

## Headspace-Solid Phase Microextraction (HS-SPME) Analysis of Korean Fermented Soybean Pastes

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**Abstract** In this study, the volatile compounds in 9 commercial fermented soybean pastes were extracted and analyzed by headspace-solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS), respectively. A total of 63 volatile components, including 21 esters, 7 alcohols, 7 acids, 8 pyrazines, 5 volatile phenols, 3 ketones, 6 aldehydes, and 6 miscellaneous compounds, were identified. Esters, acids, and pyrazines were the largest groups among the quantified volatiles. About 50% of the total quantified volatile material was contributed by 5 compounds in 9 soybean paste samples; ethyl hexadecanoate, acetic acid, butanoic acid, 2/3-methyl butanoic acid, and tetramethyl-pyrazine. Three samples (CJW, SIN, and HAE) made by *Aspergillus oryzae* inoculation showed similar volatile patterns as shown in principal component analyses to GC-MS data sets, which showed higher levels in ethyl esters and 2-methoxy-4-vinylphenol. Traditional fermented soybean pastes showed overall higher levels in pyrazines and acids contents.

**Keywords:** soybean, fermentation, volatile, headspace-solid phase microextraction (HS-SPME), gas chromatography-mass spectrometry (GC-MS)

### Introduction

Soybean has been used in the production of fermented soybean foods in many Asian countries such as soy sauce, *tempeh*, Japanese *miso* and *natto*, Thai *thua nao*, Chinese *sufu*, and Korean *doenjang* (1,2). Especially Korean fermented soybean pastes (*doenjang*) have long been consumed and recently there is increasing consumer interest for traditionally made soybean pastes that possess significant health effects (3,4). Korean traditional fermented soybean pastes (*doenjang*) have been made with *meju* which is a rectangular block of crushed cooked soybeans fermented with natural strains. On the other hand, large manufacturing companies are producing commercial fermented soybean pastes using wheat *koji* inoculated with *Aspergillus oryzae*, which has been introduced from Japanese *miso* production.

There have been many studies focusing on the flavors of Korean fermented soybean pastes (5-10). The volatile component profiles of soybean pastes vary with the micro-flora involved, as well as by the processing conditions (e.g., fermentation, drying, brining, or aging). Volatiles in fermented soybean pastes prepared using different types of strains were examined (6-8), as well as different extraction (5,9) and aging and mixing methods (10). However, volatile components in commercially and traditionally manufactured fermented soybean pastes have not been quantitatively compared.

For the volatile extraction, different methods can be utilized. Steam distillation/solvent extraction (SDE) or Likens-Nickerson (LN) extraction was widely used to collect wide ranges of volatile compounds in solid foods

(11,12). However, in this extraction method, a large sample size and a large quantity of solvent are needed. Heat is usually involved and may cause flavor changes. Thus, solid phase microextraction (SPME) is currently replacing older techniques for volatile analysis in many areas (11,12). The SPME method is rapid, less labor intensive, uses little solvent, and is relatively inexpensive. In this paper, headspace SPME combined with gas chromatography-mass spectrometry (GC-MS) was utilized for the isolation and identification of volatile compounds in various soybean pastes. For the selection of important volatiles when examining the effects of manufacturing variables, detailed information on the volatile compositions of fermented soybean products could be valuable.

The specific objectives of this study were to identify and quantify the volatile components in various traditional and mass produced fermented soybean pastes using headspace SPME coupled with GC-MS and to compare their volatile compositions.

### Materials and Methods

**Materials** Nine fermented soybean pastes (*doenjang*) were purchased from different manufacturing companies (Table 1). Six of the samples (ANJA, SOHI, CHJE, CHJA, MON, and SUJA) were collected from local companies producing Korean soybean pastes by the traditional method, using natural micro-flora. The other 3 samples (SIN, CJW, and HAE) were acquired from large manufacturing companies producing soybean pastes using wheat *koji* inoculated with *Aspergillus oryzae*. Approximate aging times for the samples were ranged from 6 months to 1 year. In addition, traditionally fermented *meju* was added to the CJW and HAE samples during production to give a traditional *doenjang* flavor.

