Aroma Components of Traditional Korean Soy Sauce and Soybean Paste Fermented with the Same Meju

SEO, JAE-SOON, HO-GEUN CHANG, WON-DAE JI, EUN-JU LEE, MYEONG-RAK CHOI¹, HAENG-JA KIM² AND JONG-KYU KIM*

Dept. of Applied Microbiology, Yeungnam University, Kyongsan 712-749, Korea

¹Dept. of Biological Engineering, Yosu Fisheries National University, Yosu 550-749, Korea

²Dept. of Economics Education, Kyeongsang National University, Jinju, Korea

We identified volatile components of traditional Korean soy sauce and soybean paste which had been manufactured with the same traditional Meju with a view to improving the quality of traditional Korean soy sauce and soybean paste. All of the volatile components were extracted by simultaneous steam distillation-extraction (SDE) apparatus. To obtain more detailed information, whole volatile components were separated into fractions. The volatile components of the whole and of each fraction were identified by GC-mass and Kovat's retention index. Sixty two and eighty six components were identified in traditional Korean soy sauce and soybean paste, respectively. Many aroma components of traditional Korean soy sauce differ from those of traditional Korean soybean paste. It was confirmed that many aroma components of traditional Korean soy sauce and soybean paste are completely different from those of Japanese fermented soy sauce (Shoyu) and soybean paste (Miso).

The unique flavors of traditional Korean soy sauce and soybean paste depend on the source materials, microorganisms and fermenting conditions.

In order to make traditional Korean soy sauce and soybean paste, a commercially available Meju which had been manufactured traditionally with natural strains was added to a salt solution, the mixture was fermented first for two or three months, and then the liquid and solid parts of the primary fermented product were separated and fermented again. Conditions for making traditional Korean soy sauce and soybean paste are the same until the end of the first fermentation, during the second fermentation, the flavors of soy sauce and soybean paste become differentiated and develop unique characteristics (13).

There have been many studies focusing on the flavors of traditional Korean soy sauce and soybean paste.

Statistical analysis of the relationship between gas chromatographic profiles of traditional Korean soy sauce and sensory evaluation have been reported (17). Some of the character impact compounds and off-flavors in traditional Korean soy sauce have been identified (7, 14, 19). Also, there have been many studies regarding the isolation of flavors from traditional Korean soybean paste by GC, and these revealed the most effective peaks

Key words: aroma components, traditional Korean soy sauce, traditional Korean soybean paste, traditional Meju

(20). There has been research reporting statistical results (1) and on the identification of volatile components of traditional Korean soybean paste (6, 8, 18).

In addition to these studies, the main microorganism producing the character impact compounds of traditional Korean soy sauce and soybean paste has been identified as the bacterial strain, *Bacillus* sp (9). Studies of the breeding of this strain have been reported (10, 22).

However, there are no reports of the difference between the flavor of traditional Korean soy sauce and soybean paste.

In this study, we identify and discuss the flavor components extracted from traditional Korean soy sauce and soybean paste manufactured with the same traditional Meju and compare them with those of Japanese fermented soy sauce (Shoyu) and soybean paste (Miso).

MATERIALS AND METHODS

Materials

Traditional Korean soy sauce and soybean paste which were fermented with commercially available traditional Meju were used in this study. That is, 10 kg of a traditional Meju and 18 liters of 20% salt solution were mixed and then fermented for 2 months for primary fermentation. The liquid and solid part of the primary fermented product were separated and then further subjected to 5 months of

^{*}Corresponding author

secondary fermentation to obtain traditional Korean soy sauce and soybean paste, respectively.

Identification of Volatile Components

To extract the volatile components of traditional Korean soy sauce and soybean paste, we used improved Nikerson and Nikens' simultaneous steam distillation and extraction apparatus (25). Purified ethyl ether was used as solvent.

According to Fujimaki *et al.*'s method (3), the extracted flavors were separated into basic, acidic, phenolic and neutral fractions. The acidic fraction was further methylated according to Schlenk's diazomethane method (24).

The identification of volatile components was conducted against the results of a computer library search based on the mass spectrum of GC/MS and from Kovat's retention index (5, 23).

The GC/MS used was a Hewlett Packard GC II 5980 combined with a Hewlett Packard 5988 MS. Analytical conditions were given in our previous reports (16, 18).

RESULTS AND DISCUSSION

Table 1 lists the volatile components identified in tradi-

Table 1. Flavor components of traditional Korean soy sauce.

