Journal of Microbiology and Biotechnology
Vol. 6. No. 3, 194-201, 1996

Volatile Components in the Soy Sauce Manufactured by *Bacillus* Species and Fused Yeast

KIM, HAENG JA[1], EUN-JU LEE, OK-SUN SHIN, WON-DAE JI, MYEONG-RAK CHOI[2] AND JONG-KYU KIM*

Department of Applied Microbiology, Yeungnam University, Kyongsan 712-749, Korea
[1]Department of Economics Education, Kyeongsang National University, Jinju, Korea
[2]Department of Biological Engineering, Yosu Fisheries National University, Yosu, Korea

To develop a method appropriate for mass production in a factory, we manufactured soy sauce with *Bacillus* species SSA3-2M1 and fused ST723-F31 at 30°C with aeration of 1/3 vvm for 40 days. The flavor components extracted from the manufactured soy sauce were fractionated to neutral, acidic, basic and phenolic fraction and identified by GC-mass. Among the 60 kinds of identified flavor components, 16 and 23 components were detected in traditional Korean soy sauce and soybean paste, respectively. There were three peak regions that smelled like soy sauce with the GC sniffing test of flavor components and 2,6-dimethyl pyrazine, benzaldehyde, 2-methoxy phenol, phenol and benzeneethanol which were identified as character impact compounds of traditional Korean soy sauce and soybean paste were identified in the region that smelled like soy sauce. It is therefore considered possible to achieve mass production of soy sauce with standard quality by *Bacillus* species SSA3-2M1 and fused ST723-F31 in the factory.

The factors affecting the fermentation of traditional Korean soy sauce are material source, fermenting microorganisms and environmental conditions. Among these factors, fermenting microorganisms and conditions are the more important factors influencing the quality of soy sauce produced during fermentation.

The flavors of traditional Korean soy sauce have been studied. There have been the studies on the characteristic volatile components of traditional Korean soy sauce (2) and on the components of the volatile neutral fraction obtained from traditional Korean soy sauce (9). Among the forty four components, eleven components are known as available components by statistical analysis of their relationship between gas chromatographic profile of Korean ordinary soy sauce and sensory evaluation (7, 13). Other studies reported that *Bacillus* species play an important part in the production of taste and aroma components of traditional Korean soy sauce manufactured with traditional and improved Meju (6, 8). There have been many studies on the flavor of Japanese soy sauce (16, 21, 22). However, there are no reports on the flavor components of soy sauce manufactured by fermenting microorganisms, because it was not shown that the main fermenting microorganisms produced the taste and aroma of traditional soy sauce.

In this study, we manufactured soy sauce with *Bacillus* species SSA3-2M1 (12, 14, 15) and fused ST723-F31 (11), fermenting microorganisms of soy sauce. *Bacillus* species SSA3-2M1 was bred from *Bacillus* species SSA3 isolated and selected from traditional Korean soy sauce. Fused ST723-F31 was obtained from cell fusion between *Zygosaccharomyces rouxii* and *Torulopsis versatilis*, the main fermenting yeasts of soy sauce.

In order to investigate the similarity between soy sauce manufactured by *Bacillus* species SSA3-2M1 and fused ST723-F31 and traditional Korean soy sauce, the volatile components were analyzed and compared with flavor components of traditional Korean soy sauce and soybean paste and Japanese fermented soy sauce. The analysis of taste components and organoleptic tests of flavor were also carried out as described in the previous paper (3).

Therefore, the final object of this study was to develop a convenient method of manufacturing soy sauce of standardized quality using a few fermenting microorganisms.

MATERIALS AND METHODS

Strains and Preparation of Soy Sauce

We used *Bacillus* species SSA3-2M1 which was isolated and bred from traditional Korean soy sauce and

*Corresponding author
Key words: soy sauce fermenation, *Bacillus* species SSA3-2M1, fused ST723-F31, flavor components

ST723-F31 cell fused between *Zygosaccharomyces* rouxii and *Torulopsis versatilis* which are fermenting yeasts of soy sauce. The preparation of soybean extract medium and the process of fermentation were the same as described in the previous paper (3). After fermentation had been conducted in the jar fermentor (B.E. Marubishi Co., Ltd) at 30°C, aeration of 1/3 vvm and agitation of 50 rmp for 8 days, the culture was stationary fermented at 30°C for 32 days.