*n*-Alkane standards (C6-C24), sodium sulfate, sodium chloride, an internal standard compound (2-methyl-1-pentanol),

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**Table 1. Materials and their ingredients**

Code	Manufacturing method <sup>1)</sup>	Producing area	Ingredients
ANJA	Traditional	Andong	Soybean 85%, salt
SOHI	Traditional	Youngju	Soybean 90%, salt 10%
CHJE	Traditional	Cheongju	<i>Meju</i> (soybean) 99%, salt
CHJA	Traditional	Geosan	Soybean 95%, salt 5%
MON	Traditional	Sunchang	Soybean 88%, salt
SUJA	Traditional	Sunchang	Soybean 97%, salt
SIN	Modern	Cheonan	Soybean 28.35%, flour, salt, wheat ( <i>koji</i> ), ethanol
CJW	Modern	Sunchang	Soybean 53.4%, <i>meju</i> 16%, salt, ethanol, <i>koji</i>
HAE	Modern	Gongju	<i>Meju</i> 58%, soybean, flour, salt, wheat ( <i>koji</i> )

<sup>1)</sup>Traditional method using fermentation by natural micro-flora, while the modern method used wheat *koji* inoculated with *Aspergillus oryzae*.

methanol, and authentic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). The authentic chemical standards were obtained from suppliers as follows: compounds as ethyl 3-methylbutanoate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl nonanoate, ethyl benzoate, ethyl phenylacetate, 2-phenylethyl acetate, ethyl dodecanoate, ethyl tetradecanoate, ethyl pentadecanoate, ethyl hexadecanoate, 1-butanol, 3-methyl-1-butanol, 1-octen-3-ol, furfuryl alcohol, phenethyl alcohol, acetic acid, propanoic acid, 2-methyl propanoic acid, butanoic acid, 3-methyl butanoic acid, hexanoic acid, 2,5-dimethyl pyrazine, trimethyl pyrazine, 2,5-dimethyl-3-ethyl-pyrazine, tetramethyl pyrazine, 2-methoxy-phenol, 2-methoxy-4-vinylphenol, benzaldehyde, 3-octanone, 3-hydroxy-2-butanone, 2-pentyl-furan, 1,3-dimethoxy-benzene, and acetyl pyrrole from Sigma-Aldrich, 4-ethyl-phenol and maltol from Paltz & Bauer, Inc. (Waterbury, CT, USA).

**Headspace-solid phase microextraction (HS-SPME) analysis** Four commercially available SPME fibers from Supelco (Bellefonte, PA, USA) were examined for this study. These were polydimethylsiloxane (PDMS; 100  $\mu$ m), carboxen/polydimethylsiloxane (CAR/PDMS; 85  $\mu$ m), polymethylsiloxane/divinylbenzene (PDMS/DVB; 65  $\mu$ m), and divinylbenzene/carboxen/PDMS (DCP, 50/30  $\mu$ m), which showed different affinities to diverse compounds. The DCP (50/30  $\mu$ m) SPME fiber was selected because this fiber showed a higher affinity toward wide range of volatile compounds in soybean paste samples. The different extraction parameters that influence the HS-SPME analyses were studied respectively: sample ionic (sodium chloride) strength (none, 3, and 7 g), sample volume (5, 10, and 15 g), extraction time (10, 30, and 60 min), and temperature (40, 60, and 100°C) of the sample during the process. The experiments made to evaluate the influence of each of these factors were carried out with the SIN sample selected. The optimal conditions were as follows: for each analysis, 10 g of sample was placed into a 30-mL glass vial with 7 mL of distilled water, 1 mL of internal standard (IS) [2-methyl-1-pentanol (10  $\mu$ g/mL in methanol)], 3 g of NaCl, and a little magnetic stir bar. Then, the vial was tightly capped with a silicon septum and was pre-equilibrated for 30 min at 60°C in a thermostatic bath. Afterward, the stainless steel needle, where the fiber is housed, was pushed through the vial septum, then the fiber was pushed out of the housing and exposed for 30 min at

60°C to the headspace generated in the sample vial. After extraction, the fiber was pulled into the housing, and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption of the analytes at 230°C for 5 min.