Identified compounds	Molecular formula	Shoyu*	TKSP*
Hydrocarbon (1)			
Tridecane	$C_{13}H_{28}$		
Alcohols (4)			
Ethanol	C_2H_6O	+	+
3-Methyl-1-butanol	$C_sH_{10}O$	+	+
Benzenemethanol	C ₇ H ₈ O	•	•
2-(2-Ethoxyethoxy)-ethanol	$C_6H_{14}O_3$		
Esters (19)			
Formic acid, ethyl ester	$C_3H_6O_2$	+	+
Acetic acid, ethyl ester	$C_4H_8O_2$	+	+
Propanoic acid, methyl ester	$C_4H_8O_2$	•	•
2-Methyl-propanoic acid, methyl ester	$C_5H_{10}O_2$		+
Butanoic acid, methyl ester	$C_5H_{10}O_2$		+
2-Methyl-butanoic acid, methyl ester	$C_6H_{12}O_2$		
3-Methyl-butanoic acid, methyl ester	$C_6H_{12}O_2$		
Pentanoic acid, methyl ester	$C_6H_{12}O_2$		
4-Methyl-pentanoic acid, methyl ester	$C_7H_{14}O_2$		
Hexanoic acid, methyl ester	$C_7H_{14}O_2$		
Heptanoic acid, methyl ester	$C_8H_{16}O_2$		
Octanoic acid, methyl ester	$C_9H_{18}O_2$		
3-Nonenoic acid, methyl ester	$C_{10}H_{18}O_{2}$		
4-Oxo-nonanoic acid, methyl ester	$C_{10}H_{18}O_3$		
2,4,6-Trimethyl-nonanoic acid, methyl ester	$C_{13}H_{26}O_2$		
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		+
10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$		
9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_{2}$		+
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		+
Aldehydes (6)	, , , , , , , , , , , , , , , , , , , ,		
Acetaldehyde	C_2H_4O	+	
Benzeneacetaldehyde	C_8H_8O	+	+
Benzaldehyde	C_7H_6O	+	+
3-Hydroxy-benzaldehyde	$C_7H_6O_2$		
Benzenepropanal	$C_9H_{10}O$		
α -Ethylidene-benzeneacetaldehyde	$C_{10}H_{10}O$		+
Acids (6)			
Butanoic acid (=butyric acid)	$C_4H_8O_2$	+	+
3-Methyl-2-butanoic acid	$C_6H_{10}O_2$		
Nonanoic acid	$C_9H_{18}O_2$		
Tetradecanoic acid	$C_{14}H_{28}O_2$		
Hexadecanoic acid (=palmitic acid)	$C_{16}H_{32}O_2$	+	+
(Z,Z)-9,12-Octadecadienoic acid (=linoleic acid)	$C_{18}H_{32}O_2$	+	

280 SEO ET AL. J. Microbiol. Biotechnol.

Table 1. Continued.

Identified compounds	Molecular formula	Shoyu*	TKSP*
Phenols (6)			
Phenol	C_6H_6O	+	
2-Methoxy-phenol (=guaiacol)	$C_7H_8O_2$	+	+
2,6-Dimethoxy-phenol	$C_8H_{10}O_3$	+	
2,3,5,6-Tetramethyl-phenol	$C_{10}H_{14}O$		
3-tert-Butyl-4-methoxyphenol	$C_{11}H_{16}O_2$.		
Dodecyl-phenol	C ₁₈ H ₃₀ O		
Furans (6)			
2-Furancarboxaldehyde (=2-furfural)	$C_5H_4O_2$	+	+
2-Furanmethanol (=furfuryl alcohol)	$C_5H_6O_2$	+	+
Tetrahydro-2-furanmethanol (=tetrahydrofurfuryl alcohol)	$C_5H_{10}O_2$	+	
5-Methyl-2-furancarboxaldehyde (=5-methyl-2-furfural)	$C_6H_6O_2$	+	+
Furfuryl acrolein	$C_8H_{10}O$		
3-Phenyl-furan	$C_{10}H_8O$	+	
Pyrazines (5)	•		
Methyl-pyrazine	$C_5H_6N_2$	+	+
Ethenyl-pyrazine	$C_6H_6N_2$		
2,3-Dimethyl-pyrazine	$C_6H_8N_2$	+	+
2,5-Dimethyl-pyrazine	$C_6H_8N_2$	+	
Trimethyl-pyrazine	$C_7H_{10}N_2$	+	+
Miscellaneous nitrogen-containing compounds (8)			
1H-Pyrrole	C_4H_5N		+
1H-Pyrrole-2-carboxaldehyde	C ₅ H ₅ NO		
1-(1H-Pyrrol-2-yl)-ethanone	C_6H_7NO		
1H-Indole	C ₈ H ₇ N		
Nitro-benzene	$C_6H_5NO_2$	***	
3-Quinolinamine	$C_9H_8N_2$		
2,4-Dimethyl-quinazoline	$C_{10}H_{10}N_2$		
1-Phenyl-2(1H)-pyridinone	$C_{11}H_9NO$		
Miscellaneous compounds (1)			
1,1'-Oxybis-ethane	$C_4H_{10}O$		

^{*} Japaness fermented soy sauce; ** Traditional Korean soybean paste.

tional Korean soy sauce.

