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RESEARCH NOTE

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Optimal Manufacturing Conditions for Korean Soybean Paste and Soy Sauce, Using *Aspergillus oryzae* AJ 100 as a Flavor Improver

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Abstract Previously, it has been reported that *Aspergillus oryzae* can efficiently degrade unpleasant odor components such as butyric acid and 3-methyl butanoic acid from *meju*, a major ingredient in both Korean soybean paste (*doenjang*) and soy sauce. In this study, the optimal manufacturing conditions for the production of superior quality Korean soybean paste and soy sauce were determined. Specifically, *A. oryzae* AJ 100 was utilized to improve the flavor of these products. Mixtures of Korean soybean paste and *A. oryzae* AJ 100 culture (2 : 1), and of Korean soy sauce and *A. oryzae* AJ 100 culture (5 : 1), were incubated for 2 weeks at 30°C, and showed improved flavor. Butyric acid and 3-methyl butanoic acid were clearly degraded under these culture conditions.

Keywords: optimal manufacturing condition, improving flavor, traditional soybean paste, soy sauce, *Aspergillus oryzae*

Introduction

Recently, studies have been carried out on such aspects as the functional properties and distribution of isoflavones in Korean soy fermented foods (1-4), the enhancement of anti-cancer effects by fermenting organisms (5,6), and the formation of flavor components by fermenting micro-organisms (7-11).

Traditional Korean *meju* harbors several microorganisms such as 95-99% *Bacillus subtilis* strains, and 1-5% molds, including *Aspergillus*, *Mucor*, *Rhizopus*, and *Penicillium* species (7,12). It was determined that *B. subtilis* generates butyric acid and 3-methyl butanoic acid during the fermentation process after the later stage of endospore formation (8); both of these compounds are principal components of the unpleasant flavors sometimes associated with fermented foods. As *B. subtilis* was shown to exist at levels in excess of 10^4 CFU/g during *koji* (Japanese *meju*) manufacture, the Japanese soybean paste and soy sauce made from the *koji* were found to contain butyric acid (13). *Koji* is generally made with *Aspergillus oryzae*; however, *meju* is generally made using *B. subtilis*.

Traditional Korean soybean paste (*doenjang*), soy sauce, and *kochujang* made from *meju*, also contain butyric acid and 3-methyl butanoic acid (7,9,10). *Cheonggukjang*, which is prepared with only *B. subtilis*, is also shown to contain butyric acid and 3-methyl butanoic acid (11).

As mentioned above, the majority of fermented foods made with *B. subtilis* tend to harbor high levels of butyric acid and 3-methyl butanoic acid, 2 major components of the unpleasant flavors often associated with these foods. Therefore, the consumption of these foods by current younger generations is showing a significant decline. Previously, Park (8) demonstrated that *A. oryzae*, a minor component of *meju*, can efficiently degrade butyric acid and 3-methyl butanoic acid.

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In this study, a method for the decomposition of butyric acid and 3-methyl butanoic acid from Korean soy sauce and soybean paste, using *A. oryzae* AJ 100 culture, which resulted in improved flavor was described.

Materials and Methods

Materials The traditional Korean soybean paste (*doenjang*) and soy sauce were prepared at the Jain National Agricultural Cooperative Federation, Gyeongbuk, Korea.
***B. subtilis PM3 doenjang*:** *B. subtilis* PM3 was cultured for 3 days in boiled and crushed soybeans at 30°C. NaCl (7%) was added to the culture, which contained 55% water. The culture was then allowed to mature for 30 days at 30°C.
***A. oryzae AJ 100 culture*:** *A. oryzae* AJ 100 was cultured for 7 days in boiled soybeans at 30°C. The water content of the culture was 55% water.

Analysis of volatile organic acids The volatile organic acids were extracted and analyzed according to the method developed by Kageyama *et al.* (14).

Sensory evaluation The sensory panel consisted of 12 trained graduate and undergraduate students. The trained panelists scored the flavors of the samples via a 5-point scale as follows: 'very bad' was 1 point, 'bad' was 2 points, 'moderate' was 3 points, 'good' was 4 points, and 'very good' was 5 points (15).

Statistical analysis *t*-Tests and Duncan's multiple range tests were conducted using the SPSS program (SPSS Inc., Chicago, IL, USA).

