Journal of Microbiology and Biotechnology
Vol. 3, No. 4, 270-276, 1993

Character Impact Compounds in Flavors of Korean Soy Sauce Manufactured with the Traditional and the Improved Meju

KIM, JONG-KYU*, HO-GEUN CHANG, JAE-SOON SEO AND SANG-JUN LEE

Department of Applied Microbiology, Yeungnam University, Kyongsan 712-749, Korea

We characterized the character impact compounds of flavors of the fermented Korean soy sauce manufactured with both the traditional and the improved Meju made with different strains. The whole flavor samples were obtained by extracting each volatile flavor phase from both the traditional and the improved soy sauce. To get more detailed information, each whole volatile flavor was further fractionated into the basic, acidic, phenolic and neutral fractions. Each separated peak from the whole and fractionated flavor samples on gas chromatogram was identified by GC/MS and Kovat's retention index, and likewise the aroma of each peak was investigated by a sniffing test with the exercised panel. We were able to identify 15 groups of ingredients with the characteristic soy sauce aroma from the soy sauce made with the traditional Meju and 6 groups from the soy sauce manufactured with the improved Meju made with *Aspergillus oryzae*. The character impact compounds the two soy sauces were different from each other.

Traditionally, Korean soy sauce has been manufactured with a traditional Meju which was molded with the cooked and crushed soy bean in a global or hexahedron form and then exposed to natural strain conditions. From 1960 to 1970, there has been a trend toward making soy sauce with improved Meju made with *Aspergillus oryzae* and other strains. Generally the flavors of fermented products are changed by the factors of the used material source which are applied, microorganism and the fermenting process. Therefore, it was presumed that the traditional soy sauce manufactured with the traditional Meju has a diverse spectrum of volatile ingredients according to the diversity of microflora in Meju. Although, most of the volatile flavors of soy sauce can affect the aroma of the soy sauce, it has been identified that microorganisms producing the flavor character impact compounds during the fermentation of traditional Korean soy sauce are the yeasts *Torulopsis versatilis*, *Torulopsis dattila*, and *Zygosaccharomyces rouxii* and the bacteria *Bacillus* species (10, 12, 14). A soy sauce with the similar flavor character impact compounds of traditional Korean soy sauce was manufactured by using the three microorganisms, only (8). We learned that the flavor of traditional Korean soy sauce depended upon many volatile components. These components were identified by a gas chromatogram and by analyzing the data from the sniffing test (9). But, for the character impact compounds, it has been known that the mixture of 0.6ppm of dimethyl trisulfide and 30 ppm of benzeneacetaldehyde represents the soy sauce flavor (13), and that the flavor character impact compounds are dimethyl trisulfide, benzeneactealdehyde and benzeneethanol (5).

By obtaining gas chromatograms in conjunction with the sniffing tests for the components of each peak, after extracting and concentrating the volatile ingredient of the traditional Korean soy sauce, we were able to confirm that many more peaks had the unique flavors of soy sauce (7). On this point, we identified and discussed the flavor character impact compounds of flavors of soy sauce manufactured with both traditional and improved Meju, respectively, in this report.

MATERIALS AND METHODS

Materials

We used commercially available traditional Meju which was modeled into hexagonal bricks and then exposed to the natural strains, and also used commercially availa-

*Corresponding author

Key words: character impact compounds, traditional Meju, improved Meju, soybean paste

ble, improved Meju manufactured with *Aspergillus oryzae*.

Fermentation

For the primary fermentation, a Meju corresponding to 10 kg of soybean and 18L of 20% salt solution were mixed and then fermented for 2 months, and the aqueous layer of the primary fermented products was further subjected to a second fermentation of three months at room temperature to manufacture soy sauce. In quality, the soy sauce made with the traditional Meju had the traditional Korean soy souce flavors, whereas the soy sauce made with the improved Meju did not have any of the typical traditional Korean soy sauce flavor.