The identified volatile components are sixty two different compounds, which can be classified based on their functional group as follows: one hydrocarbon, four alcohols, nineteen esters, six aldehydes, six acids, six phenols, six furans, five pyrazines, eight other nitrogen-containing components and one miscellaneous component.

Of the identified components, nineteen components such as 2-(2-ethoxyethoxy)-ethanol, butanoic acid methyl ester, 2-methyl-butanoic acid methyl ester, 3-methyl-butanoic acid methyl ester, 2,4,6-trimethyl-nonanoic acid methyl ester, benzeneacetaldehyde, benzaldehyde, 3-hydroxy-benzaldehyde, butanoic acid, phenol, 2-methoxy-phenol, 2,3,5,6-tetramethyl-phenol, 3-tert-butyl-4-methoxy-phenol, 2-furancarboxaldehyde, 2-furanmethanol, tetrahydro-2-furanmethanol, 5-methyl-2-furancarboxaldehyde, 1H-pyrrole and 1H-pyrrole-2-carboxaldehyde have been reported as character impact compounds in the flavor of traditional Korean soy sauce (7, 16). These character

impact compounds are considered to contribute to the the peculiar odor of traditional Korean soy sauce. On the other hand, of the volatile components found in this experiment some components reported as off-flavor were also found (7, 14, 19). These were 3-methyl-1-butanol, indole and butyric acid. In order to produce a high quality traditional Korean soy sauce, the production of these components must be suppressed.

The volatile components identified in traditional Korean soybean paste are listed in Table 2.

The identified components are eighty six compounds. These components can be classified based on their functional group as follows: twenty six esters (formic acid ethyl ester, etc.), twelve alcohols (methanol, etc.), ten hydrocarbons (1,4-dimethyl-benzene, etc.), seven aldehydes (3-methyl-butanal, etc.), six ketones (3-pentene-2-one, etc.), six pyrazines (methyl-pyrazine, etc.), four thiazoles (2,4,5-trimethyl-thiazole, etc.), three nitrogen containing compounds (3-methyl- benzonitrile, etc.), two furanones

Table 2. Flavor components of traditional Korean soybean paste.

Identified compounds	Molecular formula	Miso*	TKSS*
Hydrocarbons (10)			
1,4-Dimethyl-benzene (=p-xylene)	C_8H_{10}		
Ethyl-benzene	C_8H_{10}		
Naphthalene	$C_{10}H_{8}$	+	
1-Methyl-naphthalene	$C_{11}C_{10}$	•	
3-Methyl-5-propyl-nonane	$C_{13}H_{28}$		
Cadinene	$C_{15}H_{24}$		
Cyclohexadecane	$C_{15}H_{24} \\ C_{16}H_{32}$		
3-Eicosene			
9-Eicosene	$C_{20}H_{40}$		
Eicosane	$C_{20}H_{40} \ C_{20}H_{42}$	•	
Alcohols (12)	20 42		
Methanol	CH₄O		
Ethanol	C₂H ₆ O	+	+
1-Propanol	C_3H_8O	+	т
2-Methyl-2-propanol		т	
	$C_4H_{10}O$		
2-Butanol	$C_4H_{10}O$	+	
2-Methyl-2-butanol	$C_5H_{12}O$		
2-Pentanol	$C_5H_{12}O$		
2-Methyl-1-penten-3-ol	$C_6H_{12}O$		
1-Methoxy-2-methyl-2-propanol	$C_5H_{12}O_2$		
1-Nonen-3-ol	$C_9H_{18}O$		
10-Undecen-1-ol	$C_{11}H_{22}O$		
9,12-Octadecadien-1-ol	$C_{18}H_{34}O$		
Esters (26)			
Formic acid, ethyl ester	$C_3H_6O_2$	+	+
Acetic acid, ethyl ester	$C_4H_8O_2$	+	+
2-Methyl-propanoic acid, methyl ester	$C_5H_{10}O_2$	+	+
Butanoic acid, methyl ester	$C_5H_{10}O_2$		+
Butanoic acid, ethyl ester	$C_6H_{12}O_2$	+	
Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$		
2-Methyl-butanoic acid, ethyl ester	$\mathbf{C}_{7}\mathbf{H}_{14}\mathbf{O}_{2}$		
3-Methyl-butanoic acid, ethyl ester	$C_7H_{14}O_2$		
Benzoic acid, methyl ester	$C_8H_8O_2$		
2-Hydroxy-benzoic acid, methyl ester	$C_8H_8O_3$		
Butanoic acid, butyl ester	$C_8H_{16}O_2$		
Haxanoic acid, ethyl ester			
Paramonatic acid, ethyl ester	$C_8H_{16}O_2$	+	
Benzeneacetic acid, ethyl ester	$C_{10}H_{12}O_2$	+	
1,2-Benzenedicarboxylic acid, dimethyl ester	$C_{10}H_{10}O_4$		
Undecanoic acid, methyl ester	$C_{12}H_{24}O_2$		
Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$		
1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$		
Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	+	
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		+
1,2-Benzenedicarboxylic acid, dipentyl ester	$C_{18}H_{26}O_4$		
9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$		
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_{2}$	+	
9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_{2}$		+
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		+
9-Octadecanoic acid, ethyl ester	$C_{20}H_{38}O_2$	+	•
Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	+	
Aldehydes (7)			
3-Methyl-butanal	$C_5H_{10}O$	+	
Benzaldehyde	C_7H_6O	<u>.</u>	+
Heptanal	C_7H_6O $C_7H_{14}O$	Ŧ	т
Benzeneacetaldehyde	C_8H_8O		I.
		+	+
2-Hydroxy-6-methyl-benzaldehyde	$C_8H_8O_2$		
Nonanal α-Ethylidene-benzeneacetaldehyde	$C_9H_{18}O \ C_{10}H_{10}O$		
	(W ()		+