Results and Discussion

In a previous study, we demonstrated that butyric acid and 3-methyl butanoic acid, 2 principal compounds responsible for the bad odors often associated with traditional Korean fermented foods, including soy sauce and soybean paste,

Table 1. Volatile organic acid composition and the sensory evaluation of *doenjang* ripened at 30 between mixtures of *doenjang* made by *B. subtilis* PM3 and the culture of *A. oryzae* AJ 100

Doenjang	Ripening time (week)	Volatile organic acid (mg/kg)		Sensory evaluation (raw *doenjang*)		
		Butyric acid	3-Methyl butanoic acid	Sensory score: Taste	Sensory score: Aroma	Result
B. subtilis PM3 (control)	1	29.4	28.8	3.0^{a}	3.5^{abc}	Weakly sweet and acidic odor
	2	29.4	23.8	3.0^{a}	3.0^{a}	Weakly acidic taste and odor
	3	33.1	30.5	3.4^{ab}	3.3^{abc}	Weakly acidic and weakly *doenjang* taste and odor
B. subtilis PM3: A. oryzae AJ100	1	24.2	22.0	4.0^{bc}	4.5^{e}	Weakly unpleasant traditional *doenjang* odor
	2	25.9	22.3	4.0^{bc}	4.0^{cde}	Weakly sweet traditional *doenjang* taste and odor
	3	27.7	15.8	4.1^{bc}	4.0^{cde}	Weakly sweet traditional *doenjang* taste and odor
	5	7.6	10.6	4.2^{c}	4.3^{de}	Sweet traditional *doenjang* taste and odor
F (*p*-value)				3.65***	4.16***	

[1)]Ratio of 1 : 1; ***$p<0.001$.

Table 2. Volatile organic acid composition and the sensory evaluation of soy sauces ripened from mixtures having various mixing ratios of traditional Korean soy sauce and the culture of *A. oryzae* AJ 100

Ripening time (week)	Mixing ratio (traditional soy sauce : *A. oryzae* AJ 100)[1)]	Volatile organic acid (mg/kg)		Sensory evaluation		
		Butyric acid	3-Methyl butanoic acid	Sensory score: Taste	Sensory score: Aroma	Result
1	1 : 0 (control)	30.4	17.2	3.0^{abc}	3.2^{bc}	A salty traditional soy sauce taste and odor
	0 : 1 (control)	63.5	15.5	2.8^{ab}	2.5^{a}	Unmatured soy sauce taste and odor
	3 : 1	7.0	21.7	4.1^{e}	4.5^{d}	Weakly traditional soy sauce taste and odor
2	1 : 0	61.1	35.2	3.1^{abc}	3.3^{bc}	A salty soy sauce taste and odor
	0 : 1	Tr.[2)]	49.8	2.4^{a}	2.7^{ab}	Unmatured soy sauce taste and acidic odor
	3 : 1	Tr.	61.9	4.0^{de}	4.5^{d}	Weakly traditional soy sauce taste and odor
F (*p*-value)				4.861***	13.592***	

[1)]1 : 0, Traditional soy sauce; 0 : 1, culture of *A. oryzae* AJ 100; ***$p<0.001$.
[2)]Trace.

were degraded by native enzymes in *A. oryzae* AJ 100 culture (8). Also, we suggested that many factors, including the reaction time and temperature of the fermentation, as well as the concentration of the *A. oryzae* AJ 100 culture in the *meju*, can influence the efficiency in which butyric acid and 3-methyl butanoic acid are degraded. In this study, we attempted to determine the optimal mixture ratio of *A. oryzae* AJ 100 culture in *meju*, in order to improve the odors of the final soy sauce and soybean paste products. The *A. oryzae* AJ 100 was cultured, and the *meju* was incubated with the *A. oryzae* AJ 100 strain for 7 days at 30°C, and then treated with NaCl (7%). A mixture of *doenjang* made with *B. subtilis* PM3 and the *A. oryzae* AJ 100 culture (1 : 1) was incubated for an additional 1 to 5 weeks at 30°C, after which the concentrations of butyric acid and 3-methyl butanoic acid were determined (Table 1). Here we found that butyric acid and 3-methyl butanoic acid decomposed to a greater degree after 5 weeks of maturation as compared to the mixture allowed to mature for 1 week; however, all of the matured mixtures presented flavors identical to those of traditional *doenjang* (Table 1).