#### Gas chromatography-mass spectrometry (GC-MS)

Volatile compounds were analyzed using on a Hewlett-Packard (HP) gas chromatograph model 6890 equipped with a split/splitless injector and a DB-WAX bonded fused capillary column (30 m  $\times$  0.32 mm i.d., film thickness=0.25  $\mu$ m, J&W Scientific Inc., Folsom, CA, USA). The detector was a mass spectrometer (HP 5973 Mass Selective Detector; Hewlett-Packard, Palo Alto, CA, USA). The analyses were performed under the following conditions: an inlet temperature of 230°C; splitless time of 1 min; a purge flow rate to the split vent at 50 mL/min for 1 min; column head pressure at 14.14 psi; a helium carrier gas flow rate of 1.3 mL/min, and an average helium gas velocity of 30 cm/sec; the oven temperature was held at 40°C for 4 min and programmed at 5°C/min until 185°C, and then held for 20 min isothermally. The mass spectra were generated in the electron impact mode (MS-EI) at 70 eV and an ion source temperature of 230°C. The spectra were taken over the *m/z* range of 40-350. The total ion chromatograms (TIC) acquired via GC-MS were used for peak area integration. HP MSD Chemstation software G1701BA ver. D.02.00 (Palo Alto, CA, USA) was used for data acquisition.

#### Compound identification and quantification

The volatile compounds were positively identified by comparing Kovats retention indices (KI) (13) and the MS fragmentation patterns with those of reference compounds, or with mass spectra in the Wiley 275 mass spectral database (Hewlett-Packard) and previously reported Kovats retention indices. The KI of unknown compounds were determined via sample injection with a homologous series of alkanes (C6-C24). The GC/MS conditions were the same as described above. To quantify the volatiles, the samples were run in duplicate, and the integrated areas based on the total ion chromatograms were normalized to the areas of the internal standard and averaged. The relative volatile concentrations in the 9 samples were determined by comparison with the concentration of the internal standard (2-methyl-1-pentanol), assuming a response factor of 1. To determine the reproducibility of the duplicate injections and which

**Table 2. Volatile components (ppm<sup>1)</sup>, w/w) identified in the headspace of 9 fermented soybean pastes by solid phase microextraction (n=2)**

KI <sup>2)</sup>	Code	Volatile compound <sup>3)</sup>	Sample									Id <sup>4)</sup>	Ref
			CJW	HAE	SIN	ANJA	SOHI	MON	SUJA	CHJE	CHJA		
Esters													
1055	et1	Ethyl butyrate	0.21	3.55	0.20	0.74	0.32	1.00	8.21	1.08	1.07	B	2,5,14,19