282 SEO ET AL. J. Microbiol. Biotechnol.

Table 2. Continued.

Identified compounds	Molecular formula	Miso*	TKSS*
Acetal (1)			
1,1-Diethoxy-ethane	$C_6H_{14}O_2$		
Ketones (6)	-		
3-Pentene-2-one	C_5H_8O		
Cyclohexanone	$C_6H_{10}O$		
2-Hexanone	$C_6H_{12}O$		
3-Methyl-2-cyclohexen-1-one	$C_7H_{10}O$		
2-Heptanone	$C_7H_{14}O$		
1-(2-Hydroxy-5-methylphenyl)-ethanone	$C_9H_{10}O_2$		
Acid (1)			
Hexadecanoic acid (=palmitic acid)	$C_{16}H_{32}O_2$		+
	C ₁₆ 11 ₃₂ O ₂		
Phenols (1)	0.11.0		
3,5-Bis(1-methylethyl)-phenol	C ₁₂ H ₁₈ O		
Furan (1)			
Tetrahydro-2-methyl-furan	$C_5H_{10}O$		
Furanones (2)			
5-Pentyldihydro-2(3H)-furanone	$C_9H_{16}O_2$		
5-Hexyldihydro-2(3H)-furanone	$C_{10}H_{18}O_2$		
Pyrazines (6)			
Methyl-pyrazine	$C_5H_6N_2$	+	+
2,3-Dimethyl-pyrazine	$C_6H_8N_2$		+
2,6-Dimethyl-pyrazine	$C_6H_8N_2$		
3-Ethyl-2,5-dimethyl-pyrazine	$C_8H_{12}N_2$	+	
Tetramethyl-pyrazine	$C_8H_{12}N_2$		
2,5-Dimethyl-3-propyl-pyrazine	$C_9H_{14}N_2$		
Pyridine (1)			
Pyridine	C_5H_5N	+	
		-	
Miscellaneous nitrogen-containing compounds (3) 3-Methyl-benzonitrile	CHN		
1-Butyl-1H-pyrrole	C_8H_7N		
	$C_8H_{13}N$		
9-Octadecanamide	C ₁₈ H ₃₅ NO		
Thiazoles (4)			
2,4,5-Trimethyl-thiazole	C_6H_9NS		
4-Ethyl-2,5-dimethyl-thiazole	$C_7H_{11}NS$		
2-Methyl-thiazole	C_8H_7NS		
4,5-Dimethyl-2-isopropyl-thiazole	C ₈ H ₁₃ NS		
Miscellaneous compounds (5)			
1-Ethoxy-butane	$C_6H_{14}O$		
Methoxy-benzene	C_7H_8O		
Ethoxy-benzene	$C_8H_{10}O$		
1-Ethyl-4-methoxy-benzene	$C_9H_{12}O$		
5-Butoxy-2-pentene	$C_9H_{18}O$		

^{*} Japanese fermented soy sauce; ** Traditional Korean soy sauce.

(5-pentyldihydro-2(3H)-furanone, etc.), one acetal (1,1-diethoxy-ethane), one acid (hexadecanoic acid), one phenol (3,5-bis(1-methylethyl)-phenol), one furan (tetrahydro-2-methyl-furan), one pyridine (pyridine) and five other miscellaneous compounds (1-ethoxy-butane, etc.). Among them, the most abundant group was esters,

and the next group was alcohols.