Analytical Methods

An improved Nikerson and Nikens' simultaneous steam distillation and extraction apparatus (18) was used to extract the volatile components. We put the sample in the sample port and the extractable solvent of ethyl ether in the solvent port, respectively, and then extracted the volatile components for two hours by increasing the temperature of the sample port to a boiling point after circulating the solvent preliminarily. The concentrated whole flavors of the extracted solution was extracted with ethyl ether by adjusting the pH according to the Fujimaki's method (3) to obtain the basic, acidic, phenolic, and neutral fractions which were then added with anhydrous Na_2SO_4 and exposed at 4℃ overnight to get rid of humidity. Among the fractions, the acidic fraction was chosen and further methylated by Schlenk's Diazomethane method (17), concentrated to about 2 ml of volume by a rotary evaporator at atmospheric pressure, transferred to vials, reconcentrated to 50 μl by using N_2 gas and then used as a sample for the sniffing tests of the components for each gas chromatograpic peak (GC sniffing test) and identification of the volatile components.

For the GC sniffing test, we used the prepared gas chromatograph which had been designed to allow parts of the volatile ingredients to flow to the column of the detector and other parts to flow to the outside of the gas chromatograph. The gas chromatography used was a Shimadzu GC-8A, the column used was a wide bore column of CBP-W12-100 (Chemically bounded fused silica capillary column), and the oven temperature was programed to increase from 60℃ to 200℃ by 10℃ /min.. The temperature of the injector and the detector was 240℃, the carrier gas was N_2 (8 ml/min.), and the detector was an FID.

On the other hand, the identification of volatile ingredients was carried out by a computer library search based on the mass spectrum of GC/MS and Kovat's retention index (4, 15). The GC-mass used was a HEWLETT PACKARD GC II 5980 combined with a HEWLETT PACKARD 5988 MS, the column used was an HP-FFAP (25 m×0.33 μm×0.2 mm), the injector temperature was 200℃, and the detector temperature was 230℃. The oven temperature was kept at 45℃ for 2 min. and increased from 45℃ to 220℃ by 10℃ /min., the carrier gas was He (0.9 ml/min.), the electron voltage was 70 eV, and the split ratio was 10 : 1.

Identifying of the Character Impact Compounds

We identified the aroma of the peak of each volatile ingredients on the gas chromatogram by using the wide bore column. We did the same peak identification with the gas chromatogram using the narrow bore column. Then by using the reciprocal pattern of gas chromatogram with the narrow bore column, we identified the volatile ingredients and by using the internal standard, butyl hydroxy toluene retention index.

RESULTS AND DISCUSSION

Character Impact Compounds of Korean Soy Sauce Manufactured with the Traditional Meju

The drawings of the gas chromatogram used with the narrow bore column for the whole flavor of the traditional soy sauce and for the basic, acidic, phenolic and neutral fractions obtained from the whole flavor of the traditional soy sauce are represented in Fig. 1~5 and the character impact compounds listed in Table 1.

In whole flavor, there were three regions that smelled like the soy sauce as shown in Fig. 1. The ingredients

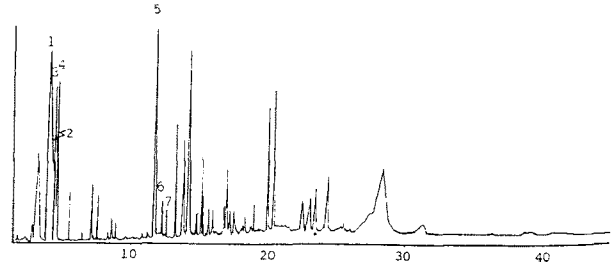

Fig. 1. Gas chromatogram of whole volatile components of Korean soy sauce produced by traditional Meju.
Data of sniffing test: Peak no. 1 and 2, a sweet and weak soy sauce odor; 3 and 4, a savory soy sauce odor; 5~7, a sweet and weak soy sauce odor. Gas chromatographic (GC) conditions: GC, HEWLETT-PACKARD 5980 II GC; column, HP-FFAP (25 m×0.33 μm×0.2 mm); inj. temp., 200℃; det. temp., 230℃; oven temp., 45℃ for 2 min., 45~220 (10℃ min.) and 220℃ for 15 min.; carrier gas, He (0.9 ml/min.); split ratio, 10 : 1.