Extraction and Fractionation of Volatile Components

After the soy sauce was fermented in the Jar fermentor for 8 days, the culture was stationary fermented again for 32 days at 30°C. An improved Nikerson and Nikens' simultaneous steam distillation and extraction apparatus was used to extract the volatile components of soy sauce (19). Purified diethyl ether was used for extraction solvent. The process of extraction was as follows; put the sample and solvent in the sample port and the solvent port, respectively, and then extracted the volatile components for more than two hours by increasing the temperature of the sample port to boiling point after circulating the solvent preliminary. The whole extracted flavors were fractionated with diethyl ether by adjusting the pH according to the Fujimaki's method (1) to obtain the basic, acidic, phenolic and neutral fractions. Anhydrous Na_2SO_4 at 4°C was added overnight to each fraction to remove moisture and the fractions were concentrated to a final 50 μl by using N_2 gas to obtain the samples of GC analysis.

GC Sniffing Test of Volatile Components

We performed the GC sniffing test to find the flavor and pattern of the peaks of each volatile component. The GC sniffing test was carried out with prepared gas chromatography which had been designed to allow a part of the volatile components to flow through the column to the detector and the other parts to flow to the outside of gas chromatography. For the GC sniffing test, the conditions of preparative gas chromatography were as follows: Instrument, Shimadzu GC-8A; column, chemically bonded fused sillica capillary column(CBP20-W12-100); injection & detection temp., 240°C; column temp., 60-200°C (10°C/min); carrier gas, N_2(8 ml/min); range, 10^3; detector, FID.

Identification of Volatile Components

After the each peak of volatile aroma components was analyzed by GC sniffing test, the mass spectrum of each of the volatile aroma components was obtained by the GC-mass and the aroma components were identified by GC-mass and Kovat's index (17, 20). The conditions of GC and GC-mass were as follows: Instrument, GC-HEWLETT-PACKARD 5890, Mass-KRATOS Inc. CENCEPT SERIES-I(England); column, HP-FFAP 30 m × 0.33 μm × 0.2 mm; injector temp., 230°C; detector temp., 250°C; temp. program, 45°C for 2 min., 45-220°C(15°C/min.) and then 220°C for 11.4 min; carrier gas, He (5 ml/min.); electron voltage, 1100 eV; split ratio, 10:1.

RESULTS AND DISCUSSION

The gas chromatogram of the whole volatile flavor components of soy sauce produced by *Bacillus* species SSA3-2M1 and fused ST723-F31 are shown in Fig. 1, and the identified flavor components by GC-mass and Kovat's index are listed in Table 1.

Among the 60 flavor components, 57 components were identified as whole volatile flavors and there were three kinds of hydrocarbons, four kinds of alcohols, eleven kinds of ketones, nine kinds of pyrazines, three kinds of amides and seventeen others.

Of the identified flavor components, seventeen and tweniy three components were the same flavor components identified in the traditional Korean soy sauce and soybean paste. In traditional Korean soy sauce and soybean paste, 62 and 60 flavor components have been identified, respectively (10).

The components identified both in traditional Korean soy sauce and soybean paste were acetic acid · ethyl ester, pentanal, pyrazine, methyl pyrazine, 2,5-dimethyl-pyrazine, 2,3-dimethyl pyrazine, trimethyl pyrazine, acetic acid, benzaldehyde, 2-methoxy phenol(guaiacol), benzeneethanol, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone

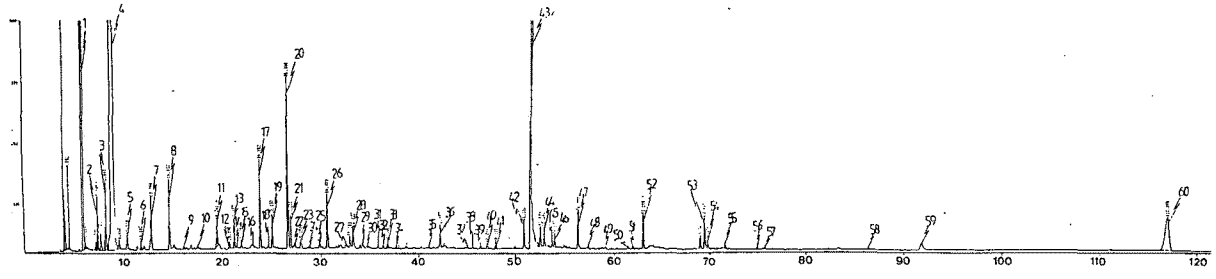

Fig. 1. Gas chromatogram of the whole flavor components obtained from soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

Table 1. Volatile flavor components in the soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

Peak No.	Identified components	Aroma	TKSS	TKSP	JPSS
1	Propanal				+
2	Acetic acid, ethyl ester		+	+	+
3	3-Methyl butanal (isovaleraldehyde)				+
4	Unknown				
5	Pentanal		+	+	+
6	3-Methyl-2-pentanone			+	+
7	1-Propanol				+
8	Hexanal			+	+
9	2-Methyl-1-propanol			+	+
10	Xylene			+	+
11	2-Heptanone			+	
12	3-Methyl-1-butanal (isopentyl alcohol)				
13	Pyrazine		+	+	+
14	N-Methyl-N-propyl-butylamine		+		
15	2-Pentyl-furan				
16	3-Octanone				+
17	Methyl-pyrazine		+	+	+
18	2,5-Dimethyl-pyrazine		+	+	
19	3-Hydroxy-2-butanone (acetoin)	savory			+
20	4,6-Dimethyl-pyramidine	and weak			
21	2,6-Dimethyl-pyrazine	soy sauce		+	+
22	Ethyl-pyrazine	aroma	+		+
23	2,3-Dimethyl-pyrazine		+	+	+
24	4-Hydroxy-4-methyl-2-pentanone				
25	2-Nonanone				
26	Trimethyl-pyrazine		+	+	+
27	3-ethyl-2,5-dimethyl-pyrazine			+	+
28	Acetic acid		+	+	+
29	2-Ethenyl-6-methyl-pyrazine				
30	3-Nonen-2-one				
31	Benzaldehyde		+	+	+
32	Propannoic acid (propionic acid)				+
33	1-Octanol				
34	Propanedioic acid, diethyl ester				+
35	Dihydro-5-methyl-2(3H)-furanone				
36	2-Acethylthiazole				
37	3-Mecraptopyridine				
38	N-(2-Methylpropyl)-acetamide		+		
39	Naphthalene			+	+
40	N,N-Dibutyl-formamide				
41	N,N-Dibutyl-acetamide				
42	2-Methoxy-phenol (guaiacol)		+	+	+
43	Unknown	savory			
44	Benzenethanol (penethyl alcohol)	and burned	+	+	
45	α-Ethylidene-benzenacetaldehyde	soy sauce		+	
46	Unknown	aroma			
47	Phenol		+		+
48	4-Hydroxy-2(5)-ethyl-5(2)-methyl-3(2H)-furanone (HEMF)		+	+	+
49	5-Methyl-2-phenyl-2-hexanal				
50	5-Hexyldihtdro-2(3H)-furanone			+	
51	2-Methoxy-4-(2-propenyl)-phenol(eugenol)	savory			
52	1-Ethoxy-4-ethyl-benzene	and offensive			
53	1,2-Benzenedicarboxylic acid, diethyl ester	soy sauce			
54	γ-Dodecalatone	aroma			
55	1H-Indole		+	+	
56	(Z)-9,17-Octadecadienal				+
57	4-Hydroxy-3-methoxy-benzaldehyde				
58	Hexanedioic acid, dioctyl ester				
59	Tridecanoic acid				
60	1,2-Benzenedicarboxylic acid, bis (2-ethyl-)				

TKSS, Traditional Korean Soy Sauce; TKSP, Traditional Korean Soybean Paste; JPSS, Japanese Soy Sauce; +, Present.