Six compounds of naphthalene, ethanol, 2-butanol, benzaldehyde, α-ethylidene-benzeneacetaldehyde, tetramethylpyrazine were previously identified in the flavor of traditional Korean soybean paste (6). Also, many of the identified compounds were reported as character impact compounds in the flavor of traditional Korean soybean paste (18).

On the other hand, 1,4-dimethyl-benzene, ethyl-benzene, 1-propanol, formic acid ethyl ester, acetic acid ethyl ester, 3-methyl-butanal, benzaldehyde, heptanal, benzeneacetaldehyde, 2-heptanone, methyl-pyrazine, 2,3-dimethyl-pyrazine and 2,6-dimethyl-pyrazine were reported as flavor components of raw and roasted soybeans (2). Among them, 1-propanol and ethyl-benzene were identified in the headspace volatiles obtained from raw and 10 min-roasted soybean, and pyrazines and ethyl formate were also major components in 20 and 30 min-roasted soybean.

Besides the synthesis of flavors by chemical reaction, flavors can be formed by metabolic activity of bacterial strains such as *Bacillus* sp. That is, in the flavors of traditional Korean soybean paste and of Japanese natto, pyazines were produced by *Bacillus* sp. SPB1 (12) and *Bacillus natto* (26), respectively. Also, it has been reported that *Bacillus subtilis* and a mutant of *Corynebacterium glutamicum* was able to produce tetramethylpyrazine (11, 21)

The identified volatile components in traditional Korean soy sauce were compared with the components of traditional Korean soybean paste manufactured with the solid portion of the primary fermented product using traditional Meju and with the already reported components of traditional Korean soybean paste.

Fourteen components of ethanol, formic acid ethyl ester, acetic acid ethyl ester, 2-methyl-propanoic acid methyl ester, butanoic acid methyl ester, hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester, octadecanoic acid methyl ester, benzeneacetaldehyde, benzaldehyde, \alpha-ethylidene-benzeneacetaldehyde, hexadecanoic acid, methyl-pyrazine and 2,3-dimethyl-pyrazine were present in the traditional Korean soybean paste manufactured with the solid portion of the primary fermented product using traditional Meiu. Nine components of 3-methyl-1-butanol, butanoic acid, 2-methoxyphenol, 2-furancarboxaldehyde, 2-furanmethanol, 5-methyl-2-furancarboxaldehyde, trimethyl-pyrazine, 1H-indole and 1H-pyrrole were present in the already reported components of traditional Korean soybean paste (6, 14). Considering these results, it can be proposed that the common odor of traditional Korean soy sauce and soybean paste is due to the volatile components which were present in both traditional Korean soy sauce and soybean paste.

Of the volatile components listed in Table 1, thirty nine components such as tridecane, benzenemethanol, 2-(2-ethoxyethoxy)-ethanol, propanoic acid methyl ester, 2-methyl-butanoic acid methyl ester, 3-methyl-butanoic acid methyl ester, 4-methyl-pentanoic acid methyl ester, hexanoic acid methyl ester,

heptanoic acid methyl ester, octanoic acid methyl ester, 3nonenoic acid methyl ester, 4-oxo-nonanoic acid methyl ester, 2,4,6-trimethyl-nonanoic acid methyl ester, 10,13octadecadienoic acid methyl ester, acetaldehyde, 3-hydroxy-benzaldehyde, benzenepropanal, 3-methyl-2-butanoic acid, nonanoic acid, tetradecanoic acid, (Z.Z)-9,12octadecadienoic acid, phenol, 2,6-dimethoxy-phenol, 2,3, 5,6-tetramethyl-phenol, 3-tert-butyl-4-methoxyphenol, dodecyl-phenol, tetrahydro-2-furanmethanol, furfuryl acrolein, 3-phenyl-furan, ethenyl-pyrazine, 2,5-dimethylpyrazine, 1H-pyrrole-2-carboxaldehyde, 1-(1H- pyrrol-2yl)-ethanone, nitro-benzene, 3-quinolinamine, 2,4-dimethyl-quinazoline, 1-phenyl-2(1H)-pyridinone and 1,1'oxybis-ethane were present only in the traditional Korean soy sauce manufactured with the liquid portion of the primary fermented product using traditional Meju.

