

Taste Components of Soy Sauce Manufactured by *Bacillus* Species SSA3-2M1 and Fused ST723-F31

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In order to investigate fermenting conditions and the microorganisms necessary for factory production of traditional Korean soy sauce, we manufactured soy sauce made by *Bacillus* species SSA3-2M1 and fused ST723-F31 with aeration (1/30 vvm, 1/3 vvm and 2/3 vvm) at 30°C for 40 days. This method was chosen to investigate the changes of dissolved oxygen, pH, cell number, flavor and the taste components during fermentation. When air was supplied (2/3 vvm) to the fermentor during fermentation, the flavor of the soy sauce and the composition of taste components (free amino acids, free sugars and organic acids) were similar to that of traditional Korean soy sauce after 22 days. The results of our experiments indicate that the mass production of traditional Korean soy sauce is possible using *Bacillus* species SSA3-2M1 and fused ST723-F31 given sufficient aeration.

Traditional Korean soy sauce has been home made and consumed domestically as an important seasoning. It is manufactured by first soaking the Meju in saline solution and then fermenting the separated liquid part. However, this traditional method of preparation is very difficult and fermentation periods of about 6 to 12 months are required.

Microorganisms existing in the material source (Meju) and in the environment during the first and second fermentations influence the taste and flavor of traditional Korean soy sauce. It is difficult to control the production or balance of aroma and taste components because of the naturally inflowing microorganisms, and as a result it is difficult to adjust the taste and flavor of soy sauce. It is also difficult to mass produce traditional Korean soy sauce.

To solve these problems, it is required to fix fermentation microorganisms. If we can manufacture traditional soy sauce with a single or a few microorganisms, it would be easier to control the microorganisms, and produce soy sauce of consistent quality.

There are many studies examining the taste of traditional Korean soy sauce. These studies include the distribution of taste components (5, 9, 10-13); sensory evaluation (1); the characteristics of taste (16); and the analysis of major effective factors on the taste (8, 11, 16);

but there are no studies on the taste components of soy sauce produced by fermentation microorganism, except the study on the taste components of soy sauce produced by *Bacillus* species SSA3-2M1 (4).

In this experiment, fermentation bacteria and yeast of soy sauce, *Bacillus* species SSA3-2M1 and fused ST723-F31 were used to produce soy sauce in the conditions of aeration (2/3 vvm, 1/3 vvm and 1/30 vvm) at 30°C for 40 days. *Bacillus* species SSA3-2M1 was bred to increase proteinase activity from *Bacillus* species SSA3 which was isolated from traditional Korean soy sauce (15, 17, 18). Fused ST 723-F31 was obtained by cell fusion between *Zygosaccharomyces rouxii* and *Torulopsis versatilis*, the main fermentation yeasts of soy sauce (14). The fusant has the characteristics of both strains.

We investigated and compared the characteristics of manufactured soy sauce by organoleptic test and analysis of taste components (free amino acids, free sugars and organic acids), then the results were compared with the flavor and taste of soy sauce produced by *Bacillus* species SSA3-2M1 and traditional Korean soy sauce. We would like to propose a convenient method by which traditional Korean soy sauce can be fermented in a factory.

MATERIALS AND METHODS

Strains

We used *Bacillus* species SSA3-2M1 which was iso-

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lated from traditional Korean soy sauce and bred for the fermentation of soy sauce, and fused ST723-F31 cell fused between *Zygosaccharomyces rouxii* and *Torulopsis versatilis*, the main fermentation yeast.

Manufacture of Soy Sauce

The medium for manufacture of soy sauce was soybean extract medium. The soybean extract medium was prepared as follows; 1300 g of soybean was soaked in water overnight and crushed, then 8 volumes of water was added and the mixture was boiled for 3 h and filtered twice. Finally, NaCl was added and the concentration adjusted to 10%, then autoclaved at 121°C for 20 min. The preculture solution of *Bacillus* species SSA 3-2M1 and fused ST723-F31 was inoculated for 8 days to soybean extract medium of 3 liter to adjust its concentration to one percent, and then fermented in the three Jar fermentors (B. E. MARUBISHI Co., LTd). The temperature and agitation of Jar fermentors were 30°C and 50 rpm. The aeration conditions of Jar fermentor number 1, 2 and 3 were 2/3 vvm, 1/3 vvm and 1/30 vvm, respectively.