1065	et2	Ethyl 2-methylbutanoate	0.57	0.00	0.02	1.42	0.02	0.13	4.84		1.39	B	2,11,14
1074	et3	Ethyl 3-methylbutanoate	0.34	0.18	0.02	3.60	0.02	0.24	2.22		1.46	A	
1113	et4	Isoamyl acetate <sup>5)</sup>				0.13					0.08		
1214	et5	Ethyl hexanoate	0.68	0.38	0.91		0.75	0.26	0.66		0.05	A	2,6,14
1328	et6	Ethyl lactate	1.00	0.03	0.47		0.25	0.03				A	2,14
1408	et7	Ethyl octanoate	0.85	2.43	1.31	0.35	1.44	0.53	1.01	0.06	0.15	A	
1523	et8	Ethyl nonanoate	0.10	0.55	0.34	0.05	0.34	0.11	0.10			A	
1546	et9	Ethyl 3-(methylthio)propionate <sup>5)</sup>	0.20	0.12				0.06	0.31			B	
1569	et10	Methyl decanoate <sup>5)</sup>				1.22						B	
1631	et11	Ethyl benzoate	3.98	0.32	0.57	0.68	0.24	0.29	0.48			A	6,9,14,15
1731	et12	Methyl benzenacetate		0.14		0.59	0.47		0.54	0.17	0.81		
1760	et13	Ethyl phenylacetate	0.55	0.74	0.52	4.34	0.27	0.53	6.27	0.14	1.22	A	9,11,15
1785	et14	2-Phenylethyl acetate		0.16	0.13	0.38	0.10	0.19	0.13	0.02	0.14	A	2
1824	et15	Ethyl dodecanoate <sup>5)</sup>	0.26	0.83	0.74				0.23			A	2,6
2029	et16	Ethyl tetradecanoate	1.17	0.70	1.64	0.07	0.99	0.11	0.35			A	2,6
2095	et17	Ethyl heptadecanoate <sup>5)</sup>	1.43	0.50	0.30				0.25			B	
2128	et18	Ethyl pentadecanoate <sup>5)</sup>	1.96	0.76	0.31		0.25					A	15
2187	et19	Methyl hexadecanoate <sup>5)</sup>	0.59	0.31	1.73	4.69	0.51	0.99	1.15	0.57	0.85	B	2,19
2224	et20	Ethyl hexadecanoate	21.25	10.74	29.28	2.29	10.29	1.01	1.58	0.21	2.88	A	2,15,19
2247	et21	Ethyl-9-hexadecanoate	0.77	0.46	0.60		0.48					B	19
Alcohols													
1139	al1	1-Butanol <sup>5)</sup>						0.22	0.16	0.04		A	2,6,14
1203	al2	3-Methyl-1-butanol	0.15	0.23	0.61	0.65	0.81	0.49	0.80		0.65	A	
1345	al3	1-Hexanol	0.03	0.05	0.07	0.04	0.02	0.07					
1437	al4	1-Octen-3-ol	0.19	0.85	0.64	0.94	0.75	1.52	2.20	1.56	2.19	A	
1511	al5	2-Nonanol <sup>5)</sup>				0.55		0.38				B	
1639	al6	Furfuryl alcohol <sup>5)</sup>			0.11	1.05				1.61		A	2,15,19
1883	al7	Phenethyl alcohol	0.35	0.35	1.01	4.56	0.61	1.12	1.25	0.38	2.03	A	2,14,15
Acids													
1430	ac1	Acetic acid	4.24	2.01	6.18	13.88	3.12	5.22	11.40	9.95	8.43	A	6,14,19
1526	ac2	Propanoic acid	0.04	0.13	0.24	0.85		0.05	0.28	1.27	0.43	A	15
1551	ac3	2-Methyl propanoic acid	0.87	0.89	0.31	4.92	0.87	0.44	1.15	1.02	3.18	A	
1600	ac4	Butanoic acid	0.01	2.38	0.10	9.89	6.74	2.23	4.50	24.58	2.77	A	
1646	ac5	2/3-Methyl butanoic acid	2.43	6.42	2.14	34.29	5.49	1.75	7.50	7.36	13.62	A	
1781	ac6	4-Methyl pentanoic acid <sup>5)</sup>	0.10			0.16	0.44		0.17	0.25		B	
1822	ac7	Hexanoic acid <sup>5)</sup>				0.94	0.47	0.36	0.07	0.35	0.27	A	9,11,15

