

Characteristic Flavors of Korean Soybean Paste

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We confirmed the character impact compounds of the flavors of the soybean paste manufactured with the traditional and improved Meju, respectively, by using the following methods: gas chromatography (GC), sniffing tests and GC-mass. The soybean paste made with the traditional Meju had 12 compound groups that smelled like the soybean paste flavor, whereas the soybean paste made with the improved Meju had 7 compound groups of soybean paste flavor smell. We were easily able to determine that there is a difference of soybean paste flavor compounds between the soybean pastes made with either the traditional or the improved Mejus because the two soybean paste flavors are very different from each other.

Meju was put in a salt water and subjected to the primary fermentation, and then the fermented product was divided into liquid and solid portions, which were processed for the secondary fermentation to make Korean soy sauce from the liquid portion and to make Korean soybean paste from the solid portion, respectively. The Korean soy sauce and soybean paste manufacturing process are the same up to the primary fermentation, but the flavors of the soy sauce and the soybean paste are completely different after secondary fermentation. Although the flavors change according to the temperature of the fermented soybean paste, the salt concentration and the microorganisms, all Koreans can recognize the smell of boiling soybean paste even without seeing it, which means that all the soybean pastes have common flavors. It is well known that Korean soybean paste has unique flavors which are completely different from those of Korean Chungkookjang, Japanese Natto, and Japanese Miso. By analyzing the flavor ingredients by gas chromatogram and the data of sniffing test, we found out that the unique flavor ingredients are composed of many compounds, not one compound (1). Thereafter, some of the volatile flavor ingredients were identified (4). After identifying that the bacteria of the *Bacillus* species produced the unique flavor ingredients of Korean soybean paste (12), it was confirmed that only *Bacillus subtilis* PM3 and SS9 produced the unique flavor and taste of Korean soybean paste (5). It had been

known that the neutral proteinase, with a molecular weight of 40,000 daltons, of *Bacillus subtilis* PM3, produced the taste of soybean paste (7).

However, there has been no report on the character impact compounds of Korean soybean paste. This paper is on the character impact compounds of the soybean paste manufactured with the traditional Meju (made with the natural strains) and the improved Meju (made mainly by *Aspergillus oryzae*), respectively, in this study.

MATERIALS AND METHODS

Materials

A commercially available, traditional Meju which had been molded into bricks and then manufactured with natural strains and a commercially available, improved Meju manufactured with *Aspergillus oryzae* were used in this study.

Fermentation

18 L of 20% salt solution was added to the Meju corresponding to 10 kg of soybean, the mixture was subjected to two months of primary fermentation, and the solid part of the primary fermented product was further subjected to 5 months of secondary fermentation to manufacture the soybean paste.

Preparation of Volatile Flavors of Soybean Paste

The soybean paste was subjected to an improved Nikerson and Niken's simultaneous steam distillation and extraction apparatus (11). Purified ethyl ether was used to extract the volatile flavors. The extracted whole flavor

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was fractionated into the basic, acidic, phenolic and neutral fractions by adjusting the pH according to Fujimaki's method (2). They were then dried over anhydrous Na₂SO₄ at 4°C overnight. The acidic fraction was further methylated by Schlenk's Diazomethane method (10).

Gas Chromatography (GC) and GC-MS Analysis

GC analysis was carried out with a Shimadzu GC-8A equipped with an FID detector. The column was a wide bore capillary column of CBP-W12-100. The temperature was programmed to increase from 60°C to 200°C at the rate of 10°C/min. The temperatures of the injector and detector were maintained at 240°C. The flow rate of the carrier gas N₂ was 8 ml/min. GC-MS analysis was carried out with a HEWLETT PACKARD GC II 5980 combined with a HEWLETT PACKARD 5988 MS equipped with a SPB-5 (30 m×0.2 μm×0.25 mm) column. The oven temperature was programmed to increase from 45°C to 280°C at the rate of 6°C/min. The injector temperature was 280°C and the detector temperature was 300°C. The carrier gas was He (0.5 ml/min.), the electron voltage was 70 eV and the split ratio was 10:1. Most peaks were identified by using the computer library which was based on the mass spectrum of GC/MS and Kovat's retention index (3, 9).

