Volatile Components in Fermented Soybean (*Glycine max*) Curds

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Volatile components of three commercial fermented soybean (*Glycine max*) curds (FSC) (A−C) were extracted using a simultaneous steam distillation and extraction apparatus, and extracts were analyzed by gas chromatography/mass spectrometry. A total of 111 compounds was found. Sixty-three compounds were common to all three brands. Brands A, B, and C contained 90, 90, and 82 components, respectively. Major classes of compounds included alcohols (32) and esters (25). Similarities and differences existed in the composition of the FSC samples. Twelve common compounds including six esters, four alcohols, one ketone, and one miscellaneous compound had a dry weight of >1000 μg/kg of sample. Quantitative differences existed among the commercial brands ($p < 0.05$). The quantity of ethanol detected might produce large numbers of ethyl esters that contribute to the desirable fruity and floral notes for the final products.

Keywords: *Volatiles; soybean fermentation; sufu; curd; Asia*

INTRODUCTION

The use of soybean (*Glycine max*) as a main ingredient in processed food dates back to 160 A.D. in China (Hsu, 1994). Soybean has been used in the production of fermented soybean curd (FSC) and other products (Macrae et al., 1993). FSC is also known as Chinese sufu, Chinese cheese, bean-curd preserved, bean-curd cheese, soybean cheese, tofuru, etc. (Macrae et al., 1993; Hsu, 1994; Rao et al., 1996). It is a tofu with molds of the genus *Actinomucor* and *Mucor* purposely infested on it (Steinkraus, 1983).

Production of FSC can be roughly divided into two major parts: (1) preparation of tofu and (2) inoculation of mold, brining, and aging (Wang and Hesseltine, 1970; Su, 1986; Campbell-Platt, 1987; Chen and Ho, 1989). Various kinds of FSC could be produced depending on the processing step and the ingredients used. Major variation exists at the brining step when different ingredients may be added before aging (Hsu, 1994). Nutritionally, FSC contains less moisture and fat, but more protein, fiber, and ash, than tofu (Macrae et al., 1993). All soy proteins in the FSC have been hydrolyzed to form smaller fragments below 10000 Da (Rao et al., 1996). FSC is often used as a main dish as well as a condiment. The product has a soft texture and a very distinct flavor. Although the cooked soybean retains a strong beany flavor, fermented products contain much less of the undesirable flavor (Macrae et al., 1993). Moreover, some natural antinutritional elements are reduced during the fermentation process (Macrae et al., 1993).

Wang and Hesseltine (1970) described the taste of molded tofu as bland and suggested that both the flavor and the aroma of FSCs were developed in the brining and aging steps. During brining, the salt helped to release the mycelia-bound proteases from fungi, making them readily available to penetrate and to digest the protein in the matrix of a tofu (Wang, 1967; Wang and Hesseltine, 1970). Chou and Hwan (1994) observed the increase in free fatty acids of molded tofus during aging in the brine only in the absence of alcohol. Similar observations were reported by Wai (1968), particularly during the early stage of aging. Both the brining and aging produced a pool of products. Some of them would develop into components providing characteristic flavor (Wang and Hesseltine, 1970; Su, 1986). When alcohol was added as a part of the dressing mixture in the aging period, it would react with the free fatty acid substrates to produce more a desirable aroma in the FSC (Wang and Hesseltine, 1970; Su, 1986). Recently, the importance of the presence of ethanol in the qualitative and quantitative productions of volatile components during the aging period of FSCs was proved by Hwan and Chou (1999).

FSC is consumed in China, Taiwan, Hong Kong, and other Asian countries, but information on the volatile components of this fermented product is scarce (Ho et al., 1989; Hwan and Chou, 1999). Therefore, the objectives of this study were (1) to determine the volatile components in the steamed commercial fermented soybean curds and (2) to identify those common volatile components among these steamed products.

MATERIALS AND METHODS

Sample Preparation and Collection of Volatile Components. Three brands of FSCs commonly available and consumed in Hong Kong were chosen in local markets. Samples of each brand were picked randomly from the market shelves. Two brands (B and C) were locally prepared, and one (A) was imported from Taiwan. FSCs were kept in their own containers and transported back to the laboratory immediately. Because FSCs can be consumed after steaming, the individual sample was cut into smaller cubes (1 cm × 1 cm × 1 cm) and placed on a stainless steel mesh above 400 mL of boiling, double-distilled water in a 5-L round bottle flask to simulate the conditions during steaming. A total of 60 g of sample was used for each extraction. One milliliter of 2,4,6-trimethylpyridine, used as an internal standard (I.S., 10 μg/

