Volatile Compounds Produced from Peanut Oil Heated with Different Amounts of Cysteine

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Volatile compounds produced in the headspace of peanut oil heated with cysteine (0.5–10.0 g) at 200 °C for 5 h were collected by a simultaneous purging and solvent extraction apparatus. Volatiles in the extracts were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Ninety-eight compounds were positively identified among over 150 gas chromatographic peaks. The majority of these were sulfur-containing compounds such as thiophenes, thiazoles, thiazolines, thiazolidines, and cyclic polysulfides. The two most abundant sulfur-containing compounds were 2-methylthiazolidine and 2-methyl-2-thiazoline. Decarboxylation of cysteine at high temperatures and subsequent reaction with aldehydes was proposed as a formation mechanism of both 2-alkylthiazolidines and 2-alkyl-2-thiazolines.

Keywords: *Peanut oil; volatile compounds; cysteine; heterocyclic compounds*

INTRODUCTION

Various constituents in foods undergo a multitude of interactions during cooking. The products formed by such thermal reactions change the flavor, color, and taste of cooked foods. The primary pathway responsible for these phenomena is the nonenzymatic Maillard reaction, which involves the reaction between amines and carbonyl compounds. This reaction scheme is frequently exploited and applied in the food industry to modify the color and flavor of commercial products.

In the past three decades a considerable amount of research has focused on simple model systems consisting of an amino acid and a sugar. Recently, studies utilizing amino acid/fatty acid model systems have attracted the attention of flavor chemists. Macku and Shibamoto (1991a) reported on the volatile compounds generated from the thermal interaction between cysteine and corn oil, one of several refined vegetable oils. Many volatile heterocyclic compounds were also reported from heated corn oil/glycine model systems (Macku and Shibamoto, 1991b).

Mottram and Edwards (1983) demonstrated that the removal of phospholipids and triglycerides from freeze-dried beef prior to cooking resulted in a loss of "meaty" aroma and the subsequent appearance of "toasted" or "biscuit-like" odors. Similar results were found in model systems containing an amino acid, a reducing sugar, and a phospholipid (Salter et al., 1988). In a recent study, Farmer and Mottram (1990) found that the addition of triglyceride or phospholipid to Maillard model reaction systems produced distinctively different aromas and volatile products, suggesting that different types of lipids display different reaction mechanisms in cooked food. The remarkably different profiles of volatile products produced in these experiments suggested that lipids play a crucial role in flavor and aroma generated by the Maillard reaction.

In the present study, the volatile compounds generated from the thermal interaction between cysteine and peanut oil were isolated by a simultaneous purging and solvent extraction apparatus and identifed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Furthermore, efforts were made to determine the formation mechanisms of volatile sulfur-containing compounds in peanut oil and to identify novel aroma compounds produced in lipid-rich cooked foods.

EXPERIMENTAL PROCEDURES

Materials. Refined peanut oil was purchased from a local market. The oil contained no added antioxidants (only endogenous tocopherols). Cysteine and cysteamine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO). Dichloromethane and chloroform were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). All authentic chemicals were obtained from reliable commercial sources.

Sample Preparation. Peanut oil (100 g) and different amounts of cysteine (0.5, 1.0, 2.0, 5.0, and 10.0 g) were mixed and placed in a 500-mL two-neck round-bottom flask, which was interfaced to a simultaneous purging and solvent extraction (SPE) apparatus developed by Umano and Shibamoto (1987). The mixture was heated at 200 °C for 5 h while stirring. The headspace volatiles were purged into 250 mL of deionized water by a purified nitrogen stream at a flow rate of 10 mL/min. The volatiles trapped by the water were continuously extracted with dichloromethane (50 mL) for 5 h. The water temperature was kept at 10 °C by a Brinkman RM6 constant-temperature water circulator. The dichloromethane extract was dried over anhydrous sodium sulfate overnight, and the extract was then concentrated to 2 mL by distillation with a Vigreux column. The concentrated extracts were placed in vials and stored under argon at −4 °C until analyzed.