Table 2. Volatile flavor components of the neutral fraction in the soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

Peak No.	Identified components	Aroma	TKSS	TKSP	JPSS
1	Unknown				
2	1,1-Diethoxy-ethane				+
3	3-Methyl-1-butanal (isovaleraldehyde)			+	
4	Methanethial, homopolymer				
5	Unknown				
6	Hexanal (carproic acid)			+	+
7	Undecane				
8	2-Methoxy-methanol (methyl cellosolve)				
9	2-Heptanone				
10	3-Methyl-1-butanol (isoamyl acohol)		+	+	+
11	2-Pentyl-furan				
12	1-Octane-3-one				
13	3-Hydroxy-2-butanone (acetoin)				+
14	3,4-Dimethyl-1-pentanol				+
15	Acetic acid		+	+	+
16	Unknown				
17	Benzenacetaldehyde	soy sauce aroma	+	+	+
18	Unknown				
19	Benzenethanol (penethyl alcohol)			+	
20	1,2-Benzenedicarboxylic acid, diethyl ester		+		
21	(Z,Z)-9,12-Octadecanoic acid				+
22	Unknown				
23	1,2-Benzenedicarboxylic acid, bis (2-ethyl)				

TKSS, Traditional Korean Soy Sauce; TKSP, Traditional Korean Soybean paste; JPSS, Japanese Soy Sauce; +, Present.

Table 3. Volatile flavor components of the acidic fraction in the soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

Peak No.	Identified components	Aroma	TKSS	TKSP	JPSS
1	Propanal				+
2	Acetic acid, ethyl ester		+	+	+
3	Unknown				
4	Methyl-benzen (toluene)				+
5	Acetic acid	soy sauce aroma	+	+	+
6	Benzaldehyde		+	+	+
7	Propanoic aicd				+
8	Propanedioic aicd, diethyl ester				+
9	Unknown				

TKSS, Traditional Korean Soy Sauce; TKSP, Traditional Korean Soybean paste; JPSS, Japanese Soy Sauce; +, Present.

(HEMF) and 1H-indole (21, 22). 27 components were the same flavor components identified in Japanese soy sauce. Japanese soy sauce had been identified 267 types of flavor components. Among the components, HEMF was identified as a character impact compound of Japanese soy sauce which has a particular sweet flavor similar to caramel (14). Because the primary fermentation of soy sauce yeasts, *Zygosaccharomyces rouxii* and *Torulopsis* species produce it, it was considered that HEMF was produced by fused ST723-F31. The components in traditional Korean soy sauce and soybean paste as well as Japanese soy sauce were acetic acid · ethyl ester, pentanal, pyrazine, methyl pyrazine, 2,3-dimethyl pyrazine, benzaldehyde, 2-methoxy phenol (guaiacol) and HEMF.

We investigated the peak region that smelled like traditional Korean soy sauce flavor using the GC sniffing test using preparative GC. There were three regions that smelled like soy sauce. The ingredients of one region were 2-methoxy phenol(guaiacol) of peak no. 42, unknown of peak no. 43, benzeneethanol of peak no. 44, α-ethyliene benzeneacetaldehye of peak no. 45, unknown of peak no. 46, phenol of peak no. 47 and HEMF of peak no. 48. Among the components, HEMF, benzeneethanol, 2-methoxy phenol and phenol were founded as character impact compounds of traditional Korean

Table 4. Volatile flavor components of the basic fraction in the soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

Peak No.	Identified components	Aroma	TKSS	TKSP	JPSS
1	Propanal				
2	Acetic acid, 1-methylethyl ester				+
3	Unknown				
4	1-Propanal				+
5	Undecane				
6	2-Methoxy-ethanol (methyl cellosolve)				
7	Trimethyl oxazole				
8	Pyrazine		+	+	
9	Methyl-pyrazine		+	+	+
10	2,4-Dimethyl pyramidine				
11	2,6-Dimethyl pyrazine		+	+	+
12	Ethyl pyrazine		+		+
13	2,3-Dimethyl pyrazine	savory	+	+	+
14	4-Hydroxy-4-methyl-2-pentanone	soy sauce			
15	2,4,5-Trimethyl thiazole	aroma			
16	2-Ethyl-6-methyl-pyrazine				+
17	Trimethyl pyrazine		+	+	+
18	Ethenyl-pyrazine		+		
19	3-Ethyl-2,5-dimethyl-pyrazine			+	+
20	Tetramethyl-pyrazine			+	+
21	2-Acetylthiazole				
22	Unknown				
23	1,2-Benzenedicarboxylic acid, diethyl ester				
24	1,2-Benzenedicarboxylic acid, bis (2-ethyl)				

TKSS, Traditional Korean Soy Sauce; TKSP, Traditional Korean Soybean Paste; JPSS, Japanese Soy Sauce; +, Present.