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Table 1. Proximate Analyses (Wet Percent) of Three Commercial Fermented Bean (*G. max*) Curds (A−C)[a]

brand	moisture	protein	fat	ash	carbohydrate[b]
A	78.8 ± 0.3	6.1 ± 0.2	9.5 ± 0.2	4.1 ± 0.0	1.5
B	77.9 ± 0.2	7.5 ± 0.2	7.8 ± 0.1	4.9 ± 0.0	1.9
C	70.3 ± 0.1	10.4 ± 0.0	7.9 ± 0.0	9.5 ± 0.0	2.0

[a] Data are expressed as mean ± standard deviation of $n = 2$ or 3. [b] Carbohydrate % = 100% − (moisture + protein + fat + ash)%.

mL in distilled water), was added to the sample before extraction. A Likens and Nickerson (1964) type simultaneous distillation and extraction (SDE) apparatus (Model 523010-000, Kontes, NJ) was used for the extraction of volatile components. Each sample was extracted with 40 mL of redistilled dichloromethane. Extraction time was set for 2 h after the distilled water started to boil in the sample flask. Extracts were then concentrated to 15 mL with a gentle stream of nitrogen gas (99.995% purity), dried with 2.3 g of anhydrous sodium sulfate, and further concentrated to 0.05 mL. Extracts were kept in a freezer (−70 °C) until further analyzed. Triplicate extractions were prepared for each brand.

GC/MS Conditions. Qualitative and quantitative analyses of extracts were carried out with a Hewlett-Packard 6890 gas chromatograph (GC) coupled with a Hewlett-Packard 5973 mass selective detector (MSD). GC and MS conditions were similar to those described by Chung and Cadwallader (1994). Separations of the volatile compounds were performed on a Supelcowax 10 column (60 m length × 0.25 mm. i.d. × 0.25 μm d_f; Supelco, Inc., Bellefonte, PA). Five microliters of each extract was injected into the GC. Other GC conditions were as follows: pulse splitless valve delay for 30 s, injector temperature at 220 °C, helium carrier gas at 30 cm/s. Temperature program was as follows: initial temperature of 35 °C for 5 min, ramp rate at 2 °C/min, and final temperature of 195 °C for 90 min. MSD conditions were as follows: ion source temperature at 230 °C, MS quadrupoles temperature at 106 °C, interface temperature at 200 °C, ionization voltage at 70 eV, mass range at 33−450 amu, scan rate at 6.52 scans/s, and electron multiplier voltage at 1106 V.

Compound Identification and Quantification. Compound identification and confirmation were done by comparing the retention indices (RI) (van den Dool and Kratz, 1963) and mass spectra of an unknown with those of authentic standards under identical analytical conditions. Tentative identifications were based on matching mass spectra of unknown compounds with those in the Wiley library of mass spectral database (Hewlett-Packard Co., 1995). Quantification of compounds in each sample was determined by the standard curve method using the peak area of a specific fragment of a compound to that of the I.S. (fragment chosen was m/z 121). Relative abundance of a tentatively identified compound was estimated by the ratio of the relative area of a specific fragment of the tentatively identified compound to the area of the internal standard.

Proximate Analysis. Moisture, protein, fat, and ash analyses were carried out according to the AOAC official methods (AOAC, 1980).

Statistical Analysis. Compounds from triplicate samples were analyzed by one-way analysis of variance (ANOVA) and compared by the Tukey test at $p < 0.05$ level of significance (Ott, 1988).

RESULTS AND DISCUSSION

Table 1 shows the data from the proximate analysis of brands A−C. All brands have very high moisture content (70.3−78.8%). The percentage ranges of protein, fat, and carbohydrate are 6.1−10.4, 7.8−9.5, and 1.5−2.0%, respectively. The amounts of ash found (4.1−9.5%) are higher than those of carbohydrate in all brands.

Table 2 and Figure 1 show the volatile components identified and the total ion chromatograms. Brands A, B, and C have totals of 90, 90, and 82 components, respectively. With a combined total of 111 compounds identified in the three brands, 21 compounds were tentatively identified. Among the 63 common compounds, a majority of them belonged to alcohols (19) and esters (15). The rest of the common components included pyrazines (7), ketones (7), pyridines (5), aldehydes (4), miscellaneous compounds (3), sulfur-containing compounds (2), and furan (1).