Table 1. Character impact compounds of Korean soy sauce manufactured with traditional Meju

Peak no.	Retention time (min.)	Components	Contents (ppm)	Aroma
1	4.06	Butanoic acid, methyl ester	44.70	a sweet and weak soy sauce odor
2	4.35	Unknown	+(?)	
3	4.55	Butanoic acid, 2-methyl-, methyl ester	15.70	a savory soy sauce odor
4	4.67	Butanoic acid, 3-methyl-, methyl ester	23.40	
5	11.78	2-Furancarboxaldehyde	30.40	a sweet and weak soy sauce odor
6	12.24	Unknown	+(?)	
7	12.57	Benzaldehyde	1.30	
8	9.55	2-Furancarboxaldehyde	0.10	a weak soy sauce odor
9	10.61	2-Furancarboxaldehyde, 5-methyl-	0.01	a weak soy sauce odor
10	10.76	Benzaldehyde, 3-hydroxy-	0.01	
11	10.88	Ethane, 1-(2-pyridinyl)-*	0.01	
12	11.25	2-Furanmethanol	0.01	
13	13.69	Ethanol, 2-(2-ethoxyethoxy)-*	0.07	a sourish and weakly soy sauce odor
14	13.82	Benzoic acid, methyl ester*	0.02	
15	14.12	2-Furanmethanol	0.01	
16	15.51	2-Furanmethanol, tetrahydro-	0.08	a weakly sourish and savory soy sauce odor
17	15.73	Nonanoic acid, 2,4,6-trimethyl-, methyl ester	0.01	
18	15.83	Unknown	0.01	
19	13.51	Butanoic acid	0.02	a weak soy sauce odor
20	14.04	Unknown	0.01	
21	14.19	2-Furanmethanol	0.04	
22	16.64	Phenol, 2-methoxy-	0.03	a weak soy sauce odor
23	18.15	Phenol	0.02	a bran soup and weak soy sauce odor
24	18.53	1H-Pyrrole-2-carboxaldehyde*	0.01	
25	20.27	Phenol, 2,3,5,6-tetramethyl-	0.07	a soy sauce odor
26	4.47	Unknown	0.18	an unpleasant soy sauce odor
27	12.24	1H-Pyrrole	0.01	a weakly savory and soy sauce odor
28	12.53	Benzaldehyde	0.01	
29	13.17	2-Furancarboxaldehyde, 5-methyl-	0.06	
30	14.15	Benzeneacetaldehyde	0.23	a weak soy sauce odor
31	18.55	1H-Pyrrole-2-carboxaldehyde	0.01	a boiled soy sauce odor
32	18.93	3-Tert-butyl-4-methoxyphenol	0.02	
33	19.00	Unknown	+(?)	

*: Assumed components by GC-mass, +(?): Incomputable contents. The content of each component was calculated on the basic of the peak area on gas chromatogram.

were mainly the derivatives of butanoic acid, and 2-furancarboxaldehyde and benzaldehyde also played an important role.

In the basic fraction, there were two regions that smelled like the soy sauce (see Fig. 2) and the ingredient of one region was 2-furancarboxaldehyde alone, and the ingredients of the other region were 2-furancarboxaldehyde, 5-methyl-, benzaldehyde, 3-hydroxy-, ethane, 1-(2-pyridinyl)- and 2-furanmethanol represented the soy sauce aroma as shown in Table 1.