Measurement of DO, pH and Cell Number and Organoleptic Test

DO and pH were measured by DO meter and pH meter, and cell numbers were measured by the conventional plating count method on the nutrient agar medium. The organoleptic test was carried out by panel according to Jonhston's methods (2).

Isolation and Purification of Free Amino Acids, Free Sugars and Organic Acids

In order to analyze taste components, 50 ml of the fermented soy sauce was obtained according to a time table. After the filtration of 50 ml samples by Whatman No.2 filter paper, the filtrate was concentrated under vacuum, desalted by adding 90% methanol and dried completely under vacuum. Desalted and dried samples were added to 50 ml of water to purify taste components.

The purification of free amino acids, free sugars and organic acids was performed by ion-exchange chromatography using Setsuko's method (19, 21). Cation exchange resin and anion exchange resin, Amberlite IR-120 and Amberlite IRA-400 were packed in a 35×2 cm column. Then the sample was slowly passed through both columns and eluted by 100 ml distilled water. The euent passing through the cation and anion resin was sampled for analysis of free sugars. The free sugars were analyzed by high performance liquid chromatography (HPLC) as follows: Instruments, Waters Associates Inc. HPLC Milford, MA01757, USA; detector, differential refractometer R410; flow rate, 1.0 ml/min; column, C18; mobile phase, CH₃CN : H₂O=80 : 20. The free amino acids adsorbed to the cation resin were eluted slowly with 100 ml of 2-N NH₄OH solution, then were concentrated under vacuum. The free amino acid dried for

analysis was added to 3 ml of 0.1 M citrate buffer (pH 2.2) and was analyzed using an amino acid autoanalyzer (LKB4150). The organic acids adsorbed to anion resin were eluted slowly with 100 ml of 1.5 N (NH₄)₂CO₃ and concentrated and dried under vacuum. The moisture in the organic acids was removed overnight by a desiccator containing P₂O₅ and then dissolved with 20 ml of diethyl ether. Methylation was carried out to purified non-volatile organic acids using the diazomethane method of Schlenk for GC analysis (20). The conditions of gas chromatography for organic acid analysis were as follows: Instruments, Shimadzu GC-8A; column, chemically bonded fused silica capillary column (CBP20-W12-100); detector, FID; injector (inj.) & detector (det.) temperature (temp.), 200°C; carrier gas, N₂ (25 ml/min.); column temp., 60~150°C (increase 8°C/min.); range, 10²; attenuation, 1. According to the method of Kageyama (7), filtered soy sauce solution was added to 2% H₂SO₄ solution to a final concentration of 0.1% for the analysis of volatile organic acids. The GC analysis conditions of the volatile organic acids were as follows: Instrument, Shimadzu GC-8A; column, 10% PEG 6000 in glass column 2 m; inj. & detec. temp., 200°C; carrier gas, N₂ (40 ml/min.); column temp., 150°C; range, 10²; attenuation, 64.

RESULTS AND DISCUSSION

Changes of DO, pH, Cell Number and Flavor Depending on Fermentation Conditions

When soy sauce was fermented, aeration was 2/3 vvm, 1/3 vvm and 1/30 vvm. Table 1 shows the changes of DO, pH, cell number and flavor depending on aeration and fermentation time.

The fermented soy sauce after 40 days was obtained after growing microorganisms in a fermentor with aeration for 8 days, and stationary fermenting to age the flavor and taste components for 32 days. The cell number of bacteria and yeasts was from 10⁶/ml to 10⁷/ml and the growth of bacteria was faster than that of yeast.

DO decreased from 7.3 mg/l to 0.1 mg/l but increased again. It was presumed that DO decreased at log phase because of the rapid growth of microorganisms and again increased during the stationary phase. When 2/3 vvm of aeration was supplied, the growth of microorganisms was fast, and the change of DO was fast aslo. By contrast, when 1/30 vvm of aeration was supplied, the growth of microorganisms and changes in DO were very slow because of the slow growth of microorganisms.

When 2/3 vvm of aeration was supplied, pH was decreased from 5.8 to pH 4.0 and the increased again to pH 7.1. pH gradually decreased to pH 4.2 when little (1/30 vvm) aeration was supplied. It was considered that it was related the growth of microorganism and changes of DO, and the pH of the manufactured soy sauce differed ac-

Table 1. DO, pH, cell number, odor and taste of soybean extract fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31 depending on air conditions.

