

Isolation and Identification of Headspace Volatiles Formed in Heated Butter

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Unsalted sweet butter was heated at 100, 150, or 200 °C for 5 h. The headspace volatiles formed were collected during heating using a simultaneous purging and solvent extraction apparatus. Among 140 peaks on a gas chromatogram of the headspace sample from butter heated at 200 °C, 77 were positively identified by gas chromatography and gas chromatography/mass spectrometry. Major compounds identified were 21 aldehydes, 12 fatty acids, 11 ketones, 10 nitrogen- and/or sulfur-containing compounds, 7 alkanes, 6 δ -lactones, and 4 furans, which constituted over 85% of total volatiles recovered. The number of volatiles formed in a headspace of butter heated at 100 or 150 °C was much less than that formed at 200 °C. However, all volatiles formed at 100 and 150 °C were found in a sample heated at 200 °C.

INTRODUCTION

Butter contains a wide variety of compounds in addition to milk fats and water, which constitute over 87% of total components (Corbin and Whittier, 1965). Over 120 compounds had been reported as flavor constituents of butter products as of 1975 (Ramsaw, 1974; Kinsella, 1975).

Determination of the flavor impact by an individual component in heated foods is very difficult due to the large number of compounds produced and their different flavor threshold values (Nawar et al., 1988). However, isolation and identification of flavor chemicals formed in foods under various heating conditions is one avenue to understanding the formation of complex flavor compounds in cooked foods.

Analysis of volatile organic compounds present in a fatty sample is not easy. Direct extraction with an organic solvent is practically impossible because most organic solvents dissolve fatty materials. Conventional steam distillation, or a more advanced simultaneous steam distillation-solvent extraction method, is most commonly used to isolate volatile components from fatty samples (Buttery et al., 1977; Ohnishi and Shibamoto, 1984). A high-vacuum cold-finger distillation has also commonly been used to recover volatile chemicals from oil samples (Fross and Holloway, 1967; Nawar et al., 1969; 1988). Recently, headspace volatiles from overheated beef fat were satisfactorily collected using a newly developed simultaneous purging-solvent extraction (SPE) apparatus (Umano and Shibamoto, 1987).

Butter is widely used not only as a spread but also for seasoning during cooking of foods, suggesting that it plays an important role in formation of flavors during heating. In the present study, therefore, headspace volatiles formed from butter heated at different temperatures were collected using an SPE and analyzed by gas chromatography/mass spectrometry to investigate the role of butter in formation of flavors by heat.

EXPERIMENTAL PROCEDURES

Materials. A commercial unsalted sweet butter was purchased from a local market. Analytical grade solvents and reagents were

obtained from reliable commercial sources and used without further treatment.

Headspace Sample. Butter (250 g) was placed in a 500-mL, two-neck, round-bottom flask. The flask was connected to an SPE and heated slowly at 100, 150, or 200 °C. The headspace volatiles were purged into 230 mL of deionized water by an air stream (10 mL/min). The volatiles dissolved in the water were simultaneously and continuously extracted with 70 mL of dichloromethane for 5 h. The water temperature was maintained at 10 °C by using a Brinkman RM6 constant-temperature water circulator.

The dichloromethane extract was dried over anhydrous sodium sulfate for 12 h. After removal of sodium sulfate, the volume of the extract was reduced to 1.5 mL by fractional distillation. Prior to GC analysis, 0.1 mL of a solution of 2,5-dimethylhexane in dichloromethane (5 mg/mL) was added to the sample as a GC internal standard for quantitative analysis of the headspace volatiles. The final sample volume was adjusted to exactly 2 mL by adding dichloromethane.