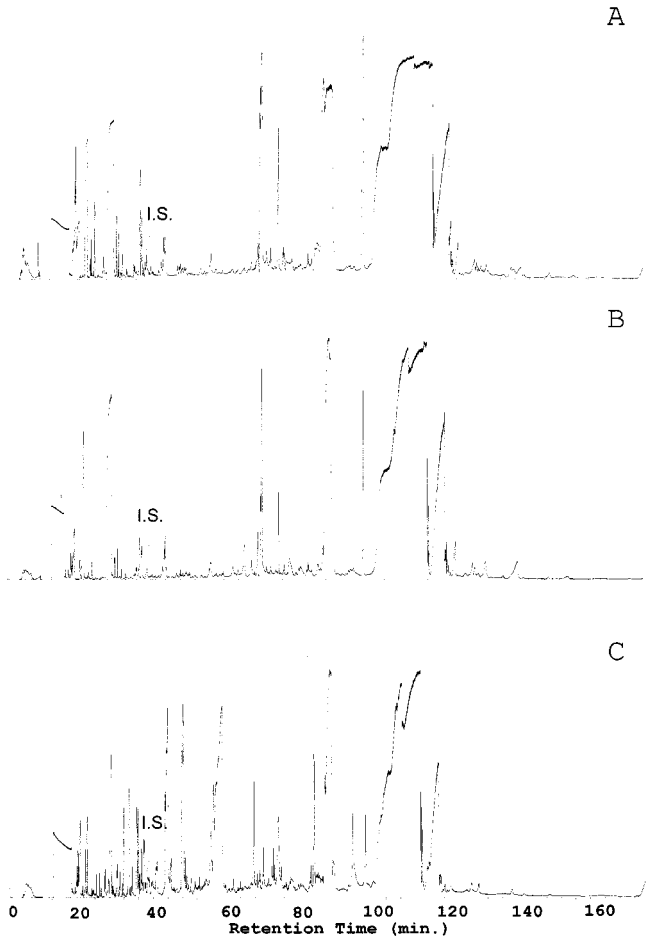

Figure 1. Typical total ion chromatograms of three commercial fermented soybean (*G. max*) curds (A−C). I.S., internal standard.

Among the 10 aldehydes, pentanal, *n*-hexanal, benzaldehyde, and 2-phenyl-2-butenal were found in all three FSCs. Very desirable aromas were reported in pentanal, benzaldehyde, and 2-phenyl-2-butenal (Aldrich, 1998), but *n*-hexanal contributed a green aroma to both the soybean milk and textured soy protein (Ames and Macleod, 1984; Kobayashi et al., 1995). Because the quantity of *n*-hexanal in the FSCs was high, it would be expected to give a strong greenish background to the products. These compounds could be produced by lipid oxidation and degradation (Ames and Macleod, 1984).

A combined total of 25 esters was detected in the FSCs, which were among the largest classes of compounds detected. Fifteen compounds were common in all samples. Although there was no acid detected in the volatile components of these samples, the presence of a large number of various esters might suggest that different acids probably existed in an earlier stage during the production process (Wang and Hesseltine, 1970). With the ample supply of ethanol in the final stage, most acids underwent esterification and formed ethyl esters (Wang and Hesseltine, 1970; Streitwieser

Table 2. Volatile Components in Three Commercial Fermented Soybean (*G. max*) Curds (A−C)