Fer. time (h)	DO			pH			Aroma			Cell number (each/ml)		
	Jar. fer. no. 1	2	3	1	2	3	1	2	3	1	2	3
0	7.5	7.3	7.3	5.8	5.8	5.8	savory soybean	savory soybean	savory soybean	3.4(6)*	2.9(6)	3.6(6)
										1.9(6)	1.5(6)	1.2(6)
3	6.6	6.1	6.5	5.8	5.8	5.7				2.4(6)	2.6(6)	3.4(6)
										1.2(6)	1.2(6)	4.4(5)
6	6.6	6.3	6.5	5.8	5.8	5.7	sweet soybean milk	sweet soybean milk	sweet soybean milk	3.6(6)	3.5(6)	3.2(6)
										1.3(6)	1.1(6)	1.0(6)
10	6.4	6.4	5.9	5.7	5.7	5.7				3.4(6)	4.8(6)	6.6(6)
										1.8(6)	2.0(6)	1.7(6)
18	0.7	0.2	0.9	5.5	5.3	5.6	sweet Meju	weak Meju		3.2(6)	2.3(6)	3.6(6)
										3.0(6)	1.5(7)	2.0(6)
23	0.2	0.1	0.6	5.3	4.9	5.5	Meju			8.1(6)	8.0(7)	6.4(6)
										7.7(6)	2.0(7)	1.1(7)
30	0.2	0.1	0.2	5.2	4.7	5.3	weak unpleasant Meju		weak Meju	5.3(6)	6.6(7)	5.8(6)
										7.7(6)	2.0(7)	1.1(7)
45	0.1	0.1	0.1	5.3	4.4	4.6				3.1(7)	1.2(7)	2.2(7)
										1.5(7)	1.7(7)	6.0(6)
68	0.1	0.1	0.1	5.1	4.6	4.3		sourish Meju	sourish Meju	4.0(7)	2.0(7)	5.3(6)
										2.2(7)	1.1(7)	4.6(7)
76	0.9	0.1	0.1	4.9	4.5	4.3	sweet Meju	unpleasant Meju		2.1(7)	2.3(7)	3.5(7)
										2.8(7)	1.4(7)	4.3(7)
96	0.8	0.9	0.1	4.5	4.3	4.3				2.5(7)	1.6(7)	5.9(7)
										2.3(7)	4.8(7)	7.8(7)
124	4.0	3.6	0.1	4.6	4.2	4.5				3.1(7)	2.7(7)	3.2(7)
										1.2(7)	1.3(7)	4.3(7)
148	4.0	4.6	0.1	4.8	4.1	4.5	soybean paste			5.9(7)	9.4(7)	7.2(7)
										2.2(7)	1.5(7)	2.1(7)
193	0.1	6.3	0.1	6.0	4.2	4.3	soybean paste weak soysauce	soybean paste		5.7(7)	5.6(7)	4.6(7)
										5.3(7)	2.5(7)	1.6(7)
242	4.9	6.9	2.5	6.8	4.1	4.1			Meju	2.2(7)	0.1(7)	1.6(7)
										1.5(7)	2.2(7)	5.9(7)
296	6.9	6.8	6.4	6.4	4.2	4.1	soy sauce			5.5(7)	2.4(7)	5.6(7)
										2.9(7)	4.8(7)	2.7(7)
338	4.8	6.9	6.4	6.9	4.2	4.1	soy sauce			7.3(7)	7.0(7)	6.1(7)
										2.9(7)	4.8(7)	2.7(7)
412	6.5	7.1	6.8	6.9	4.2	4.1				8.7(7)	6.1(7)	3.9(7)
										1.4(7)	3.8(7)	3.2(7)
528		7.1	6.8	7.1	4.2	4.2	traditional soy sauce odor & taste	weak soysauce odor & taste	unpleasant weak soy sauce odor & taste			
										6.7(7)	8.6(7)	8.2(7)
960	1.4	1.6	0.8	7.9	7.9	7.6	traditional Korean soy sauce odor & taste	traditional Korean soy sauce odor & taste		2.7(7)	1.7(7)	1.5(7)

*(6), 10⁶; (7), 10⁷. Jar fer. no. 1, 2 and 3, 2/3 vvm, 1/3 vvm and 1/30 vvm of aeration, respectively.