Of the volatile components listed in Table 2, many components such as 1,4-dimethyl-benzene, ethyl-benzene, naphthalene, 1-methyl-naphthalene, 3-methyl-5-propyl-nonane, cadinene, cyclohexadecane, 3-eicosene, 9eicosene, eicosane, methanol, 1-propanol, 2-methyl-2propanol, 2-butanol, 2-methyl-2-butanol, 2-pentanol, 2methyl-1-penten-3-ol, 1-methoxy-2-methyl-2-propanol, 1nonen-3-ol, 10-undecen-1-ol, 9,12-octadecadien-1-ol, butanoic acid ethyl ester, butanedioic acid dimethyl ester, 2-methyl-butanoic acid ethyl ester, 3-methyl-butanoic acid ethyl ester, benzoic acid methyl ester, 2-hydroxybenzoic acid methyl ester, butanoic acid butyl ester, hexanoic acid ethyl ester, benzeneacetic acid ethyl ester, 1,2-benzenedicarboxylic acid dimethyl ester, undecanoic acid methyl ester, tetradecanoic acid methyl ester, 1,2benzenedicarboxylic acid dibutyl ester, tetradecanoic acid ethyl ester, 1,2-benzenedicarboxylic acid dipentyl ester, 9,12-octadecadienoic acid methyl ester, hexadecanoic acid ethyl ester, 9-octadecanoic acid ethyl ester, octadecanoic acid ethyl ester, 3-methyl-butanal, heptanal, 2-hydroxy-6-methyl-benzaldehyde, nonanal, 1,1-diethoxyethane, 3-pentene-2-one, cyclohexanone, 2-hexanone, 3methyl-2-cyclohexen-1-one, 2-heptanone, 1-(2-hydroxy-5-methylphenyl)-ethanone, 3,5-bis(1-methylethyl)-phenol, tetrahydro-2-methyl-furan, 5-pentyldihydro-2(3H)-furanone, 5-hexyldihydro-2(3H)-furanone, 2,6-dimethyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, tetramethyl-pyrazine, 2,5dimethyl-3-propyl-pyrazine, pyridine, 3-methyl-benzonitrile, 1-butyl-1H-pyrrole, 9-octadecanamide, 2,4,5-trimethylthiazole, 4-ethyl-2,5-dimethyl-thiazole, 2-methyl-thiazole, 4,5-dimethyl-2-isopropyl thiazole, 1-ethoxy-butane, methoxybenzene, ethoxy-benzene, 1-ethyl-4-methoxy-benzene and 5-butoxy-2-pentene were present only in the traditional Korean soybean paste manufactured with the solid portion of the primary fermented product using traditional Meju.

These results obviously demonstrate that there is a difference between the volatile components of traditional

284 SEO ET AL. J. Microbiol. Biotechnol.

Korean soy sauce and soybean paste.

Although the traditional Korean soy sauce and soybean paste manufacturing process was the same during primary fermentation, the secondary fermentation process was different. Thus, the flavors of the traditional Korean soy sauce and the soybean paste are completely different after secondary fermentation. That is, after the secondary fermentation was employed, after dividing the liquid and solid portions to manufacture traditional Korean soy sauce and soybean paste, the liquid and solid portions contained completely different and unique flavors. The two portions probably have different flavors because of the difference in the microorganisms involved, or the difference in the physiological phenomenon in that one is in liquid form and the other is in solid form. When the bacteria and yeasts participate in manufacturing traditional Korean soy sauce and soybean paste; in liquid cultivation the soy sauce flavor is produced and in solid cultivation the soybean paste flavor is produced (15). The reason is as yet unknown.

We also compared the identified flavors in traditional Korean soy sauce and soybean paste with the volatile components reported from Japanese fermented Shoyu and Miso.

Of the identified components in traditional Korean soy sauce, twenty two components were present in the volatile components of Japanese fermented Shoyu (28, 29). By contrast, forty components found in traditional Korean soy sauce including tridecane, benzenemethanol, 2-(2-ethoxy ethoxy)-ethanol, propanoic acid methyl ester, 2-methyl-propanoic acid methyl ester, butanoic acid methyl ester, 2-methyl-butanoic acid methyl ester, 3methyl-butanoic acid methyl ester, pentanoic acid methyl ester, 4-methyl-pentanoic acid methyl ester, hexanoic acid methyl ester, heptanoic acid methyl ester, octanoic acid methyl ester, 3-nonenoic acid methyl ester, 4-oxononanoic acid methyl ester, 2,4,6-trimethyl-nonanoic acid methyl ester, hexadecanoic acid methyl ester, 10,13octadecadienoic acid methyl ester, 9-octadecenoic acid methyl ester, octadecanoic acid methyl ester, 3-hydroxybenzaldehyde, benzenepropanal, α-ethylidene-benzeneacetaldehyde, 3-methyl-2-butanoic acid, nonanoic acid, tetradecanoic acid, 2,3,5,6-tetramethyl-phenol, 3-tert-butyl-4methoxyphenol, dodecyl-phenol, furfuryl acrolein, ethenylpyrazine, 1H-pyrrole, 1H-pyrrole-2-carboxaldehyde, 1-(1H-pyrrol-2-yl)-ethanone, 1H-indole, nitro-benzene, 3quinolinamine, 2,4-dimethyl-quinazoline, 1-phenyl-2(1H)pyridinone and 1,1'-oxybis-ethane have not been repored in the flavor of Japanese fermented Shoyu.