				sample A		sample B		sample C	
compound[a]	RI[b]	*m/z*[c]	sig[d]	concn[e] (μg/kg)	SD[f]	concn[e] (μg/kg)	SD[f]	concn[e] (μg/kg)	SD[f]
aldehydes (10)									
pentanal	1000>	44	–	24.6	22.6	151.3	133.3	121.4	109.1
n-hexanal	1086	56	–	499.8	313.3	860.3	825.6	1319.1	295.5
(*E*)-2-octenal	1434	70	–	- - -	- - -	316.1	206.4	- - -	- - -
2-furancarboxaldehyde	1472	96	–	11.9	8.3	- - -	- - -	283.5	138.0
(*E*,*E*)-2,4-heptadienal	1499	81	–	- - -	- - -	150.2	117.0	- - -	- - -
benzaldehyde	1530	77	**	306.2^{A}	27.8	311.4^{A}	20.8	451.0^{B}	59.8
5-methyl-2-furancarboxaldehyde	1580	110	**	6.4	9.0	- - -	- - -	67.1	12.5
(*E*,*E*)-2,4-decadienal*	1816	81	–	235.5	244.8	1175.7	1229.3	- - -	- - -
2-phenyl-2-butenal*	1939	115	–	207.1	60.1	203.5	16.5	346.0	113.6
1*H*-pyrrole-2-carboxaldehyde	2032	95	–	- - -	- - -	14.0	1.4	24.8	13.0
aromatics (2)									
styrene	1261	104	–	- - -	- - -	34.1	15.9	34.4	4.5
(2-chloroethyl)benzene	1593	91	–	68.0	47.0	23.9	12.0	- - -	- - -
esters (25)									
ethyl butanoate	1039	71	**	5936.1^{A}	1601.5	1591.9^{B}	678.6	590.9^{B}	251.0
ethyl 2-methylbutanoate	1055	57	**	56.8^{A}	16.2	162.5^{A}	53.5	786.5^{B}	271.7
ethyl 3-methylbutanoate	1072	88	–	- - -	- - -	35.7	24.4	82.8	40.2
3-methylbutyl acetate	1126	88	**	541.0	478.8	9027.4	4865.1	- - -	- - -
ethyl pentanoate	1139	88	–	90.1	80.7	74.7	19.1	13.4	18.9
ethyl 2-butenoate	1165	69	–	- - -	- - -	- - -	- - -	167.4	28.3
ethyl hexanoate	1238	99	–	658.9	203.3	520.3	60.9	478.2	99.5
3-methylbutyl butyrate	1270	71	–	296.3	38.7	- - -	- - -	- - -	- - -
ethyl lactate	1347	45	**	9744.4^{A}	1148.3	5084.5^{B}	937.3	2755.7^{B}	1822.0
ethyl octanoate	1439	88	–	295.8	98.3	387.0	42.7	157.3	91.8
ethyl 3-hydroxybutanoate	1522	43	**	552.9^{A}	45.5	359.2^{A}	91.6	7947.9^{B}	996.6
ethyl 2-hydroxydecanoate*	1549	69	**	235.8	12.4	- - -	- - -	84.3	9.1
octyl methanoate*	1560	56	–	38.7	22.0	- - -	- - -	- - -	- - -
ethyl decanoate	1643	157	–	183.7	28.7	215.3	46.6	- - -	- - -
ethyl phenylacetate	1793	91	–	191.2	13.1	296.4	65.1	150.1	15.1
2-phenylethyl acetate	1823	104	–	172.2	17.3	- - -	- - -	- - -	- - -
ethyl dodecanoate	1848	157	–	214.2	32.8	- - -	- - -	- - -	- - -
ethyl 3-phenylproprionate	1892	104	**	984.8^{A}	69.9	2096.2^{B}	511.2	514.1^{A}	147.6
ethyl tetradecanoate	2053	88	–	1222.5	65.4	1675.3	339.0	1662.2	1229.8
methyl hexadecanoate	2218	74	**	5110.6^{A}	1206.0	4028.4^{A}	1785.1	135.6^{B}	81.0
ethyl hexadecanoate	2255	88	**	29024.8^{A}	1685.8	40181.3^{A}	7870.3	4214.8^{B}	2780.1
ethyl (*Z*)-9-octadecenoate	2480	310	–	41797.5	12400.3	47881.1	13019.7	26750.9	17328.4
ethyl (*Z*,*Z*)-9,12-octadecadienoate	2530	308	–	116959.9	28091.5	151097.7	25346.1	71738.4	40473.1
ethyl (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoate	2596	261	**	28033.3^{AB}	5686.6	47771.7^{A}	9766.9	15272.3^{B}	9277.7
butyl (*Z*,*Z*)-9,12-octadecadienoate*	2631	67	–	- - -	- - -	403.1	97.1	- - -	- - -
furan (1)									
2-pentylfuran	1236	81	–	130.2	84.8	277.3	132.8	334.4	35.8
miscellaneous (7)									
3-ethyl-2-methyl-1,3-hexadiene*	1421	67	–	37.4	16.5	46.1	8.5	- - -	- - -
3-methyl-1*H*-pyrrole*	1563	80	–	28.5	9.2	66.5	38.0	43.2	23.0
naphthalene	1748	128	–	306.1	312.3	65.9	9.5	54.7	7.9
thialdine*	1765	163	–	- - -	- - -	28.6	9.2	- - -	- - -
1-(2-furanylmethyl)-1*H*-pyrrole	1838	81	–	- - -	- - -	- - -	- - -	51.0	9.4
benzenepropanenitrile	2048	131	–	- - -	- - -	- - -	- - -	16.2	5.3
1*H*-indole	2444	117	**	10041.3^{A}	1354.8	11212.1^{A}	1243.8	1014.0^{B}	163.5
pyridines (5)[g]									
pyridine	1179	79	–	88.9	16.7	99.4	5.7	264.9	106.0
2-methylpyridine	1211	93	**	25.8^{A}	12.0	17.2^{A}	9.5	99.7^{B}	24.0
3-methylpyridine	1290	93	**	17.9^{AB}	15.5	21.0^{AC}	1.2	54.6^{BC}	13.8
2,4,6-trimethylpyridine (I.S.)[h]	1365	121	–						
3-methoxypyridine*	1584	109	**	4.4^{A}	2.5	6.3^{A}	4.9	62.8^{B}	23.9
3-phenylpyridine	2200	155	–	137.8	59.2	54.2	19.5	86.3	28.7
pyrazines (10)									
2-methylpyrazine	1266	94	**	338.1^{A}	11.5	577.1^{A}	158.9	1552.9^{B}	412.6
2,5-dimethylpyrazine	1322	108	–	269.3	13.3	423.7	74.0	1459.0	718.3
2,6-dimethylpyrazine	1328	108	–	210.7	28.6	347.8	50.5	1171.1	612.8
ethylpyrazine	1335	108	**	59.4^{A}	23.3	88.3^{A}	7.9	633.0^{B}	301.4
2,3-dimethylpyrazine	1346	108	**	33.9^{A}	3.0	56.7^{A}	11.5	230.0^{B}	65.8
2-ethyl-6-methyl-pyrazine*	1386	122	**	47.9^{A}	15.0	87.8^{B}	12.0	151.7^{C}	7.9
2,3,5-trimethylpyrazine	1403	122	**	96.7	32.0	192.1	33.6	- - -	- - -
3-ethyl-2,5-dimethylpyrazine*	1446	135	**	352.0^{A}	7.2	27.3^{A}	4.4	4490.4^{B}	929.9
2-ethenyl-6-methylpyrazine*	1492	120	–	- - -	- - -	- - -	- - -	45.2	35.1
isopropenylpyrazine*	1599	119	–	- - -	- - -	- - -	- - -	34.2	15.0