In the acidic fraction, there were two regions that smelled like the soy sauce flavor (see Fig. 3) and the ingredients of one region were ethanol, 2-(2-ethoxyethoxy)-, benzoic acid, methyl ester, 2-furanmethanol, and the

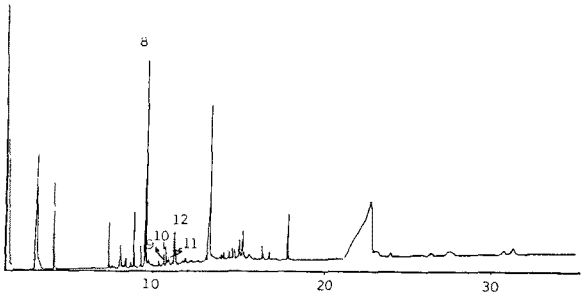

Fig. 2. Gas chromatogram of the basic fraction fractionated from whole volatile components of Korean soy sauce produced by traditional Meju.
Data of sniffing test: Peak no. 8, a weak soy sauce odor; 9~12, a weak soy sauce odor. GC conditions were same as described in Fig. 1.

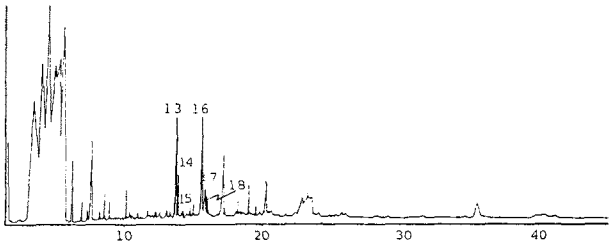

Fig. 3. Gas chromatogram of the acidic fraction fractionated from whole volatile components of Korean soy sauce produced by traditional Meju.
Data of sniffing test: Peak no. 13~15, a sourish and weakly soy sauce odor; 16~18, a weakly sourish and savory soy sauce odor. GC condition were same as described in Fig. 1.

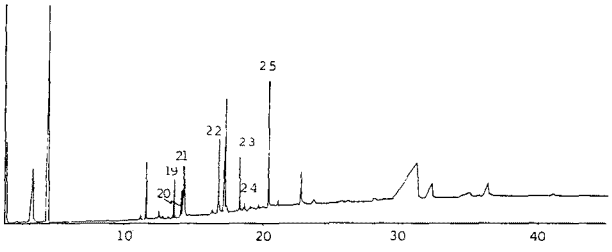

Fig. 4. Gas chromatogram of the phenolic fraction fractionated from whole volatile components of Korean soy sauce produced by traditional Meju.
Data of sniffing test: Peak no. 19~21, a weak soy sauce odor; 22, a weak soy sauce odor; 23 and 24, a bran soup and weak soy sauce odor; 25, a soy sauce odor. GC conditions were same as described in Fig. 1.

ingredients of the other region were 2-furanmethanol, tetrahydro-, nonanoic acid, 2,4,6-trimethyl-, methyl ester and one unknown compound as listed in Table 1.

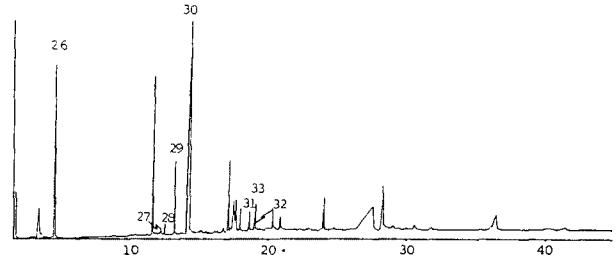

Fig. 5. Gas chromatogram of the neutral fraction fractionated from whole volatile components of Korean soy sauce produced by traditional Meju.
Data of sniffing test: Peak no. 26, an unpleasant soy sauce odor; 27~29, a weakly savory and soy sauce odor; 30, a weak soy sauce odor; 31~33, a boiled soy sauce odor. GC conditions were same as described in Fig. 1.