A portion of the *A. oryzae* AJ 100 culture was incubated with soy sauce (3 : 1) for 1-5 weeks at 30°C, after which the concentrations of butyric acid and 3-methyl butanoic acid were assayed (Table 2). The levels of butyric acid in the 5-week cultured samples were significantly less than those in the 1-week cultured samples. However, the concentration of 3-methyl butanoic acid was enhanced after 5 weeks of incubation, as compared to after 1 week of incubation. Overall, the sensory scores of the mixtures incubated for 1-2 weeks were higher than those of the controls. Thus, in the future we must conduct further tests to optimize the degradation of 3-methyl butanoic acid within 1-2 week cultures.

A mixture of *doenjang* and *A. oryzae* AJ 100 culture (3 : 1) was incubated for 1-2 weeks at 30°C (Table 3). Here, the levels of butyric acid and 3-methyl butanoic acid from the samples cultured for 2 weeks were lower than the control levels (Table 3). The sensory scores, which indicated the odor preferences among the samples, were higher in the 2-week cultured samples than the controls (Table 3). However, in the future we still need to determine the optimal mixture ratio, as all of the matured mixtures in this study continued to have bad flavors.

The mixtures of 1-5 g of *doenjang* and 1 g of *A. oryzae*

Table 3. Volatile organic acid composition and the sensory evaluation of *doenjang*s ripened from mixtures having various mixing ratios of traditional *doenjang* and the culture of *A. oryzae* AJ 100

Ripening time (week)	Mixing ratio (traditional *doenjang* : *A. oryzae*)[1)]	Volatile organic acid (mg/kg)		Sensory evaluation				
				Sensory score				Result
		Butyric acid	3-Methyl butanoic acid	Raw *doenjang*		Boiled *doenjang*		
				Taste	Aroma	Taste	Aroma	
1	1 : 0 (control)	30.4	17.2	4.2^{c}	4.3^{b}	4.0^{c}	4.5^{e}	Traditional *doenjang* taste and odor
	0 : 1 (control)	63.5	15.5	3.1^{a}	2.6^{a}	3.0^{ab}	2.2^{a}	A slightly acidic odor
	3 : 1	7.0	21.7	4.2^{c}	4.5^{b}	4.0^{c}	4.1^{de}	Weak traditional *doenjang* odor
2	1 : 0	Tr.[2)]	71.8	3.0^{a}	4.0^{b}	3.1^{c}	3.3^{bc}	Traditional *doenjang* taste and odor
	0 : 1	Tr.	49.8	3.0^{a}	2.5^{a}	2.4^{a}	2.7^{ab}	A slightly acidic odor
	3 : 1	5.3	Tr.	4.0^{bc}	4.5^{b}	4.1^{c}	4.2^{de}	Weak traditional *doenjang* odor
F (*p*-value)				4.458***	13.655***	5.633***	13.948***	

[1)]1 : 0, Traditional *doenjang*; 0 : 1, culture of *A. oryzae* AJ 100; ***$p<0.001$.
[2)]Trace.

Table 4. Volatile organic acid composition and the sensory evaluation of *doenjang* ripened at 30°C from mixtures of traditional *doenjang* and the culture of *A. oryzae* AJ 100 (I)

Sample	Ripening time (week)	Mixing ratio	Volatile organic acid (mg/kg)		Sensory evaluation (raw *doenjang*)		
					Raw *doenjang*		
					Sensory score		Result
			Butyric acid	3-Methyl butanoic acid	Taste	Aroma	
Traditional *doenjang* (control)	1	1 : 0	28.6	1.6	3.3^{abc}	3.0^{ab}	Traditional *doenjang* taste and odor
	2	1 : 0	22.3	0.8	3.0^{ab}	3.0^{ab}	Traditional *doenjang* taste and odor
Traditional *doenjang* : *A. oryzae* AJ 100	1	5 : 1	Tr.[1)]	7.2	3.9^{c}	3.5^{bc}	Sweet *doenjang* taste and odor
		2 : 1	16.6	1.2	3.9^{c}	3.4^{bc}	Sweet *doenjang* taste and odor
		1 : 1	12.5	0.0	3.3^{abc}	2.5^{a}	Mold-like *doenjang* taste and odor
	2	5 : 1	6.3	2.9	3.7^{bc}	3.0^{ab}	Weakly sweet *doenjang* taste and odor
		2 : 1	9.9	1.2	3.7^{c}	4.0^{c}	Sweet traditional *doenjang* taste and odor
		1 : 1	5.8	1.1	3.0^{ab}	3.3^{b}	Weak taste and odor
F (*p*-value)					2.300** (0.009)	3.600*** (0.000)	

[1)]Trace; **$p<0.01$, ***$p<0.001$.