Confirming the Flavor Character Compounds

We identified the character impact compounds on gas chromatogram used with the wide bore column by a sniffing test, and by comparing the reciprocal pattern of gas chromatogram with a narrow bore column based on the internal standard of retention time.

RESULTS AND DISCUSSION

The Character Impact Compounds of Soybean Paste Manufactured with the Traditional Meju and the Odor of Meju

The gas chromatogram of the whole flavors extracted from the soybean paste manufactured with the traditional Meju and the result of the sniffing tests are presented in Fig. 1 and the ingredients are listed in Table 1. There were seven regions represented in the soybean paste flavor which consisted of from 2 to 10 kinds of components on the gas chromatogram. At least 10 combined components from peak no. 1 to 10 represented the soybean paste flavor and at least 9 components from peak no. 11 to 19. At least 7 components from peak no. 20 to 26, at least 2 components from peak no. 27 to 28, plus at least 3 components from peak no. 29 to 31, next at least 7 components from peak no. 32 to 37, and at least 5 components from peak no. 38 to 42 represented the soybean paste flavor, respectively.

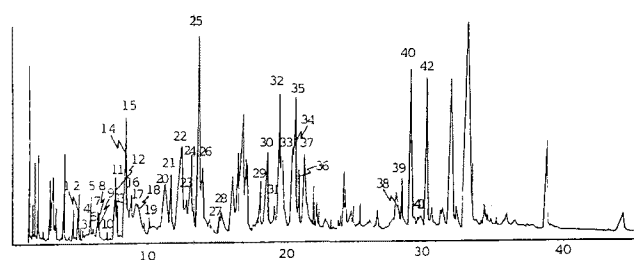


Fig. 1. Gas chromatogram of whole volatile components of Korean soybean paste produced by traditional Meju.

Data of sniffing test: Peak no. 1~10, a weakly savory and soybean paste odor; 11~19, a weakly savory and soybean paste odor; 20~26, a weakly savory and soybean paste odor; 27 and 28, a weakly fishy and soybean paste odor; 29~31, a weakly savory and soybean paste odor; 32~37, a weakly savory and hot soybean paste odor; 38~42, a weakly savory and soybean paste odor. Gas chromatographic (GC) conditions: GC, HEWLETT-PACKARD 5980 II GC; column, SPB-5 (30 m×0.2 μm×0.25 mm); inj. temp., 280°C; det. temp., 300°C; oven temp., 45°C for 2 min., 45~280°C (6°C/min.) and 280°C for 10 min.; carrier gas, He (0.5 ml/min.); split ratio, 10:1.

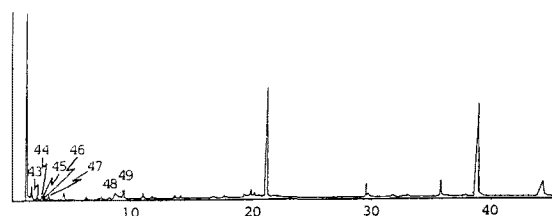


Fig. 2. Gas chromatogram of the acidic fraction fractionated from whole volatile components of Korean soybean paste produced by traditional Meju.

Data of sniffing test: Peak no. 43~47, a sourish and weak Meju odor; 48 and 49, a weakly sourish and weak soybean paste odor. GC conditions were same as described in Fig. 1.

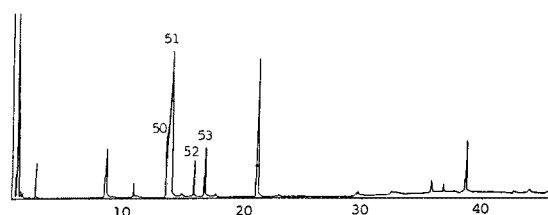


Fig. 3. Gas chromatogram of the phenolic fraction fractionated from whole volatile components of Korean soybean paste produced by traditional Meju.