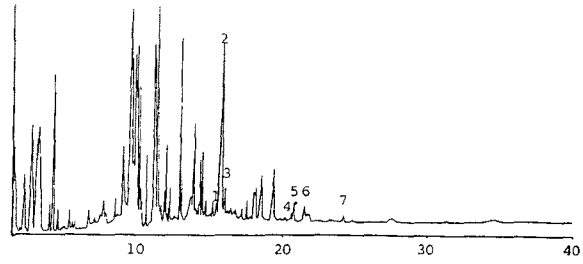

Fig. 6. Gas chromatogram of whole volatile components of Korean soy sauce produced by improved Meju.
Data of sniffing test: Peak no. 1~3, a weakly savory soy sauce odor; 4~7, a weakly unpleasant soy sauce odor. GC conditions were same as described in Fig. 1.

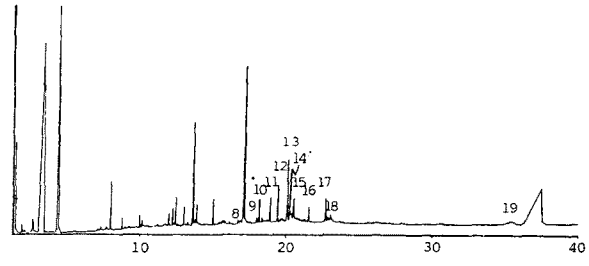

Fig. 7. Gas chromatogram of the acidic fraction fractionated from whole volatile components of Korean soy sauce produced by improved Meju.
Data of sniffing test: Peak no. 8, an unpleasant soy sauce odor; 9~18, an unpleasant soy sauce odor; 19, a weakly unpleasant soy sauce odor. GC conditions were same as described in Fig. 1.

In the neutral fraction, each of the four regions smelled like soy sauce (see Fig. 5) and the ingredient of the first region was one unknown compound alone, the ing-

redients of the second region were 1H-pyrrole, benzaldehyde, 2-furancarboxaldehyde, 5-methyl-, the ingredient of the third region was benzeneacetaldehyde alone, and the ingredients of the fourth region were 1H-pyrrole-2-carboxaldehyde, 3-tert-butyl-4-methoxyphenol, one unknown compound, respectively as listed in Table 1.

Of the ingredients that smelled like soy sauce, benzeneacetaldehyde was the only identified substance (5). The undetected substances that smelled like soy sauce in this study were dimethyl trisulfide, benzenethanol (5), and 4-hydroxy-2 (or5) ethyl-5- (or2) -methyl-3- (2H) -furanone (HEMF) (12). It has been reported that butanoic acid and dimethyl trisulfide were synthesized by *Bacillus* species (6, 11). It was supposed that butanoic acid, 3-methyl- were synthesized by a salt-resistant yeast that survived and existed in the fermenting soy sauce (11).

Character Impact Components of Korean Soy Sauce Manufactured with the Improved Meju

The whole flavor and the fractionated acidic and phenolic fractions of the whole flavor of Korean soy sauce

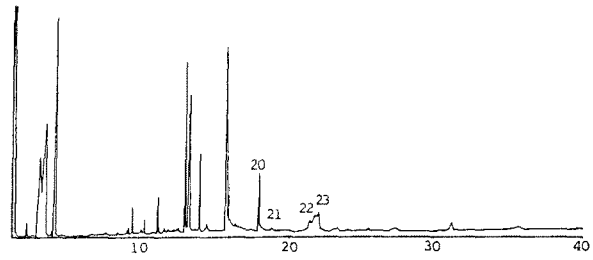

Fig. 8. Gas chromatogram of the phenolic fraction fractionated from whole volatile components of Korean soy sauce produced by improved Meju.

Data of sniffing test: Peak no. 20~23, an unpleasant soy sauce odor. GC conditions were same as described in Fig. 1.

