octane, 111-65-9; acrolein, 107-02-8; 1-octene, 111-66-0; 1,3-cyclohexadiene, 592-57-4; (*E*)-2-octene, 13389-42-9; butanal, 123-72-8; (*Z*)-2-octene, 7642-04-8; 2-butanone, 78-93-3; nonane, 111-84-2; benzene, 71-43-2; 1-nonene, 124-11-8; cyclooctene, 931-88-4; (*Z*)-3-nonene, 20237-46-1; (*E*)-2-nonene, 6434-78-2; (*Z*)-2-nonene, 6434-77-1; pentanal, 110-62-3; decane, 124-18-5; 2-methylbicyclo[3.3.0]octane, 3868-64-2; bicyclo[5.1.0]octane, 286-43-1; toluene, 108-88-3; 4-nonyne, 20184-91-2; 1-decene, 872-05-9; (*Z*)-3-decene, 19398-86-8; 3-methylbicyclo[3.3.0]octane, 32273-77-1; cyclononene, 3618-11-9; (*E*)-2-decene, 20063-97-2; (*Z*)-2-decene, 20348-51-0; butylcyclohexane, 1678-93-9; hexanal, 66-25-1; (*E*)-cyclodecene, 2198-20-1; (*E*,*E*)-2,4-nonadiene, 56700-78-8; (*E*,*E*)-1,3,6-octatriene, 22038-69-3; undecane, 1120-21-4; (*E*,*Z*)-1,3,6-octatriene, 15187-61-8; 1-pentylcyclopentene, 4291-98-9; 4,6-decadiene, 55682-65-0; ethylbenzene, 100-41-4; (*E*)-2-pentenal, 1576-87-0; camphane, 464-15-3; (*E*)-5-undecene, 764-97-6; (*E*)-3-undecene, 1002-68-2; 1-undecene, 821-95-4; (*Z*)-cyclodecene, 935-31-9; (*E*)-2-undecene, 693-61-8; (*Z*)-2-undecene, 821-96-5; 2-heptanone, 110-43-0; heptanal, 111-71-7; limonene, 138-86-3; dodecane, 112-40-3; propylbenzene, 103-65-1; (*E*)-2-hexenal, 6728-26-3; 1-hexylcyclopentene, 4291-99-0; 4-octanone, 589-63-9; 2-pentylfuran, 3777-69-3; (*E*)-3-dodecene, 7206-14-6; cyclododecane, 294-62-2; 1-dodecene, 112-41-4; (*Z*)-3-dodecene, 7239-23-8; cycloundecene, 6568-15-6; (*E*)-2-dodecene, 7206-13-5; cyclohexanone, 108-94-1; 2-octanone, 111-13-7; octanal, 124-13-0; (*Z*)-3-heptenal, 21662-18-0; (*E*,*Z*)-2,4-undecadiene, 66717-34-8; tridecane, 629-50-5; butylbenzene, 104-51-8; (*E*)-2-heptenal, 18829-55-5; 2-methyl-3-octanone, 923-28-4; (*E*)-5-undecen-3-yne, 74744-31-3; 2-hexylfuran, 3777-70-6; 1-tridecene, 2437-56-1; cyclododecene, 1501-82-2; 2-nonanone, 821-55-6; nonanal, 124-19-6; (*Z*)-3-octenal, 78693-34-2; (*E*,*Z*)-2,4-dodecadiene, 74685-27-1; pentylbenzene, 538-68-1; (*E*)-2-octenal, 2548-87-0; 2-heptylfuran, 3777-71-7; 1-octen-3-ol, 3391-86-4; heptanol, 111-70-6; 2-butenylbenzene, 1560-06-1; (*E*,*E*)-2,4-heptadienal, 4313-03-5; 2-decanone, 693-54-9; decanal, 1122-31-2; benzaldehyde, 100-52-7; (*E*)-2-nonenal, 18829-56-6; (*Z*)-2-nonenal, 60784-31-8; octanol, 111-87-5; (*E*)-2-decenal, 3913-81-3; (*E*)-2-undecenal, 53448-07-0; acetaldehyde, 75-07-0; isobutanal, 78-84-2; isopentanal, 590-86-3; 2-methylbutanal, 96-17-3; (*Z*)-3-pentenal, 53448-06-9; 2-methylpentanal, 123-15-9; (*Z*)-3-hexenal, 6789-80-6; (*Z*)-3-nonenal, 31823-43-5.

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Received for review March 28, 1986. Revised manuscript received July 25, 1986. Accepted August 28, 1986.