Table 2. Contents of amino acids in soybean extract fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31 depending on air conditions. (mg/100 ml)

Jar fer. no.	days	AA* Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met
1	3	7.71	7.23	2.85	8.40	3.12	1.59	1.41	2.43	20.34	4.44
	4	15.24	7.65	36.57	40.41	69.84	2.49	1.47	18.54	55.77	26.82
	5	6.09	18.84	4.68	29.31	20.70	1.41	1.11	3.09	22.92	11.37
	10	3.90	26.21	21.45	27.33	50.70	6.36	6.12	1.21	82.92	18.21
	20	14.31	44.40	54.69	85.50	589.62	26.35	33.42	0	133.29	27.51
	40	79.31	167.97	125.58	532.65	437.67	252.24	57.72	0	240.12	107.76
2	3	7.71	10.53	3.03	8.88	11.31	1.86	1.23	5.49	11.91	3.09
	4	10.32	19.89	3.78	14.01	11.79	2.01	0.99	9.03	17.40	6.96
	5	2.61	5.76	1.26	5.07	0	0.93	0.72	1.74	5.52	1.38
	10	9.06	31.20	7.08	25.80	35.19	3.39	3.21	5.82	25.47	11.49
	20	8.07	15.63	3.00	13.32	17.13	1.77	0.96	4.50	13.98	4.71
	40	132.96	117.42	35.73	98.13	426.84	67.35	78.36	28.89	168.09	103.05
3	3	12.12	8.97	6.48	33.21	27.27	3.12	21.45	18.72	19.65	5.97
	4	6.30	1.98	3.39	11.79	32.88	1.32	7.80	0	5.43	1.65
	5	6.57	5.10	2.46	14.70	18.60	1.71	7.44	2.43	8.58	3.30
	10	4.26	0	13.29	11.61	2.40	1.41	1.38	1.26	12.87	1.44
	20	3.87	18.30	0	14.01	12.09	1.47	0.87	0	16.17	6.27
	40	88.41	89.61	78.69	342.30	183.51	29.10	30.06	0	219.39	102.24
Jar fer. no.	days	AA* Ileu	Leu	Tyr	Phe	His	Lys	Arg	NH ₃	Total	
1	3	3.21	21.51	14.93	3.06	50.04	2.76	10.98	1.38	167.19	
	4	9.51	53.19	53.58	85.56	10.26	5.85	12.63	0.15	505.53	
	5	2.43	23.82	21.42	38.88	50.52	2.46	10.86	1.50	271.41	
	10	36.60	95.07	84.30	121.98	119.07	37.17	36.54	2.76	777.90	
	20	89.04	147.45	88.23	136.68	132.09	127.02	51.84	47.13	1828.47	
	40	226.53	299.70	147.21	339.87	90.63	127.74	130.56	9.21	3376.48	
2	3	1.17	14.82	14.73	28.23	52.35	4.08	11.49	1.41	193.32	
	4	2.52	18.54	18.45	33.51	52.92	1.11	7.53	1.35	232.11	
	5	0.87	6.84	7.08	13.23	25.59	0.63	0	1.02	80.25	
	10	7.08	24.84	28.41	39.00	65.82	5.55	12.90	0.99	342.30	
	20	4.50	19.83	15.42	22.86	38.73	0.84	6.39	0.93	192.57	
	40	153.84	198.48	140.00	276.42	110.61	137.19	44.85	6.51	2325.72	
3	3	3.93	16.67	23.55	40.35	69.69	3.75	15.66	2.10	342.66	
	4	1.44	10.86	7.50	14.43	39.12	1.83	8.19	0.93	156.84	
	5	2.46	15.33	11.37	20.40	44.16	2.49	6.03	0.93	174.06	
	10	3.42	19.80	12.99	23.34	43.02	2.34	7.01	1.08	162.93	
	20	3.45	24.51	2.77	31.66	59.94	31.14	7.08	1.17	229.77	
	40	157.89	233.52	149.34	207.09	80.97	59.28	120.75	7.83	2179.98	

*AA, amino acid. Jar fer. no. 1, 2 and 3, 2/3 vvm, 1/3 vv and 1/30 vvm of aeration, respectively.

cording to fermentation. There were reports that traditional Korean soy sauce has a broad range of pH from pH 4.3 to 7.8 (4).