Table 2. Character impact compounds of Korean soy sauce manufactrued with improved Meju

Peak no.	Retention time (min.)	Components	Contents (ppm)	Aroma
1	15.62	Hexadecanoic acid, methyl ester	+(?)	a weakly savory soy sauce odor
2	15.80	Phenol, 2,3,5,6-tetramethyl	26.91	
3	16.00	Hexadecanoic acid, ethyl ester	3.43	
4	20.53	9,12,15-Octadecatrienoic acid, methyl ester	+(?)	a weakly unpleasant soy sauce odor
5	20.74	Benzeneacetic acid	2.40	
6	21.43	Benzaldehyde, 4-hydroxy-3-methoxy-	1.40	
7	24.26	1,2-Benzenedicarboxylic acid, butyl 8-methyl propyl ester	+(?)	
8	16.63	Phenol, 2-methoxy	0.86	an unpleasant soy sauce odor
9	18.18	Phenol	2.33	an unpleasant soy sauce odor
10	18.36	Octanedioic acid, dimethyl ester	0.71	
11	18.96	Unknown	2.75	
12	19.46	Nonanedioic acid, dimethyl ester	2.57	
13	20.15	Hexadecanoic acid,methyl ester	6.37	
14	20.28	Unknown	2.77	
15	20.53	Benzeneacetic acid, alpha-hydroxy-	2.97	
16	21.58	1,2-Benzenedicarboxylic acid, dimethyl	1.78	
17	22.70	Octadecanoic acid, methyl ester	3.13	
18	23.08	9-Octadecenoic acid(Z)-, methyl ester	1.11	
19	35.43	Unknown	+(?)	a weakly unpleasant soy sauce odor
20	18.81	Dodecanoic acid	0.36	an unpleasant soy sauce odor
21	21.37	Unknown	2.86	
22	21.98	Unknown	5.39	
23	23.35	Tetradecanoic acid	0.71	

*: Assumed components by GC-mass, +(?): Incomputable contents. The content of each component was calculated on the basic of the peak area on gas chromatogram.

manufactured with the improved Meju made by *Aspergillus oryzae*, smelled like the soy sauce during the gas chromatography, and are represented in Fig. 6~8. Overall, there were six regions that smelled like the flavor of the soy sauce identified by gas chromatography. But we used the soy sauce as an analytical sample after subjecting it to two months of primary fermentation and for three months of the secondary fermentation, the flavor was very different from that of the soy sauce made by the traditional Meju.

In the overall flavor, there were two regions that smelled like the soy sauce, in the acidic fraction, there were three regions that smelled like the soy sauce, and in the phenolic fraction, only one region contained the soy sauce flavor, respectively. The results of the gas chromatogram and the sniffing tests used to identify these flavors are shown in Fig. 6, 7 and 8.

Most of the ingredients that smelled like and made up the soy sauce flavor were the derivatives of fatty acid and phenolic type substances as shown in Table 2 (2). Phenol, 2-methoxy alone represented the soy sauce flavor, which is identical with that of the soy sauce manufactured with the tradiditional Meju. Of the soy sauce flavor compounds found from this experiment only phenol was also found in the traditional soy sauce.

When we made soy sauce made with the improved Meju with *Aspergillus oryzae*, it differed from the soy sauce made with the traditional Meju, because the traditional method of making Meju reacts to whatever bacteria moves through the air at that time; however, in our improved Meju there was a less possibility that the *Bacillus* species of the strains which produce character impact compounds of Korean soy sauce, would be introduced to survive from the Meju and the surrounding environment. The amount of *Bacillus* included in the Meju determines the production speed of soy sauce (1). In the Japanese fermented soy sauce, the substance synthesized by Maillard's reaction, which was not a metabolic product or a directly synthesized product of microorganisms, smelled like soy sauce (16, 19, 20).

It was presumed when we made soy sauce with the improved Meju made by *Aspergillus oryzae* that the fermentation would be delayed by the lack of the *Bacillus* species, which would cause the production of soy sauce to be difficult and not be involved as much as they are in the traditional Meju. Therefore it would be natural that the flavor ingredients of the soy sauce manufactured with the improved Meju would be different from those of the soy sauce manufactured with the traditional Meju.

Acknowledgement

This study was supported in part by NON DIRECT RESEARCH FUND, Korea Research Foundation, 1992.

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(Received September 24, 1993)