Table 2. Continued

compound[a]	RI[b]	*m/z*[c]	sig[d]	sample A concn[e] (μg/kg)	sample A SD[f]	sample B concn[e] (μg/kg)	sample B SD[f]	sample C concn[e] (μg/kg)	sample C SD[f]
alcohols (32)									
ethanol	1000>	45	–	8.9×10^5	2.3×10^5	1.4×10^6	2.3×10^5	4.2×10^5	2.2×10^5
2-butanol	1027	45	**	2346.6^{AB}	565.4	3458.3^{A}	1133.7	406.3^{B}	40.5
1-propanol	1041	59	–	25190.0	16860.8	36132.3	6783.0	10556.8	3693.7
2-methyl-1-propanol	1094	43	**	17171.4^{A}	3993.0	26826.3^{A}	6284.3	1131.4^{B}	96.7
3-pentanol	1108	59	–	16.4	11.3	18.5	6.1	32.1	31.8
2-pentanol	1120	45	–	90.7	14.9	93.9	29.1	43.7	14.1
1-butanol	1144	56	–	1586.2	1137.9	898.7	279.2	199.9	42.9
1-penten-3-ol	1160	57	–	53.0	37.5	73.5	22.8	- - -	- - -
3-methyl-1-butanol	1209	55	–	- - -	- - -	- - -	- - -	3095.5	1498.6
1-pentanol	1250	55	–	786.3	133.3	1267.0	595.1	453.6	210.3
2-ethyl-1-butanol	1310	43	–	- - -	- - -	47.1	10.1	- - -	- - -
2-heptanol	1321	55	**	274.5	22.0	759.4	149.4	- - -	- - -
1-hexanol	1355	55	–	3318.4	370.7	3481.4	738.5	2886.3	175.5
3-ethoxy-1-propanol	1377	59	–	- - -	- - -	473.6	118.9	- - -	- - -
3-octanol	1395	59	**	338.6	46.6	151.3	67.5	- - -	- - -
2-butoxyethanol	1404	57	–	- - -	- - -	- - -	- - -	99.2	60.8
1-octen-3-ol	1454	57	–	979.4	266.7	1578.3	230.9	- - -	- - -
1-heptanol	1457	70	–	275.7	87.8	- - -	- - -	- - -	- - -
2,3-butanediol	1543	45	–	244.3	65.1	- - -	- - -	333.5	70.8
1-octanol	1560	56	–	164.8	68.9	337.1	337.6	- - -	- - -
2-furanmethanol	1666	98	**	382.5^{A}	245.7	412.7^{A}	104.9	1927.6^{B}	343.8
1-phenylethanol	1818	107	–	8.8	2.4	- - -	- - -	- - -	- - -
2-methoxyphenol	1867	109	**	276.1^{A}	2.3	450.8^{A}	86.6	1578.5^{B}	272.7
benzenemethanol	1882	79	**	107.6^{A}	4.9	138.3^{A}	7.2	211.7^{B}	20.3
benzeneethanol	1917	91	**	8720.7^{A}	1956.6	9769.2^{A}	382.0	640.7^{B}	95.1
phenol	2015	94	**	5747.9^{A}	687.4	6077.4^{A}	783.1	445.1^{B}	81.7
4-ethyl-2-methoxyphenol	2038	137	**	163.0^{A}	62.5	80.9^{AB}	6.3	44.6^{B}	6.9
4-methylphenol	2091	107	–	67.3	27.9	63.9	5.8	52.0	10.2
4-ethylphenol	2183	107	–	249.2	52.3	204.2	36.4	339.6	79.0
2-methoxy-4-vinylphenol*	2203	150	**	133.5^{A}	20.4	106.6^{A}	16.2	1511.4^{B}	591.2
1,4-naphthalenediol*	2373	160	–	- - -	- - -	- - -	- - -	24.0	0.7
4-vinylphenol*	2398	120	**	75.3^{A}	11.1	103.4^{A}	27.9	1597.1^{B}	333.4
ketones (12)									
2-heptanone	1185	43	–	972.6	502.6	718.8	93.4	601.7	77.2
3-octanone	1258	43	–	92.9	4.2	146.3	36.8	427.6	266.5
3-hydroxy-2-butanone	1288	45	–	385.3	8.7	596.8	255.5	4583.4	3159.0
2-octanone	1289	58	–	86.7	10.4	73.9	16.9	- - -	- - -
3-octen-2-one	1411	55	–	54.4	22.1	61.7	2.6	- - -	- - -
1-(2-furanyl)ethanone	1511	95	**	595.4^{A}	58.0	204.7^{B}	61.5	198.8^{B}	85.1
3-hydroxy-2-methyl-4*H*-pyran-4-one	1968	126	**	1847.5^{A}	528.6	1898.3^{A}	289.0	3540.5^{B}	272.5
1-(1*H*-pyrrol-2-yl)ethanone	1977	109	**	30.6^{A}	5.6	54.4^{A}	7.9	239.9^{B}	48.2
dihydro-5-pentyl-2(3*H*) furanone	2036	85	**	758.4^{A}	103.8	588.7^{A}	122.1	209.1^{B}	102.6
piperidinone*	2124	99	–	135.3	37.1	93.4	23.4	- - -	- - -
1(3*H*)-isobenzofuranone	2356	105	–	- - -	- - -	- - -	- - -	25.6	14.9
5-hexyldihydro-2(3*H*)-furanone*	2374	85	–	157.2	26.9	254.1	74.3	- - -	- - -
sulfur-containing compounds (7)									
5-methylisothiazole*	1282	99	–	- - -	- - -	15.0	2.6	55.7	22.7
2-(methylthio)ethanol	1534	92	**	155.9^{A}	10.2	96.3^{AB}	33.6	86.4^{B}	15.2
3-thiophene carboxyaldehyde	1687	112	–	250.0	66.1	180.8	18.9	- - -	- - -
2-thiophene carboxyaldehyde	1702	112	–	41.3	2.3	34.4	4.1	- - -	- - -
3-(methylthio)propanol	1721	106	**	88.0^{A}	11.5	189.9^{B}	47.2	61.7^{A}	16.3
2-ethyl-5-methylthiophene*	1783	111	–	- - -	- - -	- - -	- - -	27.4	10.1
3-(methylthio)-pyridine*	1791	125	–	- - -	- - -	- - -	- - -	40.3	6.7