Table 5. Volatile flavor components of the phenolic fraction in the soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

Peak No.	Compositions	Aroma	TKSS	TKSP	JPSS
1	Propanal				+
2	Acetic acid, ehtyl ester		+	+	+
3	Unknown				
4	Benzen				+
5	Unknown				
6	Undecane				
7	1-Butanol			+	+
8	2-Methoxy-ethanol (methyl collosolve)				
9	2-Methyl dodecane				
10	3-Hydroxy-2-butanone (aectoin)				+
11	2,5-Dimethyl-pyrazine	sour	+	+	+
12	Trimethyl-pyrazine	and weak		+	+
13	Acetic acid	soy sauce	+	+	+
14	2-Ethyl-1-hexanol (2-ethylhexyl alcohol)	aroma			
15	Benzaldehyde		+	+	+
16	Propanedioic acid, dithyl ester				+
17	2,3-Butanediol (2,3-butylene glycol)				+
18	Unknown				
19	Unknown				
20	2-Methoxy phenol (guaiacol)	sour	+	+	+
21	Unknown	and savory			
22	Unknown	soy sauce			
23	3-Hydroxy-2-methyl-4H-pyrane-4-one	aroma			+
24	Phenol		+	+	+
25	Unknown	offensive			
26	2-Methoxy-4-(2-propenyl)-phenol	aroma		+	
27	2,3,5,6-Tetramethyl-phenol		+		

TKSS, Traditional Korean Soy Sauce; TKSP, Traditional Korean Soybean Paste; JPSS, Japanese Soy Sauce; +, Present.

soy sauce and soybean paste (10). Another region smelled that like savory and weak offensive traditional soy sauce flavor and the gradients were 5-hexyldihydro-2(3H)-furanone, 2-methoxy-4-(2-propenyl) phenol, 1-ethoxy- 4-ethyl-benzene, 1,2-benzenedicarboxylic acid · diethyl ester, γ-dodecalactone and 1H-indole of peak no. 50-55 respectively. We presumed that the offensive soy sauce flavor was due to indole which was identified as an off-flavor of soy sauce (5). Many kinds of pyrazine were identified in the other regions that smelled like savory and weak soy sauce flavor overall and made up the ingredients of peak no. 13 to 31. These pyrazines were found to be character impact compounds of Japanese Natto (18) and were produced in small amounts while boiling soybean. There are large amounts of pyrazines in traditional Korean soy sauce and soybean paste. It has been reported that the *Bacillus* species produce many kinds of pyrazine (4). 2,6-Dimethyl pyrazine of peak no. 21 and benzaldehyde of peak no. 31 were identified as character impact compounds of traditional Korean soy sauce and soybean paste (10).

To identify flavors in detail, the whole flavor was fractionated into neutral, acidic, basic and phenolic fractions. The identified neutral, acidic, basic and phenolic fraction are listed in Table 2, 3, 4 and 5. And each gas chromatogram are shown in Fig. 2, 3, 4 and 5.

In the neutral fraction, 23 kinds of flavor components were identified and the ingredients of the peak region that smelled like sour soy sauce were benzeneacetaldehyde of peak no. 17, unknown of peak no. 18 and benzeneacetaldehyde of peak no. 19. Benzeneacetaldehyde was found to be a character impact compound in traditional Korean soy sauce and soybean paste (10).

In the acidic fraction, 9 kinds of flavor components were identified and the ingredients of the regions that smelled like soy sauce aroma were acetic acid of peak no. 5 and benzaldehyde of peak no. 6. Benzaldehyde was identified as a character impact compound that smelled like soy sauce flavor in traditional Korean soy sauce and soybean paste (10).