Data of sniffing test: Peak no. 50 and 51, a savory and weakly fishy soybean paste odor; 52 and 53, a savory and weak soybean paste odor. GC conditions were same as described in Fig. 1.

Table 1. Character impact compounds of Korean soybean paste manufactured with traditional Meju

Peak no.	Retention time (min.)	Components	Contents (ppm)	Aroma	
1	4.81	Butanoic acid, 2-methyl-, ethyl ester	0.53		
2	4.91	Butanoic acid, 3-methyl-, ethyl ester	0.77		
3	5.21	Benzene, 1,4-dimethyl-	0.25		
4	5.40	Benzene, ethynyl-	0.56		
5	5.82	2-Heptanone	1.78	a weakly savory and soybean paste odor	
6	5.94	Butanoic acid, butyl ester	0.41		
7	6.02	Pentanoic acid, ethyl ester	0.19		
8	6.29	Pyrazine, 2,6-dimethyl-	0.92		
9	6.41	Benzene, methoxy-	0.80		
10	6.95	Butanoic acid, 1-methyl-, propyl ester	0.23		
11	7.59	Benzaldehyde	2.45		
12	7.67	Trisulfide, dimethyl*	+(?)		
13	7.74	Benzene, 1,3,5-trimethyl-	1.10		
14	8.36	Unknown	3.42	a weakly savory and soybean paste odor	
15	8.46	Unknown	4.72		
16	8.79	Unknown	2.60		
17	9.21	Phenol	3.48		
18	9.36	Bornylene*	1.39		
19	9.73	Benzene, 1-propynyl-*	0.60		
20	11.25	Unknown	4.04		
21	11.67	Phenol, 2-ethyl-5-methyl-*	2.28		
22	12.32	Unknown	5.70	a weakly savory and soybean paste odor	
23	12.77	Benzene, 1,2-dimethoxy-	1.53		
24	13.12	Unknown	4.40		
25	13.67	Naphthalene*	11.21		
26	13.89	Benzaldehyde, 2-hydroxy-6-methyl	3.08		
27	15.03	Benzeneacetic acid, ethyl ester	0.59		a weakly fishy and soybean paste odor
28	15.17	Unknown	2.02		
29	18.10	Benzene, 4-ethenyl-1,2-dimethoxy-*	1.95	a weakly savory and soybean paste odor	
30	18.63	Unknown	5.87		
31	19.08	Alpha-, chamigrene*	1.20		
32	19.49	Cadinene*	5.54	a weakly savory and hot soybean paste odor	
33	19.72	Unknown	5.04		
34	20.42	Naphthalene, decahydro-1,6-dimethyl	4.06		
35	20.67	Cyclohexadecane	7.17		
36	20.80	Benzothiazole, 2-methyl	0.63		
37	21.23	Unknown	4.00		
38	28.02	Ethanol, 2, (9,12-octadecadienyloxy)-*	1.05		a weakly savory and soybean paste odor
39	28.43	9-Eicosene*	1.55		
40	29.14	Hexadecanoic acid, methyl ester	9.43		
41	29.78	9-Hexadecenoic acid, ethyl ester	0.80		
42	30.32	Hexadecanoic acid, ethyl ester	+(?)		
43	2.23	Propanoic acid, 2-methyl, methyl ester	0.38	a sourish and weak Meju odor	
44	2.62	Butanoic acid, methyl ester	0.55		
45	2.72	Ethane, 1,1-diethoxy-	0.34		
46	2.87	Disulfide, dimethyl*	+(?)		
47	3.23	2-Hexanone	0.54		
48	8.76	Phenol*	2.63		a weakly sourish and weak soybean paste odor
49	9.48	Butanedioic acid, dimethyl ester	1.48		
50	13.69	Phenol, 2-ethyl-*	+(?)	a savory and weakly fishy soybean paste odor	
51	14.13	Benzaldehyde, 2-hydroxy-6-methyl	38.36		
52	15.90	Phenol, 4-ethyl-2-methoxy-*	2.72	a savory and weak soybean paste odor	
53	16.83	Ethanone, 1-(2-hydroxy-5-methyl)-	5.71		