Instrumental Analysis. A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with a 60 m × 0.25 mm i.d. DB-Wax fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used for routine analysis of headspace volatiles. A nitrogen–phosphorus detector (NPD) or a flame photometric detector (FPD) in the sulfur mode was used for confirmation of the N- or S-containing compounds, respectively. The oven temperature was held at 60 °C for 4 min and then programmed to 220 °C at 3 °C/min and held for 30 min. The linear velocity of the helium carrier gas flow was 26.5 cm/s with a split ratio of 1:40. The injector temperature was 250 °C.

An HP Model 5890 GC interfaced to a VG Trio II mass spectrometer equipped with an INCOS MS data system (Finnigan, San Jose, CA) was used for MS identification of

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Table 1. Volatile Compounds Identified in Headspace Samples of Peanut Oil Heated with 0.5 (I), 1 (II), 2 (III), 5 (IV), and 10 g (V) of Cysteine

compound	GC peak area %[a]					RI[c]	identified by			
	I	II	III	IV	V		FID	NPD	FPD	MS
hexane	0.03	0.08	0.07	0.03	0.07	600	+			+
(*E*)-3-hexene	0.10	0.76	0.67	0.09	0.09	640	+			+
heptane	1.41	*b*	6.72	1.49	2.11	700	+			+
cyclohexane	12.94	9.94	1.48	5.78	3.92	705	+			+
(*E*)-3-heptene	0.59	*b*	4.68	0.46	3.47	714	+			+
(*Z*)-3-heptene	0.38	*b*	*b*	*b*	0.64	727	+			+
(*E*)-2-heptene	0.17	*b*	*b*	*b*	*b*	735	+			+
cyclohexene	3.47	*b*	*b*	*b*	*b*	751	+			+
(*Z*)-2-heptene	0.73	*b*	*b*	*b*	*b*	760	+			+
1-propanethiol	0.15	*b*	*b*	*b*	*b*	770	+		+	+
octane	17.14	1.60	1.84	2.64	4.85	800	+			+
2-methylfuran	0.23	0.15	2.54	6.21	*b*	817	+			+
2,3-dihydro-4-methylfuran	8.68	10.56	7.34	6.25	*b*	832	+			+
(*E*)-2-octene	4.60	9.79	3.94	2.73	*b*	852	+			+
(*Z*)-2-octene	0.13	5.30	0.03	0.10	*b*	863	+			+
nonane	2.36	2.16	1.69	2.95	*b*	900	+			+
butyl mercaptan	0.17	0.08	0.15	1.87	1.77	919	+		+	+
1-nonene	4.21	4.40	3.46	2.47	1.91	931	+			+
cyclooctene	0.54	0.43	0.33	0.41	0.34	940	+			+
(*E*)-2-nonene	0.35	0.58	0.46	0.22	0.15	956	+			+
(*Z*)-2-nonene	0.44	0.25	0.39	0.61	1.00	963	+			+
decane	1.09	0.38	0.87	0.06	0.09	1000	+			+
2-propylfuran	0.35	0.11	0.58	0.50	1.05	1011	+			+
thiophene	2.02	1.67	2.43	2.57	4.07	1023	+		+	+
toluene	0.43	0.37	1.81	0.87	2.21	1039	+			+
3-hexanone	2.11	2.70	0.12	1.04	0.70	1046	+			+
2-butylfuran	0.08	0.10	0.08	0.12	0.29	1056	+			+
2-methylthiophene	0.43	0.12	0.48	0.60	0.92	1095	+		+	+
undecane	0.82	0.35	0.53	0.54	0.98	1100	+			+
4,5-dehydro-2-methylthiacyclopentane	0.09	0.37	0.30	1.90	2.73	1108	+		+	+