peaks varied across samples, two-way analyses of variance (ANOVA; sample, injection) were performed for each volatile peak. All peaks varied significantly across samples, with only 4 varying significantly across replications.

**Statistical analysis of GC-MS data** To evaluate significant differences in the volatile components among the fermented soybean pastes, ANOVA was performed by the general line model (GLM) procedure using the Statistical Analysis System (SAS)<sup>®</sup> for Windows, version 8 (Cary, NC, USA). Principal component analysis (PCA) was performed on the

mean values of the relative volatile concentrations using the correlation matrix with no rotation on UNSCRAMBLER ver. 9.5 (CAMO A/S, Trondheim, Norway). PCA was applied to clarify the relationship among the samples prepared by different processing techniques and the volatile components that were present. The GC data set included 48 identified volatile compounds. Of the 63 compounds quantified, 15 compounds (shown in Table 2) were eliminated from the GC-MS data set because they could not be quantified or found in the majority of samples.

Table 2. Continued

KI <sup>2)</sup>	Code	Volatile compound <sup>3)</sup>	Sample									Id <sup>4)</sup>	Ref	
			CJW	HAE	SIN	ANJA	SOHI	MON	SUJA	CHJE	CHJA			
Pyrazines														
1304	py1	2,5-Dimethyl pyrazine	0.14	0.45	0.03	0.59	1.19	0.20	1.30	0.72	1.23	A	2,11,14,15	
1306	py2	2,6-Dimethyl pyrazine	0.03	0.02	0.08	0.66	0.82	0.06	0.59	0.26	0.72	B	2,14	
1321	py3	2,3-Dimethyl-pyrazine <sup>5)</sup>		0.04		0.12	0.39	0.02	0.22	0.15	0.26	B		
1381	py4	Trimethyl pyrazine	0.39	1.84	0.07	4.04	8.51	0.55	5.14	1.99	6.71	A		
1424	py5	2,5-Dimethyl-3-ethyl-pyrazine	0.12	0.23	0.02	0.32	0.69	0.09	0.36		0.18	A		
1443	py6	2-Ethyl-3,5-dimethyl-Pyrazine	0.07	0.15		0.54	0.62	0.10	0.54	0.18	0.54	B		
1460	py7	Tetramethyl-pyrazine	0.70	5.09	0.10	22.96	20.99	0.74	8.59	2.37	19.56	A	9,11,14,15	
1503	py8	2,3,5-Trimethyl-6-ethylpyrazine	0.24	0.40		1.22	2.61		1.77	0.29	0.86	B		
Volatile phenolics														
1830	phe1	2-Methoxy-phenol	0.30	0.42	0.27	1.36	0.42	0.26	0.40	0.27	0.96	A	6,11,15	
1999	phe2	4-Ethyl-2-methoxy-phenol	0.30	0.72	1.04	8.69	0.80	1.93	0.31	0.40	1.79	B	11,15	
2142	phe3	4-Ethyl-phenol	0.41	1.15	0.96	9.84	0.71	0.67	0.51	0.24	0.84	A		
2157	phe4	2-Methoxy-4-vinylphenol	0.44	1.04	1.67	0.25	1.24	0.21	0.78	0.47	0.11	A	11	
2348	phe5	4-Vinylphenol	0.36	0.33	0.44		0.35		0.41	0.24		B		
Aldehydes														
1439	ad1	2-Furancarboxaldehyde	0.28	0.04	0.16	2.49	0.62	0.36	0.13	0.15		B	2,15,19	
1498	ad2	Benzaldehyde	1.36	0.87	0.58	6.57	3.08	2.57	6.72	2.43	3.05	A	5,6,9,11,15	
1518	ad3	(E)-2-nonenal <sup>5)</sup>	0.19			0.40		0.17		0.10	0.70	B		
1601	ad4	Benzeneacetaldehyde	0.87	0.59	0.60	2.80	3.67	1.76	1.40	2.72	3.15	B	6,14,15	
1896	ad5	2-Phenyl-2-butenal	0.37	0.18	0.28	0.35	0.25	0.43	0.45	0.05	0.19	B		
2042	ad6	5-Methyl-2-phenyl-2-hexenal	0.08	0.06	0.13	0.65	0.08	0.06	0.13			B	14,15	
Ketones														
1223	ke1	3-Octanone		0.14		0.20		0.02	1.45		0.28	A	2,6,14	
1282	ke2	3-Hydroxy-2-butanone	0.01	0.02	0.12	0.25	0.05	0.02	0.07	0.04		A	2,14	
1993	ke3	5-Pentyl-dihydro-2[3H]-furanone	0.07	0.05	0.08	0.56	0.09	0.15	0.15	0.07	0.05			
Miscellaneous														
1209	ms1	2-Pentyl-furan	0.07	0.37	0.23	0.75	0.40	0.92	0.52	1.34	0.58	A	2,9,11	
1210	ms2	Ethenyl-benzene	0.03	0.14	0.50	0.58	1.57	1.71	0.85	0.64		B	6,11	
1701	ms3	1,2-Dimethoxy-benzene		0.27	0.00	0.49	2.04	0.07	0.55	0.25	1.97	B	11	
1719	ms4	1,3-Dimethoxy-benzene <sup>5)</sup>						0.22	1.04			A		
1934	ms5	Maltol	0.97	0.10	0.95	1.09	0.94	0.67	0.87	0.89	1.11	A	2	
1941	ms6	Acetyl pyrrole	0.29	0.08		0.41	0.43	0.12		0.15	0.28	A	14	

<sup>1)</sup> Average of the ppm ( $n=2$ )= (area of each compound  $\times$  amount of internal standard  $\times 10^6$ ) / (area of internal standard  $\times$  amount of sample)<sup>2)</sup> Kovats indices of unknown compounds on DB-WAX column.<sup>3)</sup> Compounds by order of their Kovats indices in a chemical class.<sup>4)</sup> Volatiles were identified based on the following criteria: A, mass spectrum and retention index consistent with those of an authentic standard; B, mass spectrum consistent with that of the Wiley 275 mass spectrum database.<sup>5)</sup> Volatiles were not used in principal component analysis.