Instrumental Analysis. A Hewlett-Packard Model 5790 gas chromatograph (GC) equipped with a 60 m \times 0.25 mm i.d. (film thickness = 0.25 μ m) DB-5 bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used for comprehensive analysis. The GC peak areas were integrated with a HP 5880A Series GC terminal. The same capillary column was also installed in a HP 5890 GC, equipped with a nitrogen-phosphorus detector (NPD), and in a HP 5890 Series II GC, equipped with a flame photometric detector (FPD). Each detector was used to determine the presence of nitrogen- and/or sulfur-containing compounds, respectively. The GC/NPD and the GC/FPD were interfaced to a Spectra-Physics 4290 instrument for peak area integration. The oven temperature was held at 40 °C for 5 min and then programmed to 250 °C at 2 °C/min. The injector temperature was 250 °C, and the detector temperatures were 300 °C for the FID and 220 °C for the NPD and the FPD. The column was operated with helium carrier gas at an average linear velocity of 30 cm/s.

A 30 m \times 0.25 mm i.d. (film thickness = 0.25 μ m) DB-Wax bonded-phase fused-silica capillary column was used to analyze the volatile fatty acids, which tend to front-tail on the DB-5 capillary column. The column was installed in the HP-5790 GC. The oven temperature was held at 70 °C for the first 5 min and then programmed to 200 °C at 2 °C/min. The injector temperature was 200 °C and the detector temperature 250 °C.

A HP 5890 GC interfaced to a VG Trio II mass spectrometer with VG 11-250 computer data system was used to obtain the mass spectra of the GC components at MS ionization 70 eV.

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Table I. Volatile Chemicals Identified in the Headspace from Butter Heated at 100, 150, and 200 °C