The results of organoleptic tests shows that the flavor

of the fermented products was changed from savory and sweet soybean flavor to Meju flavor, and soybean paste flavor and finally soy sauce flavor. When 2/3 vvm of aeration was supplied, the fermented solution smelled

like Meju flavor after 18 h, smelled like soybean paste flavor after 8 days and showed the flavor and taste of traditional Korean soy sauce after 22 days. The soy sauce fermented by *Bacillus* species SSA3-2M1 had a soy sauce flavor similar to a pupal smell, but the soy sauce manufactured by *Bacillus* species SSA3-2M1 and fused ST723-F31 had a savory traditional soy sauce flavor and taste. It was felt that the taste and flavor of soy sauce manufactured by *Bacillus* species SSA3-2M1 and fused ST723-F31 was better than that of *Bacillus* species SSA3-2M1. When 1/30 vvm of aeration was supplied, the changes and production of flavor was very slow and the soy sauce had an unripe soy sauce flavor.

Therefore, if *Bacillus* species SSA3-2M1 and fused ST723-F31 are used to manufacture soy sauce with aeration, we feel that the fermentation time of the soy sauce could be shortened because they rapidly produce their own unique taste and flavor.

Changes of Taste Components

The contents of free amino acids in the manufactured soy sauce are shown in Table 2. There were 18 kinds of free amino acids, and the more they were fermented, the more free amino acids were obtained. When enough air was supplied, the contents of the free amino acids were

more than with a limited air supply. It has been reported that the mean of free amino acids in traditional Korean soy sauce was 865.6 mg/ml with the minimum being 257.0 mg/ml and the maximum being 2126 mg/ml (11). When 2/3 vvm of aeration was supplied, the contents of free amino acids in the manufactured soy sauce after 10 days was similar to the mean in traditional soy sauce, and in soy sauce fermented for 40 days it was 1.5 times that of the maximum in traditional Korean soy sauce (11). In soy sauce fermented for 17 days, it was two times that of soy sauce produced by *Bacillus* species SSA3-2M1 independently (4). It was thought that this was due to the differences in the amount, activity and operating pH of proteinase secreted from fermentation microorganisms.

The contents of glutamic acid which is the savory taste components was the most higher. There were a lot of isoleucine, leucine and valine, and they were found as factors effecting on the taste of traditional Korean soy sauce (8, 11, 16).

The contents of organic acids are shown in Table 3. The volatile organic acids were acetic acid, propionic acid, butyric acid and 3-methyl butyric acid, and the non-volatile organic acids were oxalic acid, fumaric acid,

Table 3. Contents of organic acids in soybean extract fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31 depending on air conditions. (mg/100 ml)

Jar fer. no.	days	Volatile organic acids				Non-volatile acids					
		acetic acid	propionic acid	butyric acid	3-methyl butyrate	oxalic acid	fumaric acid	malonic acid	succinic acid	glutaric acid	citric acid
1	3	88.00	39.10	3.02	1.49	0.32	0.02	0.76	2.26	0.00	0.20
	4	147.33	16.40	0.95	1.96	0.16	0.10	0.15	1.07	0.13	0.29
	5	145.46	8.99	1.07	2.58	0.48	0.06	0.97	2.36	0.04	0.11
	10	11.00	5.90	0.73	8.82	0.36	1.55	1.37	trace	0.03	0.08
	20	17.50	2.40	4.22	10.17	0.36	0.34	0.31	0.80	0.06	0.12
	40	17.53	3.68	3.12	22.80	0.24	trace	0.00	1.39	0.00	0.21
2	3	129.00	22.66	1.27	0.96	0.08	0.10	0.20	0.39	0.02	0.00
	4	209.98	19.28	0.81	1.48	trace	0.10	0.15	1.87	0.00	0.27
	5	230.83	11.80	0.74	1.69	0.16	0.01	0.36	0.74	trace	0.03
	10	280.44	9.74	1.02	2.32	1.04	0.19	0.62	3.71	0.10	0.13
	20	235.18	6.75	0.33	1.62	0.20	0.17	trace	2.12	0.24	0.25
	40	86.09	5.48	2.32	20.65	0.24	0.17	0.37	0.09	0.00	0.25
3	3	33.00	70.68	1.24	0.70	2.28	0.17	0.36	2.36	0.34	0.57
	4	46.00	98.76	0.24	0.66	1.68	0.00	1.27	0.00	6.07	0.13
	5	68.00	93.48	1.04	0.92	4.40	0.00	0.36	2.04	0.39	0.07
	10	229.45	26.23	0.83	1.49	0.28	0.23	0.48	1.57	0.00	0.05
	20	254.53	18.06	0.71	1.67	0.32	trace	0.39	1.33	0.00	0.20
	40	51.26	5.24	0.59	17.73	0.20	0.17	0.12	1.01	0.15	0.27
TKSS*	1 year	11.28	9.95	0.00	0.74	33.12	0.00	0.65	1.51	0.09	0.11
	6 years	27.81	2.63	1.08	6.06	40.00	5.40	0.82	0.30	0.00	0.10
	10 years	11.70	1.99	0.00	7.04	2.92	0.42	0.87	0.00	0.00	0.11