Of the identified components in traditional Korean soybean paste, twenty components were present in the volatile components of Japanese Miso (4, 27). But, many compounds, which had not been reported in Japanese Miso, were identified in traditional Korean soybean paste. These were forty six compounds including 1,4-dimethyl-benzene, ethyl-benzene, 1-methyl-naphthalene, 3methyl-5-propyl-nonane, cadinene, cyclohexadecane, 3eicosene, 9-eicosene, eicosane, methanol, 2-methyl-2-propanol, 2-methyl-2-butanol, 2-pentanol, 2-methyl-1penten-3-ol, 1-methoxy-2-methyl-2-propanol, 1-nonen-3ol, 10-undecen-1-ol, 9,12-octadecadien-1-ol, butanoic acid methyl ester, butanedioic acid dimethyl ester, 2-methylbutanoic acid ethyl ester, 3-methyl-butanoic acid ethyl ester, benzoic acid methyl ester, 2-hydroxy-benzoic acid methyl ester, butanoic acid butyl ester, 1,2-benzenedicarboxylic acid dimethyl ester, undecanoic acid methyl ester, tetradecanoic acid methyl ester, 1,2-benzenedicarboxylic acid dibutyl ester, hexadecanoic acid methyl ester, 1,2-benzenedicarboxylic acid dipentyl ester, 9,12-octadecadienoic acid methyl ester, 9-octadecenoic acid methyl ester, octadecanoic acid methyl ester, heptanal, 2-hydroxy-6-methyl-benzaldehyde, nonanal, alphaethylidene-benzeneacetaldehyde, 1,1-diethoxy-ethane, 3pentene-2-one, cyclohexanone, 2-hexanone, 3-methyl-2cyclohexen-1-one, 2-heptanone, 1-(2-hydroxy-5-methylphenyl)-ethanone, hexadecanoic acid, 3,5-bis(1-methylethyl)-phenol, tetrahydro-2-methyl-furan, 5-pentyldihydro-2(3H)-furanone, 5-hexyldihydro-2(3H)-furanone, 2,3-dimethyl-pyrazine, 2,6-dimethyl-pyrazine, tetramethylpyrazine, 2,5-dimethyl-3-propyl-pyrazine, 3-methyl-benzonitrile, 1-butyl-1H-pyrrole, 9-octadecanamide, 2,4,5-trimethyl-thiazole, 4-ethyl-2,5-dimethyl-thiazole, 2-methylthiazole, 4,5-dimethyl-2-isopropyl thiazole, 1-ethoxy-butane, methoxy-benzene, ethoxy-benzene, 1-ethyl-4-methoxy-benzene and 5-butoxy-2-pentene.

Therefore, our results show that the characteristic odors of traditional Korean soy sauce and soybean paste are quite different from those of Japanese fermented Shoyu and Miso on account of the components listed above. The reason is thought to be the differences in materials, microorganisms involved, and the fermentation process.

Acknowledgement

This study was supported by a grant from Yeungnam University Research in 1995.

REFERENCES

- Chang, J. K. and J. K. Kim. 1984. Statistic analysis for the relationship between gas chroma-tographic profiles of Korean ordinary soybean paste flavor and sensory evaluation. Kor. J. Appl. Microbiol. Bioeng. 12: 153-163.
- Doi, Y., T. T. Tsugita, T. Kurata, and H. Kato. 1980. Changes of head space volatile components of soybeans during roasting. Agric. Biol. Chem. 44: 1043-1047.
- 3. Fujimaki, M., T. Tsugita, and T. Kubota. 1977. Frac-