compound	<i>I</i> _{DB-5} ^a	GC peak area %			ID		compound	<i>I</i> _{DB-5} ^a	GC peak area %			ID	
		100 °C	150 °C	200 °C	<i>I</i>	MS			100 °C	150 °C	200 °C	<i>I</i>	MS
aldehydes							fatty acids						
butanal	595	6.54	2.01	1.85	+ /	+	3-butenic acid ^d	649	c	0.31	0.81	+	+
3-methylbutanal	659	1.11	0.10	0.20	+	+	heptanoic acid	1086	c	b	b	+	+
pentanal	697	0.95	1.26	2.15	+	+	octanoic acid	1183	b	0.88	0.57	+	+
hexanal	801	0.15	0.88	6.01	+	+	nonanoic acid	1275	c	b	b	+	+
<i>trans</i> -2-hexenal	856	b	0.27	0.30	+	+	decanoic acid	1373	c	0.82	0.51	+	+
heptanal	901	0.35	0.84	0.80	+	+	propanoic acid	1529 ^e	c	0.05	0.12	+	+
<i>cis</i> -2-heptenal	956	c	0.06	0.06	+	+	dodecanoic acid	1566	c	0.14	0.16	+	+
2-ethyl-4-pentenal ^d	959	c	0.58	0.42	+	+	butanoic acid	1609 ^e	0.20	5.89	8.84	+	+
<i>trans</i> -2-heptenal	961	c	0.09	0.11	+	+	isopentanoic acid	1650 ^e	c	0.09	0.09	+	+
octanal	1002	c	0.64	0.42	+	+	pentanoic acid	1714 ^e	c	0.08	0.09	+	+
<i>trans,trans</i> -2,4-heptadienal	1010	c	0.25	0.30	+	+	tetradecanoic acid	1770	c	b	b	+	+
<i>trans</i> -2-octenal	1060	b	0.28	0.11	+	+	hexanoic acid	1828 ^e	0.37	4.28	3.16	+	+
nonanal	1104	0.15	1.07	0.44	+	+	hexadecanoic acid	1961	c	b	b	+	+
<i>trans</i> -2-nonenal	1161	c	0.17	0.05	+	+	furans						
decanal	1205	b	b	b	+	+	furfural	836	c	8.34	6.05	+	+
<i>trans</i> -2-decenal	1264	c	0.10	0.04	+	+	furfuryl alcohol	863	c	2.99	6.09	+	+
<i>trans,trans</i> -2,4-decadienal	1317	c	0.08	0.04	+	+	5-methylfurfural	965	c	1.73	2.26	+	+
<i>trans</i> -2-undecenal	1366	c	0.03	0.03	+	+	2-pentylfuran	992	c	0.3	0.19	+	+
dodecanal	1410	c	b	b	+	+	heterocyclic compounds						
tetradecanal	1614	c	b	b	+	+	thiazole	742	c	0.11	0.46	+	+
<i>trans</i> -2-tetradecenal	1673	b	b	0.03	+	+	1-methylpyrrole	747	c	0.04	0.07	+	+
hexadecanal	1834	b	b	0.02	+	+	pyridine	753	c	0.10	0.15	+	+
ketones							3- or 4-methylpyridine	868	c	b	0.09	+	+
diacetyl	616	5.35	0.40	0.20	+	+	1,2-dimethylpyrrole	876	c	0.10	0.04	+	+
2-pentanone	688	31.94	11.67	6.24	+	+	thiazolidine	903	c	c	0.06	+	+
2-hexanone	790	0.80	0.58	0.46	+	+	2-ethylpyridine	912	c	b	0.53	+	+
2-cyclohexenone ^d	885	c	1.62	1.36	+	+	2-methylthiazolidine	932	c	b	b	+	+
2-heptanone	891	28.87	24.86	15.60	+	+	2-propylthiazolidine	1136	c	b	b	+	+
2-octanone	993	c	0.30	0.20	+	+	1-methyl-2-butylpyrrole	1153	c	b	b	+	+
2-nonanone	1093	5.18	9.78	4.75	+	+	alkanes						
2-decanone	1193	c	0.05	0.04	+	+	pentane	500	1.76	0.51	0.67	+	+
2-undecanone	1294	1.34	2.83	1.29	+	+	hexane	600	0.13	0.19	0.66	+	+
2-dodecanone	1396	c	b	b	+	+	2-methylhexane ^d	696	c	1.81	1.98	+	+
2-tridecanone	1496	b	0.73	0.58	+	+	heptane	700	0.25	0.29	3.93	+	+
2-pentadecanone	1693	c	b	b	+	+	octane	800	1.61	2.51	2.45	+	+
lactones							3-ethyl-2-methylhexane ^d	896	c	b	0.04	+	+
δ-hexalactone	1286	c	0.05	0.02	+	+	nonane	900	0.15	0.36	0.34	+	+
δ-octalactone	1500	c	b	b	+	+	decane	1000	c	0.19	0.54	+	+
δ-decalactone	1716	c	b	b	+	+	undecane	1100	c	0.05	0.10	+	+
δ-undecalactone	1821	c	b	b	+	+	miscellaneous compounds						
δ-dodecalactone	1927	c	b	b	+	+	toluene	774	c	0.25	0.27	+	+
δ-tetradecalactone	2132	c	b	b	+	+	heptanol	972	c	b	b	+	+
							isomaltol	981	b	2.00	2.48	+	+
							<i>p</i> -cymene	1026	0.81	0.69	0.30	+	+
							limonene	1031	0.92	0.65	0.28	+	+
							isobutyl benzene ^d	1065	0.25	b	b	+	+
							maltol	1112	c	c	0.83	+	+

^a Kovats index on DB-5. ^b Values less than 0.02. ^c Not detected. ^d Tentatively identified. ^e Kovats index on DB-Wax. / +, GC peaks were identified by matching Kovats index (*I*) values and/or mass spectra (MS) with those of authentic compounds.

Identification of the butter volatiles was made by comparing their Kovats indices (Kovats, 1965) and mass spectra to those of authentic compounds.

RESULTS AND DISCUSSION

Volatile chemicals isolated and identified from a headspace of unsalted butter heated at 100, 150, and 200 °C are shown in Table I. Among 140 distinct peaks in a gas chromatogram of a headspace sample from butter heated at 200 °C, 77 compounds were positively identified. An additional six components were reported as tentatively identified because of lack of authentic compounds to obtain Kovats indices. All compounds formed at 100 and 150 °C were also formed at 200 °C.