Table 1. Continued

Peak no.	Retention time (min.)	Components	Contents (ppm)	Aroma
54	15.89	Phenol, 4-ethyl-2-methoxy	0.70	a savory and weak Meju odor
55	16.14	Naphthalene, 1-methyl	0.41	
56	16.30	Tridecane*	1.33	
57	16.66	1H-indole*	5.68	
58	30.23	Hexadecanoic acid, ethyl ester	5.38	a weak soybean paste odor
59	34.27	11,13-Eicosadienoic acid, methyl ester	0.48	a savory and weak soybean paste odor
60	34.38	Unknown	0.41	

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

The peak no. from 48 to 49 of the acidic fraction fractionated from the whole flavor, represented the soybean paste flavor at one place in Fig. 2 and the ingredients were phenol, butanoic acid, and dimethyl ester as listed in Table 1.

In the phenolic fraction of Fig. 3, there were two regions identified peak no. 50 and 51 which represented the soybean paste flavor. The ingredients were phenol, 2-ethyl and benzaldehyde, 2-hydroxy-6-methyl. The flavor identified was in peak no. 52 and 53, and included the ingredients phenol, 4-ethyl-2-methoxy- and ethanone, 1-2-hydroxy- as listed in Table 1.

The neutral fraction of Fig. 4 contained two regions of soybean paste flavor, in peak no. 58 in which the ingredients were hexadecanoic acid, ethyl ester, and in peak no. 59 and 60 in which the ingredients were 11, 13-eicosadienoic acid, methyl ester and one unknown substance as listed in Table 1.

The Meju flavors were contained in peaks no. 43 to 47 of the acidic fraction of Fig. 2, in which the ingredients were propanoic acid, 2-methyl, methyl ester, butanoic acid, methyl ester, ethane, 1,1-diethoxy-, disulfide,

dimethyl and 2-hexanone as shown in Table 1 and in the peaks numbering from 54 to 57 of the neutral fraction of Fig. 4, the ingredients were phenol, 4-ethyl-2-methoxy, naphthalene, 1-methyl, tridecane and 1H-indole as presented in Table 1.

Generally, the Meju odor would have declined, whereas the soybean paste flavor would have increased during manufacturing (primary and secondary fermentation). It is known that the soybean paste flavor is synthesized by the confirmed *Bacillus subtilis* (5). In fact, when a specific *Bacillus subtilis* was inoculated into the cooked soybeans to manufacture soybean paste, at the beginning the flavor of Chungkookjang was produced sometimes, but the Meju odor was produced mainly, and finally the soybean paste flavor was produced when the soybean paste was made (5).

While carrying out the gas chromatography and the sniffing test on the soy bean paste flavor together, it was understandable that the soybean paste represented the soybean paste flavor and the Meju odor. After the secondary fermentation was employed after dividing the solid and liquid portions, to manufacture soy sauce and soybean paste, respectively, the solid and liquid portions contained completely different unique flavors from each other.

Therefore, the two portions probably have different flavors because of the difference in the involved microorganisms, or the difference in the physiological phenomenon in that one is in liquid form and the other is in solid form. When the yeast and particularly the bacteria of *Bacillus* species both participate in manufacturing 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 (8).

The Character Impact Compounds of Soybean Paste Manufactured with the Improved Meju and Meju Odor

The results of the gas chromatogram and the sniffing

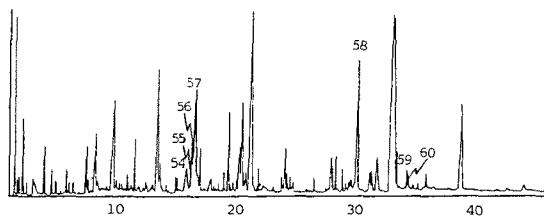


Fig. 4. Gas chromatogram of the neutral fraction fractionated from whole volatile components of Korean soybean paste produced by traditional Meju.