In the basic fraction, 24 kinds of flavor components were identified and pyrazines in the region of peak no. 8-20 had an overall savory soy sauce aroma. These pyrazines are contained in traditional Korean soy sauce, soybean paste and Japanese soy sauce and produced by *Bacillus* species(4).

In the phenolic fraction of Table 5, 27 kinds of flavor components were identified. There were two regions that

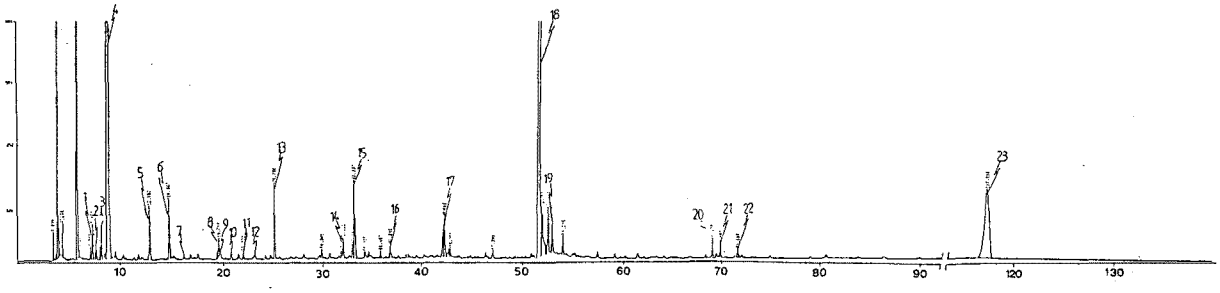

Fig. 2. Gas chromatogram of flavor components in the neutral fraction obtained from soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

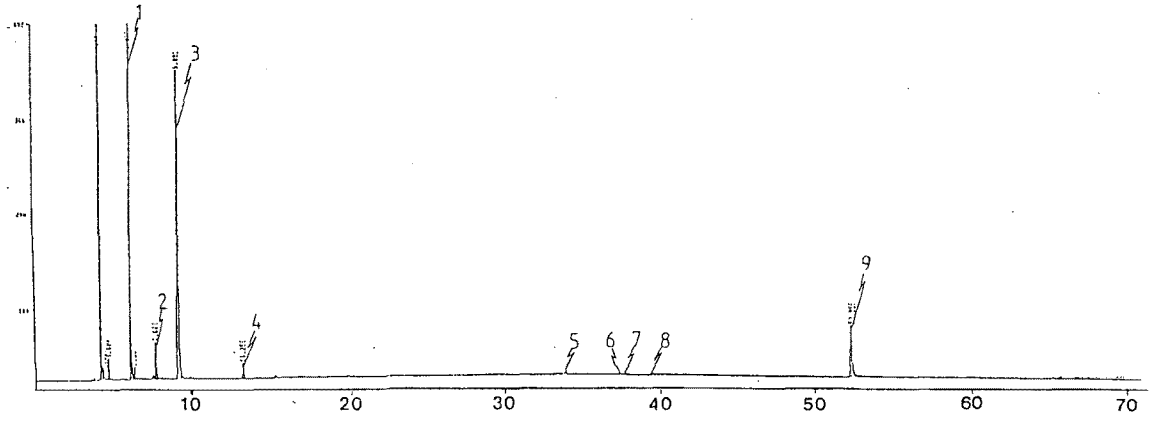

Fig. 3. Gas chromatogram of flavor components in the acidic fraction obtained from soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