Figure 1 shows relative total amounts of each chemical group formed during heating at three different temperatures. The values were relative to the amount of internal standard (2,5-dimethylhexane) and were calculated without considering FID response factors.

Aldehydes and ketones increased significantly when the heating temperature was raised to 200 °C, suggesting that lipid degradation was the major reaction occurring in butter during heat treatment. Fatty acids must play an important role in formation of volatile chemicals in heated butter. For example, Yoo et al. (1989) reported that the formation of volatile chemicals in heated milk fat depended significantly on the concentration of fatty acids. It is generally recognized that many volatile chemicals form from a fatty acid via monohydroperoxide intermediates followed by simple cleavage reactions (Nawar et al., 1988).

Among ketones identified, methyl ketones were the most abundant. These 2-ketones were proposed to form from β-keto acids via dehydration and decarboxylation reactions (Kinsella et al., 1967; Schwartz et al., 1966). More low molecular weight aldehydes (C₄–C₇) were found than high molecular weight aldehydes (C₁₀–C₁₆), in contrast to a previous paper (Nawar et al., 1988). This may be due to the different collection methods used in these experiments.

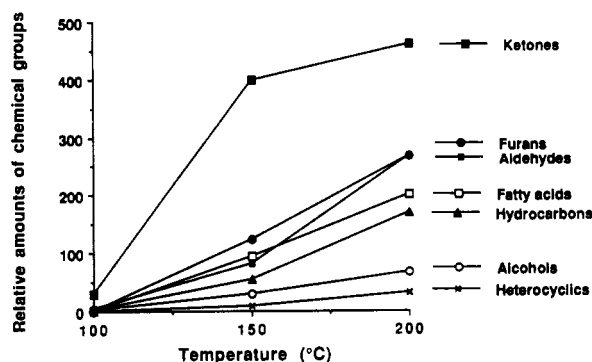


Figure 1. Relative amounts of butter volatiles for seven chemical groups formed at different temperatures. The amount of each product (W) was calculated using the equation

$W = \text{peak area of component/peak area of 2,5-DMH} \times 0.5 \mu\text{g}$ (amount of 2,5-DMH present in 2 mL of final sample) where 2,5-DMH is 2,5-dimethylhexane.

The isolation of *n*-alkanes was consistent with that in previous papers (Alencar et al., 1983; Ohnishi and Shibamoto, 1984). However, in the present study no cyclic hydrocarbons were found, whereas many series of cyclic hydrocarbons such as cyclopentanes and cyclohexanes have been reported in pyrolyzed oil samples at 300–500 °C (Alencar et al., 1983). This may be due to the use of a somewhat lower temperature (200 °C) in the present study.

Certain levels of δ -lactones, which are important to butter flavor (Urbach et al., 1972), are already present in raw butter (Kinsella, 1975). Only δ -lactones were found in the present study, although many γ -lactones are known to exist in butter (Forss, 1971). These lactones were proposed to form from δ - and γ -hydroxy acids by heat treatment (Kinsella, 1975).

Some furan derivatives, which are known as products of sugar caramelization, were identified in the present study. These furans, except for 2-pentylfuran, might form butter carbohydrates, mainly lactose residues.

Formation of heterocyclic compounds increased slightly with increasing temperature. Some heterocyclic compounds, including thiazoles, pyrroles, and pyridines, were found in butter heated above 150 °C. Even though the level of heterocyclic compounds formed was low due to low content of nitrogen or sulfur sources (such as amino acids and proteins) in butter, these compounds contribute significantly to the heated butter flavor because of their low odor threshold (Shibamoto, 1986). As mentioned above, these compounds were products of secondary reaction of aldehydes and ketones formed from lipids. For example, numerous heterocyclic compounds were formed from lipids heated with amino acids as a nitrogen source (Ohnishi and Shibamoto, 1984). Also, amounts of aldehydes found in corn oil heated with glycine were much less than those found in corn oil heated alone, suggesting that the aldehydes formed underwent secondary reactions with glycine (Macku and Shibamoto, 1991).