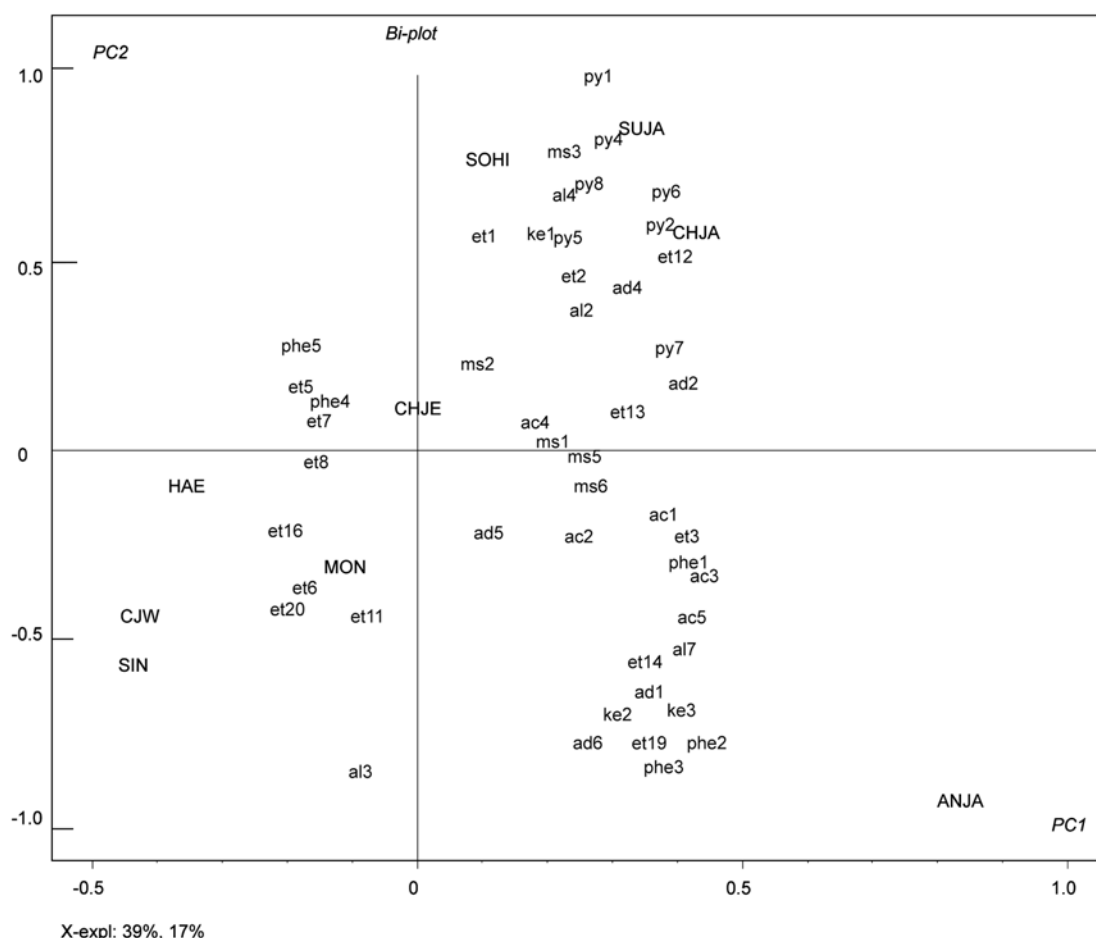
## Results and Discussion

### Headspace compositions in fermented soybean pastes

In the headspace of 9 soybean paste samples, the volatiles were isolated using SPME and then analyzed by GC-MS. Table 2 lists the identified volatile components in each of the 9 samples by their chemical classes, relative concentrations, and KIs on the DB-wax column, respectively. In the 9 soybean paste samples, a total of 63 compounds were identified, including 21 esters, 7 alcohols, 7 acids, 8 pyrazines, 5 volatile phenols, 3 ketones, 6 aldehydes, and 6 miscellaneous compounds. Generally, HS-SPME analysis of fermented soybean pastes demonstrated a

satisfactory level of extraction efficiency with wide ranges of detected volatile compounds. Volatiles were inspected by their chemical classes. Esters, acids, and pyrazines were the largest groups among quantified volatiles. The abundant compounds were ethyl hexadecanoate, acetic acid, butanoic acid, 2/3-methyl butanoic acid, tetramethylpyrazine in accordance with the literature (2,5,6,9,14,15). About 50% of the total volatile material was contributed by these 5 compounds in 9 soybean paste samples.