2-methylthiacyclopentane	0.03	1.12	2.08	0.02	0.14	1112	+		+	+
3-methylthiophene	0.08	0.13	0.35	0.41	0.62	1117	+		+	+
ethylbenzene	0.55	1.02	0.62	1.54	2.97	1124	+			+
4,5-dihydro-2-ethylthiophene	0.22	0.23	0.21	1.00	1.43	1135	+		+	+
camphane	0.10	0.88	0.56	0.04	0.08	1144	+			+
2,3-dimethylthiophene	0.67	0.43	0.71	1.04	2.20	1156	+		+	+
2-ethylthiophene	0.11	0.30	0.42	0.40	0.70	1167	+		+	+
1-ethylpyrrole	2.11	1.27	1.37	0.97	0.98	1184	+	+		+
pyridine	0.85	0.69	0.70	0.55	0.39	1195	+	+		+
dodecane	0.11	0.06	0.04	*b*	0.05	1200	+			+
propylbenzene	1.61	1.43	1.32	1.58	3.52	1204	+			+
3-ethylthiophene	0.13	0.19	0.30	0.74	1.03	1209	+		+	+
2-methylpyridine	0.33	1.42	2.15	2.30	3.10	1227	+	+		+
2-pentylfuran	0.04	0.03	0.03	0.15	0.14	1234	+			+
2-methylthiazole	0.62	0.72	1.08	1.14	1.05	1241	+	+	+	+
2-amino-4-methylthiazole	*b*	0.14	0.28	0.45	0.47	1245	+			+
thiazole	0.14	0.25	0.35	0.35	0.39	1249	+	+	+	+
2,5-dimethylthiophene	0.37	0.32	0.44	0.38	0.43	1260	+		+	+
cyclohexenyl sulfide	0.11	0.16	0.18	0.31	0.28	1272	+			+
2-methyl-2-thiazoline	0.22	0.87	1.00	3.42	4.92	1303	+	+	+	+
butylbenzene	1.45	1.92	3.11	2.68	1.97	1308	+			+
2-methyl-5-propylthiophene	0.41	0.23	0.39	0.45	1.44	1314	+		+	+
2-ethylpyrazine	*b*	*b*	*b*	*b*	0.02	1325	+	+		+
vinylthiophene	0.12	0.30	0.45	0.48	0.54	1334	+		+	+
2-butylthiophene	0.03	0.12	0.14	0.26	0.13	1351	+		+	+
2,5-diethylthiophene	*b*	*b*	*b*	0.61	0.28	1356	+		+	+
2-ethyl-2-thiazoline	*b*	0.21	0.42	0.22	0.24	1364	+	+	+	+
2-ethyl-4-methylthiazole	0.44	0.15	0.22	0.28	0.21	1368	+	+	+	+
2,2-dimethylthiazolidine	0.09	*b*	*b*	0.05	0.22	1395	+	+	+	+
4-ethyl-5-methylthiazole	*b*	0.49	1.35	2.44	1.89	1401	+	+	+	+
amylbenzene	0.18	0.48	0.59	0.73	*b*	1433	+			+
2-methylthiazolidine	0.04	5.81	17.17	12.93	17.75	1434	+	+	+	+
2-methyl-1,3-dithiolane	0.06	0.12	0.20	0.65	0.50	1438	+		+	+
2-propyl-2-thiazoline	*b*	0.42	0.68	1.22	0.98	1448	+	+	+	+
2-pentylthiophene	*b*	0.10	0.09	0.03	0.22	1452	+		+	+
thiazolidine	*b*	*b*	*b*	*b*	0.07	1460	+	+	+	+
4-methyl-5-ethylthiazole	0.24	1.34	1.17	0.64	0.58	1467	+	+	+	+
1,2-dithiane	*b*	*b*	*b*	0.02	0.05	1475	+		+	+
2-ethyl-2-methylthiazolidine	*b*	*b*	*b*	0.05	0.25	1491	+	+	+	+
pyrrole	0.10	0.24	0.36	0.32	0.32	1499	+	+		+
hexylbenzene	0.06	0.22	0.16	0.08	0.05	1513	+			+
2-ethylthiazolidine	*b*	*b*	*b*	*b*	*b*	1515	+	+	+	+
1-pentadecene	*b*	*b*	0.03	*b*	*b*	1528	+			+
2-ethyl-4-propylthiazole	*b*	*b*	*b*	0.03	0.02	1532	+	+	+	+
2-hexylthiophene	*b*	0.02	0.04	0.12	0.04	1538	+		+	+