[a] *, compound tentatively identified by mass spectrum. [b] RI, retention indices calculated on the basis of van den Dool and Kratz (1963). [c] Mass/charge value, fragment used for concentration calculation. [d] **, statistically significant difference ($p < 0.05$) among samples in a row; - - -, statistically insignificant difference ($p > 0.05$). Values of the concentration in the same row with different superscripts (A–C) are significantly different (Tukey, $p < 0.05$). [e] Average concentration of a compound (μg per kg dry sample) from three batches of samples; - - -, not detected. [f] SD, standard deviation; - - - , not determined. [g] Internal standard was excluded in total count. [h] I.S., internal standard.

and Heathcock, 1981). A large number of high molecular weight fatty acid esters, such as ethyl (*Z*)-9-octadecenoate and ethyl (*Z*,*Z*)-9,12-octadecadienoate, were detected and likely produced by the action of fungal lipases on the soybean lipid (Chou and Hwan, 1994). Most esters have characteristic odors. For example, ethyl butanoate, ethyl lactate, ethyl octanoate, ethyl phenylacetate, etc., are all described as having a fruity odor with other desirable aromas such as pineapple and honey (Bauer and Garbe, 1985). High molecular weight esters may also contribute to the overall aroma such as ethyl tetradecanoate, ethyl hexadecanoate, and ethyl (*Z*)-9-octadecenoate; these have an orris odor, a faint waxy odor, and a faint, floral note, respectively (Bur-

dock, 1994). Salo et al. (1972) indicated that the threshold values of the previous three compounds and the ethyl (*Z*,*Z*)-9,12-octadecadienoate were 5.7, 14.0, 0.87, and 0.45 mg/L, respectively, under the same testing conditions. Although these high molecular weight esters generally have larger threshold values than their smaller molecular weight esters, their concentrations in the fermented soybean curds are much higher than that of the latter. This may result in a more significant contribution to the overall aroma than that by an individual component. Had these esters not been formed, volatile acids would have been the dominating species in the samples, and the desirable fruity, floral flavor from the esters would have been replaced by the odor from the acids.

Five pyridines were detected in the three samples. None of them was reported in other soybean products such as soybean milk (Kobayashi et al., 1995), unflavored textured soy protein (Ames and Macleod, 1984), and soy sauce (Lee and Kwok, 1987; Nunomura et al., 1984). These pyridines could be products of the Maillard reaction (Fors, 1983).