* Traditional Korean soy sauce. Jar fer. no. 1, 2 and 3, 2/3 vvm, 1/3 vv and 1/30 vvm of aeration, respectively.

Table 4. Contents of free sugars in soybean extract fermented by *Bacillus* species SSA3-2M1 and fused ST723-F31 depending on air conditions. (mg/100 ml)

Jar fer. no.	days	Glucose	Fructose	Sucrose
1	3	0.00	0.00	0.00
	4	0.00	0.74	0.00
	5	0.03	0.18	1.80
	10	0.00	0.00	0.00
	20	0.00	2.76	0.00
	40	0.02	0.49	0.00
2	3	0.00	0.00	0.00
	4	0.00	1.07	0.00
	5	0.00	1.56	0.00
	10	0.03	2.49	0.00
	20	0.00	0.25	0.00
	40	0.02	0.02	0.00
3	3	0.00	0.00	0.00
	4	0.00	0.13	0.00
	5	0.00	5.51	0.00
	10	0.24	1.58	0.00
	20	0.00	0.29	0.00
	40	0.00	0.00	0.00
TKSS*	1 year	0.03	0.55	0.00
	6 years	2.37	3.57	0.00
	10 years	0.88	2.35	1.99

*, Traditional Korean soy sauce. Jar fer. no. 1, 2 and 3; 2/3 vvm, 1/3 vvm and 1/30 vvm of aeration.

malonic acid, succinic acid, glutaric acid and citric acid. There were more volatile organic acids than that of traditional soy sauce, but lactic acid was not detected which, it was reported previously, there is a lot of in traditional Korean soy sauce (5), but the contents were different according to the measuring method (7). Butyric acid, glutaric acid and citric acid were not detected in the soy sauce manufactured by *Bacillus* species SSA3-2M1, also the contents of detected organic acids were low. Acetic acid produced by anaerobic fermentation was detected in large quantities the initial stage of microbial growth, increasing cell numbers and decreasing DO value with limited aeration. The more fermentation was advanced, the more the contents of acetic acid decreased. It was considered that acetic acid degraded to the level of the other products, or naturally volatilized. The butyric acid found as an off-flavor in traditional Korean soy sauce was increased, but because the contents were low, it did not influence the overall flavor of the soy sauce. On the contrary, it contributed to creating a similar flavor to traditional Korean soy sauce. There were reports that organic acids having acid taste in the soy sauce had little influence on the taste of soy sauce (8, 16).

The types of free sugars in manufactured soy sauce are

shown in Table 4. Sucrose, fructose and glucose were detected and the contents were low compared with traditional Korean soy sauce. The type of free sugars was similar to traditional Korean soy sauce. The quantity of fructose was high, the next highest was glucose and sucrose. It was thought that this difference in the contents and kinds of free sugars was due to the type of soybeans used because the carbohydrate contents is dependent on the type of soybeans used.

The characteristic taste of soy sauce was made up of many taste components and determined by the amount and type of each component. In this study, soy sauce manufactured by *Bacillus* species SSA3-2M1 and fused ST723-F31 for 40 days was found to be similar to traditional Korean soy sauce in terms of flavor and taste by organoleptic test and the contents of taste components.

Therefore, we demonstrated that the fermentation time of soy sauce could be shortened and that we could produce traditional Korean soy sauce when the soy sauce was fermented with *Bacillus* species SSA3-2M1 and fused ST723-F31 with sufficient aeration.

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