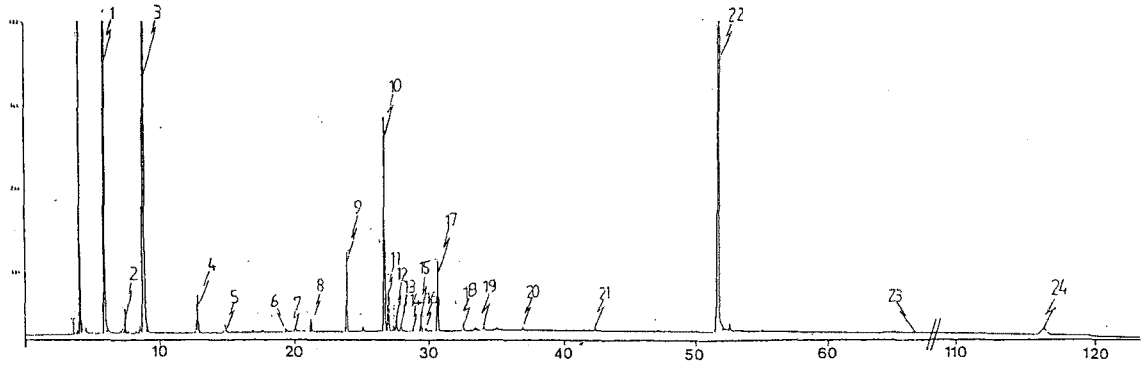

Fig. 4. Gas chromatogram of flavor components in the basic fraction obtained from soy sauce fermented by *Bacillus* species SSA 3-2M1 and fused ST723-F31.

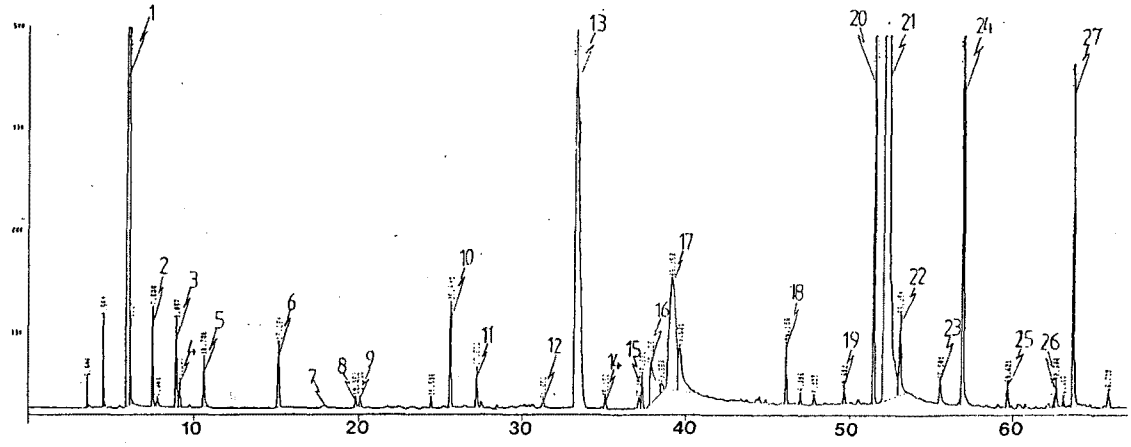

Fig. 5. Gas chromatogram of flavor components in the phenolic fraction obtained from soy sauce fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31.

smelled like soy sauce. The ingredients of one region were 2,5-dimethyl pyrazine of peak no. 11, trimethyl pyrazine of peak no. 12, acetic acid of peak no. 13, benzaldehyde of peak no. 15 and 2-ethyl-1-hexanal of peak no. 14. and smelled like sour and weak soy sauce. The ingredients of the other region were 2-methoxy phenol (guaiacol) of peak no. 20, unknown components of peak no. 21 and 22. The guaiacol is in traditional Korean soy sauce (10) and it's structure is similar to 4-ethyl guaiacol which is produced from ferulic acid by *Torulopsis* species as character impact compound of Japanese soy sauce.

When we manufactured soy sauce using *Bacillus* species SSA3-2M1 and fused ST723-F31, an aroma like traditional Korean soy sauce was produced, and benzaldehyde, 2-methoxy phenol and benzeneethanol identified as character impact compounds of traditional Korean soy sauce were produced. Thses results and our previous paper on taste components indicate that both *Bacillus* species SSA3-2M1 and fused ST723-F31 could be used to get the mass production of soy sauce in a factory.

Acknowledgement

This study was supported by a grant from the Korean Research Foundation.

REFERENCE

1. Fujimaki, M., T. Tsugita, and T. Kurata. 1977. Fractionation and identification of volatile acids and phenols in the steam distillate of rice bran. *J. Agric. Biol. Chem.* **41**: 1721-1725.
2. 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.
3. Kim, H. J., E. J. Lee, O. S. Shin, R. M. Choi, and J. K. Kim. 1996. The characteristics components of traditional Korean soy sauce manufactured by *Bacillus* species SSA3-2M1 and fused ST723-F31. *J. Microbiol. Biotechnol.* submitted for Publication.