A total of 21 esters were detected, and were among one of the largest classes of detected compounds. All the detected esters were previously found in various fermented soybean foods (2,5,6,9,14,15). Ethyl butanoate, ethyl 2-



**Fig. 1.** PCA loadings for 48 volatiles (small letters) and scores for 9 soybean paste samples (capital letters). (PC1 is 39% and PC2 is 17% of variation). The codes of volatile compound are defined in Table 2.

methylbutanoate, ethyl 3-methylbutanoate, ethyl phenylacetate, and ethyl hexadecanoate were the major compounds detected. The concentrations of these ester compounds varied much across 9 samples; their coefficients of variation ranged from 110 to 160%. The most abundant compound was ethyl hexadecanoate which likely produced by the action of fungal lipase on soybean lipids (16). The ethyl hexadecanoate levels in 4 samples (CJW, HAE, SIN, and MON) were much higher than those in the other samples.

Among 7 alcohol compounds identified, 3-methyl-1-butanol, 1-hexanol, 1-octen-3-ol, and phenethyl alcohol were detected in most of samples. Considering the low levels of detected alcohols, their aroma contributions might have been minimal on fermented soybean samples that possess overall briny, soy sauce, and cooked soybean flavors. Acids were major volatile compounds in 9 fermented soybean paste samples, which comprised about 30% of total quantified volatiles. Especially soybean pastes made with the traditional method such as ANJA, SOHI, SUJA, CHJE, and CHJA showed much higher levels of these acids than those in the other samples. Regarding to cheesy notes of these acids like butanoic acid (cheesy, sharp, rancid, sweaty, and sour) and 2/3-methylbutanoic acid (cheese, rancid, and sweaty) (17), these acids were considered as important contributors of flavor.

Pyrazines and volatile phenols are also important in the aroma as reported in *miso* (11), Korean *doenjang* (6,9), and soy sauce (8). Pyrazines, particularly alkylpyrazines, are generally described as having a nutty aroma (17). Eight pyrazines were identified in the 9 samples, and 2,5-dimethylpyrazine, tetramethyl pyrazine, and trimethyl pyrazine were the major compounds detected. The amount of total pyrazines was greatly higher in the ANJA, SOHI, SUJA, and CHJA, which all were manufactured by the traditional method. Cooking and aging process of fermented soybean pastes related to pyrazine formation (11), could affect the levels of pyrazines in these samples. Five volatile phenols were detected, while 2-methoxyphenol, 4-ethyl-2-methoxy-phenol, 4-ethyl phenol, and 2-methoxy-4-vinylphenol were detected in all samples. Various sweet note-related Maillard reaction products were also identified such as furans, maltol, and 2-acetyl pyrrole (18).

**Comparison of the headspace composition among the fermented soybean pastes** To give an overall picture of the distribution of the volatiles with the separation of the samples, principal component analysis was performed (Fig. 1). The first principal component (PC1) explained 39% of the variation across the samples, while the second PC explained 17% of the variance. By inspecting the

distribution of samples on this plot (Fig. 1), the samples made by the traditional method using natural micro-flora, scattered over the first 2 principal components. However, sample clustering (CJW, SIN, and HAE) was observed on the negative side of both PC1 and 2; these samples were made with the modernized method by the fermentation using *A. oryzae* inoculation. In these 3 samples, levels of 2-methoxy-4-vinylphenol and ethyl esters like ethyl hexanoate, ethyl tetradecanoate, and ethyl hexadecanoate were significantly higher, as compared to their respective levels in other samples. In the lower side of PC1, ANJA, which showed a distinctive volatile pattern, was located far from other samples. Acids (ac1, ac3, and ac5), phenethyl alcohol (al7), and volatile phenols (phe1, phe2, and phe3) were associated with this sample which had the highest concentrations of these compounds among the tested samples. Other traditional soybean pastes such as SOHI, SUJA, and CHJA were closely located in upper side of PC1 with the same direction as pyrazines (py1, py2, py4, py5, py6, py7, and py8). On the opposite lower side of the PC1, CJW, SIN, and HAE were located in proximity with the longer chain esters (et6, et8, et16, and et20).

In conclusion, differences in the volatile component compositions of various fermented soybean pastes (*doenjang*) were observed. The samples made with natural micro-flora from different areas of Korea showed large differences in their volatile profiles. These may be explained by differences in microorganism utilization within the natural environment. In contrast, the CJW, SIN, and HAE samples showed volatile profiles that were more similar than expected.

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