Data of sniffing test: Peak no. 54~57, a savory and weak Meju odor; 58, a weak soybean paste odor; 59 and 60, a savory and weak soybean paste odor. GC conditions were same as described in Fig. 1.

Table 2. Character impact compounds of Korean soybean paste manufactured with improved Meju

Peak no.	Retention time (min.)	Components	Contents (ppm)	Aroma
1	11.53	Pyrazine, tetramethyl	+ (?)	a sweet and weak soybean
2	11.60	Phenol, 2-methoxy	+ (?)	paste odor
3	24.63	Tridecanoic acid, 12-methyl-, methyl ester	+ (?)	a sweet and weak soybean
4	25.35	Tetradecanoic acid, methyl ester	+ (?)	paste odor
5	29.31	Hexadecanoic acid, methyl ester	+ (?)	
6	29.74	1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester	+ (?)	a sweet and weak soybean paste odor
7	30.16	Hexadecanoic acid, ethyl ester	+ (?)	
8	32.23	9,12-Octadecadienoic acid(Z, Z)-, methyl ester	+ (?)	
9	32.48	Octadecanoic acid, methyl ester	+ (?)	a sweet soybean paste odor
10	32.95	Unknown	+ (?)	
11	33.03	Unknown	+ (?)	
12	35.94	2-Diphenylmethyl pyridine	+ (?)	a sweet and weakly
13	36.53	Unknown	+ (?)	burning soybean paste odor
14	36.61	Unknown	+ (?)	
15	38.88	1,2-Benzenedicarboxylic acid, 3-nitro-	+ (?)	a sweet and weakly savory soybean paste odor
16	38.91	1,2-Benzenedicarboxylic acid, 3-nitro-	92.96	a sweet popcorn-like and weak soybean paste odor
17	9.66	Butanedioic acid, dimethyl ester	18.88	
18	10.45	Butanedioic acid, methyl-dimethyl ester	2.08	a weakly sourish Meju odor
19	11.09	Phenol, 2-methoxy	10.72	
20	20.00	1,2-Benzenedicarboxylic acid, dimethyl ester	21.32	
21	20.15	2,5-Cyclohexadiene-1,4-dione, 1,6-bis(1,1-dimethylethyl)-	2.60	
22	20.26	Unknown	3.24	
23	20.41	Unknown	+ (?)	a maturing Meju odor
24	20.49	Unknown	5.60	
25	20.60	Benzenesulfonic acid, 4-methyl-methyl ester	11.44	
26	21.39	Phosphoric acid, tributyl ester	11.76	
27	21.83	Unknown	3.16	
28	31.70	9,12-Octadecadienoic acid(Z, Z), methyl ester	1.24	
29	31.81	7-Octadecenoic acid, methyl ester	3.12	a weakly sourish Meju odor
30	32.22	Octadecanoic acid, methyl ester	3.12	
31	38.89	1,2-Benzenedicarboxylic acid, 3-nitro-	62.28	a weakly sourish and savory soybean paste odor
32	2.36	Butane, 1-ethoxy	8.40	a weak soybean paste odor
33	2.75	Ethane, 1,1-diethoxy-	0.68	
34	2.92	Disulfide, dimethyl	4.12	a savory roasted soybean and weak Meju odor
35	3.06	Unknown	0.64	
36	11.14	Phenol, 2-methoxy	23.88	an unpleasant Meju odor
37	17.05	Unknown	88.40	
38	17.77	Phenol, 2-methoxy-4-(2-propenyl)-	1.96	a weak Meju odor
39	21.29	Phosphoric acid, tri-butyl ester	7.76	
40	22.99	Unknown	4.04	a weak Meju odor