4. Kim, H. S. and Kim, J. K. 1991. Pyrazine in traditional Korean soybean paste and soybean paste produced by *Bacillus* sp. *J. Environ. Research of Yeungmam Univ.* **11**: 37-45.
5. 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. *J. Enviro. Research of Yeungmam Univ.* **11**: 31-36.
6. Kim, J. K., H. G. Chang, J. S Seo, and S. J. Lee. 1993. Character impact compounds in flavors of Korean soy sauce manufactured with the traditional and the improved Meju. *J. Microbiol. Biotechnol.* **4**: 270-276.
7. Kim, J. K., J. K. Chang, and B. K. Lee. 1984. Statistical analysis for relationship between gas chromatographic profile of Korean ordinary soy sauce and sensory evaluation. *J. Food Sci. Technolnol.* **16**: 242-250.
8. Kim, J. K., S. Y. Chung, J. Y. Song, and J. G. Jang. 1986. Characteristics of aroma produced by microorganisms during fermentation of ordinary Korean soy sauce. *J. of Resource Develop. of Yeungnam Univ.* **5**: 83-39.
9. Kim, J. K., S. Y. Kim, W. K. Ki, and S. Y. Chung. 1990. Components of volatile neutral fraction obtained from traditional Korean soy sauce. *Rural Develop. Review Kyeongsang Nat. Univ.* **8**: 41-53.
10. Kim, J. K., W. O. Ji, E. J. Lee, H. J. Kim, J. S. Seo, and H. G. Jang 1996. Aroma components of traditional Korean soy sauce and soybean paste fermented with the same Meju. *J. Microbiol. Biotechnol.* Submitted for publication.
11. Kim, J. K. and E. J. Lee. 1993. Development of a novel yeast strain which ferments soy sauce by protoplast fusion. *J. Microbiol. Biotechnol.* **3**: 24-30.
12. Kim, J. K. and S. D. Kim. 1988. Genetic Breeding of Korean soy sauce fermenting *Bacillus* by UV mutation. *J. Korean Agric. Chem. Soc.* **31**: 346-350.
13. Kim, J. K. and S. T. Kim. 1984. Principle components analysis for the relationship between gas chromatographic profile of Korean ordinary soy sauce and sensory evaluation. *J. of Kyeongsang Nat. Uni.* **23**: 81-86.
14. Kwon, O. J., J. K. Kim, and Y. G. Chung. 1986. The characteristics of bacteria isolated from ordinary Korean soy sauce and soybean paste. *J. Korean Agric. Chem, Soc.* **29**: 422-428.
15. Kyung, K. W., J. K. Kim, D. H. Kang, and Y. U. Cho. 1987. Development of exellent mutants for manufacture of ordinary Korean soy sauce and soybean paste. *Kor. J. Appl. Microbiol. Biotechnol.* **15**: 21-28.
16. Nobutake, N., M. Sasaki, Y. Asao, and T. Yokotsuka. 1976. Isolation and identification of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-furanone as a flavor components in shoyu(soy sauce). *J. Agr. Biol. Chem.* **40**: 491-495.
17. Sadtler. 1986. *The Sadtler standard gas chromatography retention index library*. Sadlter Research Lab. 1-4.
18. Tetsuo I., E. Sugawara Y. Sakurai, S. Takeyama H. Uchizawa, and S. Odagiri. 1987. Cluture media for pyrazines production by a commercial Natto *Bacillus. Nippon Nogeikagaku Kaishi* **61**: 963-965.
19. Thomas H. S., A. F. Robert, T. R. Mon, S. B. Eggling, and R. Teranish. 1977. Isolation of volatile components from a model system. *J. Agric. Food Chem.* **25**: 446-449.
20. Walter, J. and T. Shibamoto. 1980. *Qulatative analysis of flavor fragrance volatiles by glass capillary gas chromatography retention index*. Academic press.
21. 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.
22. 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)