- tionation and identification of volatile acids and phenols in the stream distillate of rice brane. *Agric. Biol. Chem.* **41**: 1721-1725.
- Iwabuchi, S. and K. Shibasaki. 1973. Studies on the aroma of miso Part II. Characterization of neutral and acidic compounds. Nippon Shokuhin Kogyo Gakkaishi 20: 48-53.
- Jennings, W. and T. Shibamoto. 1980. Qualitative analysis of flavor and fragrance volatile by glass capillary gas chromatography. Academic press Inc., London.
- Ji, W. D., E. J. Lee, and J. K. Kim. 1992. Volatile flavor components of soybean pastes manufactured with traditional Meju and improved Meju. J. Korean Agric. Chem. Soc. 35: 248-253.
- Ji, W. D., E. J. Lee, S. Y. Kim, and J. K. Kim. 1992. Characteristic volatile components of traditional Korean soy sauce. J. Korean Agric. Chem. Soc. 35: 346-350.
- Ji, W. D., S. H. Yang, M. R. Choi, and J. K. Kim. 1995.
 Volatile components of Korean soybean paste produced by Bacillus subtilis PM3. J. Microbiol. Biotechnol. 5: 143-148.
- Ji, W. D., S. J. Lee, and J. K. Kim. 1993. Dimethyl trisulfide produced by *Bacillus* sp. in cooked soybean. *J. Mi*crobiol. Biotechnol. 3: 61-63.
- Jo, Y. L., J. K. Kim, and D. J. Kwoen. 1992. Construction of expression vector of *Bacillus* sp. SSA3 strain. *Kor. J. Appl. Microbiol. Biotechnol.* 20: 637-641.
- Kempler, G. M. 1983. Advances in applied microbiology. Academic press Inc., London.
- Kim, H. S. and J. K. Kim. 1991. Pyrazines in traditional Korean soybean paste and soybean paste produced by Bacillus sp. SPB1. Environ. Research of Yeungnam Univ. 11: 37-45
- Kim, J. K. 1990. Breeding of fermenting soy sauce by cell fusion. Bulletin of Food Technol. 3: 34-45.
- Kim, J. K., E. J. Lee, S. M. Park, and G. D. Sin. 1991. Indole contents and microorganisms producing indole of traditional Korean soybean paste. *Environ. Research of Yeungnam Univ.* 11: 31-36.
- Kim, J. K., E. J. Lee, W. D. Ji, and H. J. Kim. 1996. The screening method of microorganisms producing traditional Korean soy sauce and soybean paste. J. of Resource Develop. of Yeungnam Univ. 15: 29-37.
- Kim, J. K., H. G. Chang, J. S. Seo, and S. J. Lee. 1993. Character impact compounds in flavor of Korean soy sauce manufactured with the traditional and improved Meju. J. Microbiol. Biotechnol. 3: 270-276.
- 17. Kim, J. K., J. K. Chang, and B. K. Lee. 1984. Statistic

- analysis for relationship between gas chromatographic profiles of Korean ordinary soy sauce and sensory evaluation. *Kor. J. Food Sci. & Technol.* **16**: 242-250.
- Kim, J. K., J. S. Seo, H. G. Chang, and S. J. Lee. 1993. Characteristic flavors of Korean soybean paste. *J. Microbiol. Biotechnol.* 3: 277-284.
- Kim, J. K., S. K. Jang, S. Y. Kim, S. M. Park, and K. S. Kim. 1990. Distribution of volatile organic acids in traditional Korean soy sauce and microorganisms producing the organic acids. J. of Resource Develop. of Yeungnam Univ. 9: 63-69.
- Kim, S. T. and J. K. Kim. 1984. Principal component analysis for the relationship between gas chromatographic profiles of the Korean ordinary soybean paste flavor and sensory evaluation. J. of Gyengsang Nat. Univ. 23: 87-92.
- Kosuge, T. and H. Kamiya. 1962. Discovery of pyrazine in a natural product: tetramethylpyrazine from cultures of a strain of *Bacillus subtilis*. Nature 193: 776.
- Park, H. K., Y. L. Jo, J. K. Kim, and S. Y. Jeong. 1993. Transformation of *Bacillus* sp. SSA3 strain for Korean soy sauce fermentation by plasmid. *J. of Resource Develop. of* Yeungnam Univ. 12: 89-95.
- Sadtler. 1986. The sadtler standard gas chromatography retention index library, Sadtler research laboratories, Division of Biorad Laboratories Inc., U.S.A..
- Schlenk, J. and L. Gellerman. 1960. Esterification of fatty acids with diazomethane on a small scale. *Analytical Chem.* 32: 1412-1414.
- Schultz, T. H., R. A. Flath, T. R. Mou, S. H. Eggliug, and R. Teranishi. 1977. Isolation of volatile components from a model system. J. Agric. Food Chem. 25: 446-449.
- Sugawara, E., T. Ito, S. Odagiri, K. Kubota, and A. Ko-bayashi. 1985. Comparison of compositions of odor components of natto and cooked soybeans. *Agric. Biol. Chem.* 49: 311-317.
- Sugawara, E., T. Ito, S. Odagiri, K. Kubota and A. Kobayashi. 1990. Comparison of miso aroma components in preparations by different methods. *Nippon Nogeikagaku Kaishi* 64: 171-176.
- Yokotsuka, T., M. Sasaki, N. Nunomura, and Y. Asao. 1980. Shoyu no Kaori(1) [The flavor of Shoyu (1)]. Nippon Zycuzou Kyoukai Zasshi 75: 516-522.
- Yokotsuka, T., M. Sasaki, N. Nunomura, and Y. Asao. 1980. Shoyu no Kaori(2) [The flavor of Shoyu (2)]. Nippon Zycuzou Kyoukai Zasshi 75: 717-728.

(Received April 11, 1996)