Table 1 (Continued)

compound	GC peak area %[a] I	II	III	IV	V	RI[c]	identified by FID	NPD	FPD	MS
2-pentylpyridine	0.04	0.19	0.14	0.06	0.03	1554	+	+		+
2-methyl-2-propylthiazolidine	b	0.31	0.24	0.22	0.09	1566	+	+	+	+
2,5-dimethylpyrrole	b	b	b	b	b	1589	+	+		+
3,5-dimethyl-1,2,4-trithiolane	b	0.10	0.10	0.10	0.06	1602	+		+	+
2-propylthiazolidine	b	b	0.02	0.04	0.06	1610	+	+	+	+
heptylbenzene	b	b	b	b	b	1612	+			+
2-ethyl-2-propylthiazolidine	b	0.08	0.06	0.06	0.04	1650	+	+	+	+
2-heptylthiophene	b	b	b	b	b	1652	+		+	+
2-butyl-2-methylthiazolidine	b	b	b	b	b	1675	+	+	+	+
2-butylthiazolidine	b	b	b	b	b	1719	+	+	+	+
octylbenzene	b	b	b	b	b	1721	+			+
2,4,6-trimethylperhydro-1,3,5-dithiazine	0.19	1.95	1.96	1.72	0.32	1750	+	+	+	+
2-isopentylthiazolidine	b	b	b	b	b	1752	+	+	+	+
2-butyl-2-ethylthiazolidine	b	b	b	0.02	b	1778	+	+	+	+
2-pentylthiazolidine	b	b	b	b	b	1838	+	+	+	+
3-methyl-1,2,4-trithiane	b	0.03	b	0.11	0.11	1852	+		+	+
2-hexylthiazolidine	b	b	b	b	b	1949	+	+	+	+
2-heptylthiazolidine	b	0.48	0.59	0.96	1.66	2083	+	+	+	+
myristic acid	0.04	b	0.04	0.02	b	2724	+			+
palmitic acid	0.23	0.37	0.29	0.33	0.10	2910	+			+
stearic acid	b	b	b	0.18	0.09	3181	+			+
oleic acid	b	0.81	0.47	0.36	0.10	3184	+			+
linolenic acid	b	b	b	0.10	0.04	3292	+			+

[a] Solvent peak is excluded. [b] Peak area % less than 0.02. [c] Kovats index on DB-Wax.

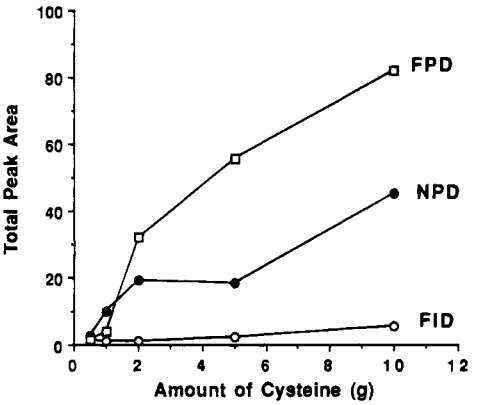

Figure 1. Total GC peak areas of volatile compounds produced from peanut oil with different amounts of cysteine measured with FID, NPD, and FPD.

the GC components using the same column and oven conditions described above. Mass spectra were obtained by electron impact ionization at 70 eV and a source temperature of 165 °C. The spectral data were recorded on a VG 11-250 computer data system.

All compounds were identified using Kovats indices (Kovats, 1965) by a combination of FID, NPD, and FPD (sulfur mode) and by MS fragmentations.

RESULTS AND DISCUSSION

The experimental conditions used in the present study may not be consistent with the conditions used in home-cooking practices in this country. However, the conditions used for deep fat frying in many Asian countries, such as China and Korea, are quite similar to those used in the present study. Moreover, in some restaurants in this country, cooking oils are kept at 150–200 °C for a prolonged time for deep fat frying.