Both pyrazines and sulfur-containing compounds were important to the aroma of food products. Pyrazines were generally described to have a nutty aroma, particularly alkylpyrazines (Ho and Carlin, 1989). Seven common pyrazines among the samples were found. They could be generated naturally during the aging process or artificially during the extraction. With 30 min of continuous distillation and extraction (SDE) of soybean milk, Kobayashi et al. (1995) did not report the presence of pyrazines in the extract. However, pyrazines were found in the extruded, unflavored, textured soy protein after 1 h of extraction with the SDE method, and in the fermented soybeans with *Bacillus subtilis* using the dynamic headspace method (Ames and Macleod, 1984; Owens et al., 1997). With the presence of fungal enzymes to disintegrate the tofu matrix and to release the necessary substrates for chemical reactions during the aging of FSC, pyrazines could be produced. Two common sulfur-containing compounds were found including 2-(methylthio)ethanol and 3-(methylthio)propanol. 3-(Methylthio)propanol was previously reported in soy sauce (Nunomura et al., 1984; Lee and Kwok, 1987). Its aroma was described to have a powerful sweet soup or meat-like odor and flavor even in high dilution (Burdock, 1994).

Alcohols are another large class containing 32 compounds. Nineteen alcohols are common among the three brands. 1-Propanol, 1-penten-3-ol, 1-pentanol, 1-hexanol, 1-octen-3-ol, and 1-octanol identified in some of the present FSCs were previously reported in soybean milk (Kobayashi et al., 1995). Besides, 2-methyl-1-propanol, 3-pentanol, 3-methyl-1-butanol, benzenemethanol, benzeneethanol, phenol, and 4-ethylphenol were also reported in soy sauce (Lee and Kwok, 1987). 2-Methoxy-4-vinylphenol and 4-vinylphenol, characterizing the cooked soybean, were detected in all fermented samples (Greuell, 1974). These two compounds together with 2-methoxylphenol were thought to be the thermal degradation products of the lignin-related phenolic carboxylic acids (van den Ouweland and Schutte, 1978). Most alcohols detected in the FSCs have characteristic aromas (Burdock, 1994; Aldrich, 1998) including 1-propanol (alcoholic, sweet), 1-butanol (sweet, balsamic), 1-penten-3-ol (butter, mild green), 1-pentanol (sweet, balsamic), 1-hexanol (fruity, aromatic), 3-octanol (nutty, herbaceous, melon, citrus), 1-phenylethanol (mild hyacinth-gardenia-like), 4-ethylphenol (woody, phenolic, sweet), 2-methoxy-4-vinylphenol (spicy, clove-like), 4-vinylphenol (vanilla-like), etc. Several common alcohols have a very high concentration (>1000 μg/kg). These include ethanol, 1-propanol, 2-methyl-1-propanol, and 1-hexanol. Their threshold values are 29, 2.4, 0.83, and 0.04 ppm, respectively (Devos et al., 1990). With one of the highest number of components among all classes, with various unique odor characters of individual components, and with so many components having very high concentrations among other classes, the alcohols could be an important class contributing to the flavor of the FSCs.

Twelve ketones were identified and seven common ones were found in the three brands. Ketones could be generated by fungal enzymatic actions or by Maillard reaction (Wang and Hesseltine, 1970; Fors, 1983; Su, 1986). Half of the components were heterocyclic compounds; the rest were aliphatic ketones. In the analyses of soybean milk volatiles, Wilkens and Lin (1970) reported the presence of 2-heptanone, 3-octanone, and 2-octanone. Aliphatic ketones such as 2-heptanone, 3-octanone, and 3-hydroxy-2-butanone were detected in the 18-h fermentation of soybean by *B. subtilis* (Owens et al., 1997). Neither the soybean milk nor the fermented soybean contained heterocyclic compounds of the ketone class. Apparently, most heterocyclic compounds of the ketone class detected here were not naturally occurring products but were thermally generated during steaming (Fors, 1983). For some aliphatic ketones such as 2-heptanone, 3-octanone, and 3-octen-2-one, they could be products of lipid oxidation or degradation (Ames and Macleod, 1984) assisted by enzymes from the inoculated mold (Wang and Hesseltine, 1970; Su, 1986). Although the presence of common compounds among the three samples was considered to be important to the character of FSCs, the presence of other compounds, including 2-octanone, 3-octen-2-one, piperidinone, 1(3*H*)-isobenzofuranone, and 5-hexyldihydro-2(3*H*)-furanone, was important only for characterizing the individual brand. The flavor notes of ketones were generally desirable in FSCs. 2-Heptanone has a fruity, spicy, cinnamon-like aroma, and 3-octanone has a floral, green, herbaceous, fruity-like note (Aldrich, 1998). 3-Hydroxy-2-butanone has a buttery note and could be found as a byproduct in the fermentation of molasses (Bauer and Garbe, 1985; Aldrich, 1998). 1-(2-Furanyl)ethanone was detected in the pyrolysis products of *N*-acetylglucosamine (Chen et al., 1998), and 1-(1*H*-pyrrol-2-yl)ethanone was identified among the volatile components in corn totilla chips (Buttery and Ling, 1998). Dihydro-5-pentyl-2(3*H*)furanone, identified in lychee by GC/olfactometry (Ong and Acree, 1998), has a powerful green, vegetable odor (Burdock, 1994). 3-Hydroxy-2-methyl-4*H*-pyran-4-one, having a malt, toasted flavor, was the most concentrated component within the ketone group and among all brands (Aldrich, 1998). Compounds such as 2-heptanone and 3-octanone were also identified in other food products prepared from soybeans (Ames and Macleod, 1984; Kobayashi et al., 1995).