It is somewhat unusual that pyrazines were not found in this system, because they are the most abundant flavor chemicals formed in cooked foods. Nawar et al. (1988), who identified 152 compounds in heated butter oil, also found no pyrazines. On the other hand, a lipid sample that contained proteins or amino acids, such as milk, produced many pyrazines upon heat treatment (Shibamoto et al., 1980).

The role of volatile chemicals formed by heat in cooked butter is not yet well understood, whereas the flavor of raw butter has been studied intensively (Ramshaw, 1974;

Kinsella, 1975). The results of the present study provide the profile of volatiles formed in the headspace of butter heated at different temperatures.

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Registry No. Butanal, 123-72-8; 3-methylbutanal, 590-86-3; pentanal, 110-62-3; hexanal, 66-25-1; *trans*-2-hexenal, 6728-26-3; heptanal, 111-71-7; *cis*-2-heptenal, 57266-86-1; 2-ethyl-4-pentenal, 5204-80-8; *trans*-2-heptenal, 18829-55-5; octanal, 124-13-0; *trans,trans*-2,4-heptadienal, 4313-03-5; *trans*-2-octenal, 2548-87-0; nonanal, 124-19-6; *trans*-2-nonenal, 18829-56-6; decanal, 112-31-2; *trans*-2-decenal, 3913-81-3; *trans,trans*-2,4-decadienal, 25152-84-5; *trans*-2-undecenal, 53448-07-0; dodecanal, 112-54-9; tetradecanal, 124-25-4; *trans*-2-tetradecenal, 51534-36-2; hexa-

decanal, 629-80-1; diacetyl, 431-03-8; 2-pentanone, 107-87-9; 2-hexanone, 591-78-6; 2-cyclohexenone, 930-68-7; 2-heptanone, 110-43-0; 2-octanone, 111-13-7; 2-nonanone, 821-55-6; 2-decanone, 693-54-9; 2-undecanone, 112-12-9; 2-dodecanone, 6175-49-1; 2-tridecanone, 593-08-8; 2-pentadecanone, 2345-28-0; δ -hexalactone, 823-22-3; δ -octalactone, 698-76-0; δ -decalactone, 705-86-2; δ -undecalactone, 710-04-3; δ -dodecalactone, 713-95-1; δ -tetradecalactone, 2721-22-4; 3-butenoic acid, 625-38-7; heptanoic acid, 111-14-8; octanoic acid, 124-07-2; nonanoic acid, 112-05-0; decanoic acid, 334-48-5; propanoic acid, 79-09-4; dodecanoic acid, 143-07-7; butanoic acid, 107-92-6; isopentanoic acid, 503-74-2; pentanoic acid, 109-52-4; tetradecanoic acid, 544-63-8; hexanoic acid, 142-62-1; hexadecanoic acid, 57-10-3; furfural, 98-01-1; furfuryl

alcohol, 98-00-0; 5-methylfurfural, 620-02-0; 2-pentylfuran, 3777-69-3; thiazole, 288-47-1; 1-methylpyrrole, 96-54-8; pyridine, 110-86-1; 3-methylpyridine, 108-99-6; 1,2-dimethylpyrrole, 600-29-3; thiazolidine, 504-78-9; 2-ethylpyridine, 100-71-0; 2-methylthiazolidine, 24050-16-6; 2-propylthiazolidine, 24050-10-0; 1-methyl-2-butylpyrrole, 71707-51-2; pentane, 109-66-0; hexane, 110-54-3; 2-methylhexane, 591-76-4; heptane, 142-82-5; octane, 111-65-9; 3-ethyl-2-methylhexane, 16789-46-1; nonane, 111-84-2; decane, 124-18-5; undecane, 1120-21-4; toluene, 108-88-3; heptanol, 111-70-6; isomaltol, 3420-59-5; *p*-cymene, 99-87-6; limonene, 138-86-3; isobutyl benzene, 538-93-2; maltol, 118-71-8; 4-methylpyridine, 108-89-4.