Table 2. Continued

Peak no.	Retention time (min.)	Components	Contents (ppm)	Aroma
41	2.00	Butanal, 3-methyl-	6.51	a sweet and weak soybean paste odor
42	2.34	Propane, 1-ethoxy-2-methyl-	4.96	
43	2.39	Ethene, trichloro	+ (?)	
44	2.47	Methane, bromodichloro-	0.74	
45	2.73	Ethane, 1,1-diethoxy	1.02	a sweet popcorn-like and sweet soybean paste odor
46	2.90	Unknown	2.52	
47	3.02	1-Butanol, 3-methyl-	7.37	
48	3.25	Benzene, methyl-	4.42	
49	3.71	Methane, dibromochloro-	0.42	
50	38.94	1,2-Benzenedicarboxylic acid, 3-nitro-	+ (?)	a weakly savory soybean paste odor

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

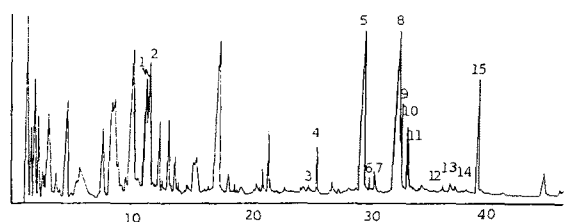


Fig. 5. Gas chromatogram of whole volatile components of Korean soybean paste produced by improved Meju. Data of sniffing test: Peak no. 1 and 2, a sweet and weak soybean paste odor; 3 and 4, a sweet and weak soybean paste odor; 5~7, a sweet and weak soybean paste odor; 8~11, a sweet soybean paste odor; 12~14, a sweet and weakly burning soybean paste odor; 15, a sweet and weakly savory soybean paste odor. GC conditions were same as described in Fig. 1.

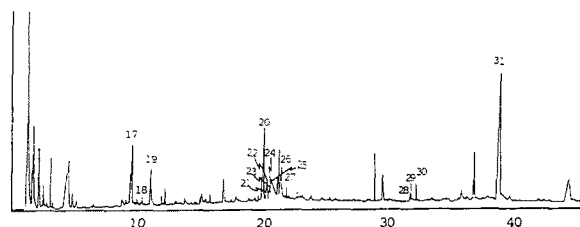


Fig. 7. Gas chromatogram of the acidic fraction fractionated from whole volatile components of Korean soybean paste produced by improved Meju. Data of sniffing test: Peak no. 17~19, a weakly sourish Meju odor; 20~27, a maturing Meju odor; 28~30, a weakly sourish Meju odor; 31, a weakly sourish and savory soybean paste odor. GC conditions were same as described in Fig. 1.

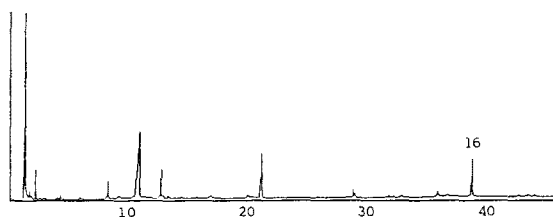


Fig. 6. Gas chromatogram of the basic fraction fractionated from whole volatile components of Korean soybean paste produced by improved Meju. Data of sniffing test: Peak no. 16, a sweet popcorn-like and weak soybean paste odor. GC conditions were same as described in Fig. 1.

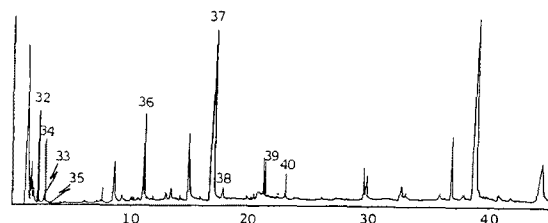


Fig. 8. Gas chromatogram of the phenolic fraction fractionated from whole volatile components of Korean soybean paste produced by improved Meju. Data of sniffing test: Peak no. 32, a weak soybean paste odor; 33~35, a savory roasted soybean and weak Meju odor; 36, an unpleasant Meju odor; 37 and 38, a weak Meju odor; 39 and 40, a weak Meju odor. GC conditions were same as described in Fig. 1.