The volatile compounds identified in the dichloromethane extracts of heated peanut oil/cysteine mixtures at five different cysteine concentrations are listed in Table 1. Ninety-eight compounds were identified among over 150 gas chromatographic peaks. These included 50 sulfur-containing compounds, 31 hydrocarbons, 7 nitrogen-containing compounds, 5 furans, 5 fatty acids, and 1 ketone. The sulfur-containing heterocyclic compounds were the most predominant of all the volatile compounds. Hydrocarbons were the second most predominant class of compounds identified. The sulfur- and nitrogen-containing heterocyclic compounds identified in the present study are well-known to be formed as a result of the amino–carbonyl reaction and have been reported by many researchers (Farmer et al., 1989; Yeo and Shibamoto, 1991).

The hydrocarbons and furans identified in the present study are primarily derived from the thermal oxidation of fatty acids including linoleate, oleate, and palmitate (Frankel, 1985) and their respective methyl esters. These three fatty acids comprise 94% of the total fatty acids found in peanut oil (Worthington, 1977). The fatty acids identified in the present study are formed as a result of thermal cleavage of mono-, di-, and triglycerides.

The total peak area of samples obtained under five different cysteine concentrations as measured by FID, NPD, and FPD is shown in Figure 1. The total peak area measured by FID shows a gradually increasing slope. However, the total peak areas of nitrogen- (by NPD) and sulfur-containing compounds (by FPD) increase at a much more rapid rate. The elevation of cysteine concentration resulted in a rapid increase in the number and quantity of heterocyclic compounds formed via intermediates from cysteine. The FID displays the merit of detecting all organic compounds in the headspace samples but also displays a corresponding demerit in the lower sensitivity toward nitrogen- and sulfur-containing compounds as compared to the NPD and FPD.

The most important and critical step in the proposed formation mechanisms of the major sulfur-containing heterocyclic compounds is the thermally induced decarboxylation of cysteine to yield cysteamine (Fujimaki et al., 1969). 2-Alkylthiazolidines are formed from the condensation reaction between cysteamine and alde-

hydes produced from the thermal degradation of lipids (Alencar et al., 1983; Ohnishi and Shibamoto, 1984; Yasuhara and Shibamoto, 1989). 2-Alkylthiazolidines are further oxidized to 2-alkylthiazoles via 2-alkyl-2-thiazolines. Increasing the amount of cysteine in the heated peanut oil sample resulted in a subsequent increase in the yield of the sulfur-containing heterocyclic compounds discussed above.

2-Alkylthiophenes identified in the present study have also been found in several fatty acid/cysteine model systems (Farmer et al., 1989). These compounds are probably formed from the interaction between fatty aldehydes and hydrogen sulfide (van der Ouweland et al., 1989). Boelens et al. (1974) reported that 2-ethylthiophene was thermally generated from a mixture containing 2-hexenal and hydrogen sulfide. A proposed formation mechanism of these 2-alkylthiophenes was recently reported by Macku and Shibamoto (1991a). Also, butyl mercaptan (1-butanethiol) is probably a product of the reaction between hydrogen sulfide and 1-butanol (Chang et al., 1978).

Few heterocyclic compounds containing only nitrogen and carbon, such as pyrazines and pyridines, were identified in the present study as compared to the highly abundant sulfur-containing compounds. However, the most predominant compound among those identified was 2-methylpyridine (2-picoline). This compound is produced from a reaction between ammonia and 2-hexenal or hexanal. Several other 2-alkylpyridines are formed from the corresponding unsaturated or saturated *n*-aldehydes with ammonia under heated conditions (Buttery et al., 1977).

Several thiazolidine compounds that contribute to meaty aromas were detected in relatively high amounts. These particular compounds have previously been overlooked as potentially significant aroma and flavor compounds. Further studies with thiazolidine derivatives in food systems are necessary to better understand their role in aroma and flavor. The addition of cysteine to peanut oil prior to cooking, then, may be a possible method of flavor enhancement during cooking and processing.

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