Three common miscellaneous compounds identified included 3-methyl-1*H*-pyrrole, naphthalene, and 1*H*-indole. 3-Methyl-1*H*-pyrrole has a "powerful smoky, slightly woody and herbaceous" aroma (Aldrich, 1998). 1*H*-Indole has an "unpleasant, cadaverous, fecal, putrid"

note in high concentration, but it has a floral note when in low concentration (Aldrich, 1998). Because 1*H*-indole had a much higher concentration than other miscellaneous components in the same class, it could have an undesirable effect on the background flavor of the FSCs. Naphthalene, described to have a mothball-like odor, could be a contaminant from the environment (McElroy et al., 1989; Budavari, 1996).

Quantitatively, 12 components, mostly from alcohols and esters, had a dry weight of >1000 μg/kg in all samples including ethanol, 1-propanol, 2-methyl-1-propanol, 1-hexanol, 3-hydroxy-2-methyl-4*H*-pyran-4-one, ethyl lactate, ethyl tetradecanoate, ethyl hexadecanoate, ethyl (*Z*)-9-octadecenoate, ethyl (*Z*,*Z*)-9,12-octadecadienoate, ethyl (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoate, and 1*H*-indole. Besides, both alcohols and esters were the major classes of compounds found in FSCs. These results showed that the background volatile components of FSCs were dominated by the fruity and sweet flavor from a majority of the esters and alcohols. Although FSCs retained the characteristic green bean note due to the presence of 2-pentylfuran and *n*-hexanal (Sessa and Rackis, 1977; Hsieh et al., 1981; Kobayashi et al., 1995) as well as the cooked soybean note from 2-methoxy-4-vinylphenol and 4-vinylphenol (Greuell, 1974), the high concentration and number of some of the esters and alcohols were probably able to mask the undesirable notes in the background (Mueller, 1965). Although both esters and alcohols contributed more to the background characteristic flavor of these commercial FSCs, the subtle flavor of individual samples was likely determined by the quality and quantity of other components that did not belong to the common ones.

The presence of these volatile components was partly due to the fungal actions on the tofu after their inoculation. Fungi were introduced to the tofu substrate for the growth and development of mycelia. Proteases and lipases produced by the fungi during the incubation periods generated large amounts of peptides and amino acids, as well as fatty acids (Steinkraus, 1983). This provided a pool of substrates for further biological and chemical reactions. Some volatile flavor components were produced in this period. Wang (1967) suggested that the subsequent brining step not only served to inhibit mold growth but also helped to release the mycelium-bound enzyme into the tofu matrix for further digestion activities. This allowed more low molecular weight components to be produced (Steinkraus, 1983).

Comparison among the three brands (A−C) showed variations in their compositions of volatile components. Although brand A was an imported product and brand B was a local product, 80 common compounds were found between them, whereas only 67 common compounds were found between brands A and C. The number of common compounds found between the two local brands (B and C) was 67, which was much lower than those found in brands A and B. For those significant ones from all three brands, slightly over two-thirds of these 34 compounds in brands A and B were statistically similar in quantity, whereas most compounds in brand C were different from either brand A or brand B. This might suggest that brands A and B were quite similar in both quality and quantity but that brand C was different from both brands A and B. Such differences were likely due to some variations in the proprietary processing steps used by each manufacturer.

The present investigation is concerned with the volatile components, particularly those common ones, without giving much consideration to the actual compounds that contributed to the aroma of the FSC. Subsequent experiments involving GC/olfactometry are necessary to screen out those important odorous components characterizing the common aroma in FSCs.

In conclusion, among the 111 combined volatile components identified in the steamed commercial FSCs, a large number of them belonged to alcohols and esters. These two classes of compounds together with other common components probably contribute more to the characteristic background odor of all brands investigated. Less predominant groups including pyrazines, sulfur-containing compounds, aldehydes, ketones, etc. might serve an important role in the subtle aroma of different FSCs.

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