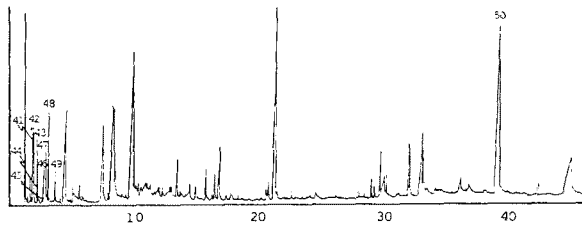


Fig. 9. Gas chromatogram of the neutral fraction fractionated from whole volatile components of Korean soybean paste produced by improved Meju.

Data of sniffing test: Peak no. 41~44, a sweet and weak soybean paste odor; 45~49, a sweet popcorn-like and sweet soybean paste odor; 50, a weakly savory soybean paste odor. GC conditions were same as described in Fig. 1.

tests to confirm the character impact compounds of soybean paste manufactured with improved Meju (made with *Aspergillus oryzae*), mainly, are represented in Fig. 5~9 and the ingredients are listed in Table 2. The gas chromatogram of the whole flavor extracted from the improved soybean paste had 6 regions representing soybean paste flavors (Fig. 5); the gas chromatogram of the basic fraction fractionated from the whole flavors (Fig. 6), the acidic fraction (Fig. 7), and the phenolic fraction (Fig. 8) represented one region of the soybean paste flavor, each; and the gas chromatogram of the neutral fraction from the whole flavor (Fig. 9) represented 3 regions of soybean paste flavors.

The ingredients of the soybean paste flavor were pyrazine, tetramethyl and phenol, 2-hydroxy, and tridecanoic acid, 12-methyl-, methyl ester and tetradecanoic acid, methyl ester as shown in Table 2. Also, three ingredients of hexadecanoic acid, methyl ester, 1,2-benzene, dicarboxylic acid, butyl 2-methyl propyl ester, and hexadecanoic acid, ethyl ester represent the soybean paste flavor; 9,12-octadecanoic acid (Z, Z)-, methyl ester, octadecanoic acid, methyl ester and two unknown compounds represented the soybean paste flavor; and 2-diphenyl methyl pyridine and two unknown compounds represented the soybean paste flavor. On the other hand, 1,2-benzenedicarboxylic acid, 3-nitro only, and butane, 1-ethoxy only, represented the soybean paste flavor. Four ingredients of butanal, 3-methyl-, propane, 1-ethoxy-2-methyl-, ethane, trichloro, and methane, bromodichloro- represented the soybean paste flavor; and five ingredients of ethane, 1,1-diethoxy, 1-butanol, 3-methyl-, benzene, methyl- and methane, bromodichloro-, plus one unknown substance represented the soybean paste flavor. But a single group of ingredients could not represent the unique flavor of soybean paste.

The character impact compounds of the soybean paste

manufactured with both the improved and the traditional Meju were totally different. These difference were postulated to be caused by the difference in the microflora which are involved in both the primary and secondary fermentation.

The Meju odor represented in the three regions of the acidic fraction of Fig. 7 and in the four regions of the phenolic fraction of Fig. 8, is in total represented in 7 ingredient groups. The ingredient of the Meju odor was mostly different from that of the Meju had been manufactured with the traditional Meju. Although peak no. 36 of phenol, 2-methoxy in Table 2 represents the Korean soy sauce flavor ingredients, in this study and phenol, 2-methoxy represented the flavor of the Meju odor in the accompanying soy sauce paper (6). In view of this point, we considered that the major ingredient of peak no. 36 is phenol, 2-methoxy, but there is a possibility that a few unanalyzed substances may also be contained in this peak.

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