Table 5. Sensory evaluation of *doenjang* ripened at 30°C from mixtures of traditional *doenjang* and the culture of *A. oryzae* AJ 100 (II)

Sample	Ripening time (week)	Mixing ratio	Sensory evaluation (boiled *doenjang*)		
			Sensory score		Result
			Taste	Aroma	
Traditional *doenjang* (control)	1	1 : 0	2.9^{bc}	2.7^{ab}	Traditional *doenjang* taste and odor
	2	1 : 0	1.8^{a}	3.0^{abc}	A salty taste and unpleasant odor
Traditional *doenjang* : *A. oryzae* AJ 100	1	5 : 1	3.1^{c}	3.0^{abc}	Weakly sweet *doenjang* taste and odor
		2 : 1	3.3^{c}	3.4^{bc}	Weakly traditional *doenjang* taste and odor
		1 : 1	3.0^{bc}	3.0^{abc}	Weak mold-like *doenjang* taste and odor
	2	5 : 1	2.3^{ab}	3.3^{bc}	Sweet taste and savory *doenjang* odor
		2 : 1	2.9^{bc}	3.4^{bc}	Sweet taste and savory *doenjang* odor
		1 : 1	2.3^{ab}	3.1^{abc}	Weak taste and odor
F (*p*-value)			5.449*** (0.000)	1.712 (0.066)	

***$p<0.001$.

Table 6. Sensory evaluation of soy sauce ripened from mixtures of traditional Korean soy sauce and the culture of *A. oryzae* AJ 100

Sample	Ripening time (week)	Mixing ratio	Sensory evaluation		
			Sensory score		Result
			Taste	Aroma	
Traditional soy sauce (control)	1	1 : 0	2.9[a]	3.0[ab]	An unpleasant soy sauce taste and odor
	2	1 : 0	3.2[ab]	3.1[ab]	An unpleasant soy sauce taste and odor
Traditional soy sauce : *A. oryzae* AJ 100	1	5 : 1	3.1[ab]	3.3[ab]	Weakly sweet soy sauce taste and odor
		2 : 1	3.1[ab]	3.7[b]	Sweet traditional soy sauce taste and odor
		1 : 1	2.9[a]	3.6[b]	Mold-like soy sauce taste and odor
	2	5 : 1	3.3[ab]	3.7[b]	Weakly sweet soy sauce taste and odor
		2 : 1	3.4[ab]	3.6[b]	Weak mold-like soy sauce taste and odor
		1 : 1	3.3[ab]	3.7[b]	Mold-like soy sauce taste and odor
F (p-value)			1.644 (0.082)	1.803* (0.049)	

*$p<0.05$.

AJ 100 culture, after 1-2 weeks of incubation at 30°C, showed lower levels of butyric acid and 3-methyl butanoic acid than were measured in the controls (Table 4). Table 4 and 5 show the sensory scores of raw and boiled *doenjangs*, respectively, after incubation with the *A. oryzae* AJ 100 culture. In this study, the optimum ratio of Korean soy bean paste to *A. oryzae* AJ 100 culture was found to be 2 : 1. The soy sauce (1-5 g) incubated for 1-2 weeks with *A. oryzae* culture (1 g) at 30°C clearly had desirable flavor (Table 6). In addition, the soy sauce incubated with the *A. oryzae* AJ 100 culture (5 : 1) had a better odor than the other samples cultured under different conditions.

References

1. Nam HY, Min SG, Shin HC, Kim HY, Fukushima M, Han KH, Park WJ, Chio KD, Lee CH. The protective effects of isoflavone extracted from soybean paste in free radical initiator treated rats. Food Sci. Biotechnol. 14: 586-592 (2005)
2. Yang SO, Chang PS, Lee JH. Isoflavone distribution and β-glucosidase activity in *cheonggukjang*, a traditional Korean whole soybean-fermented food. Food Sci. Biotechnol. 15: 96-101 (2006)
3. Jang CH, Lim JK, Kim JH, Park CS, Kwon DY, Kim YS, Shin DH, Kim JS. Change of isoflavone content during manufacturing of *cheonggukjang*, a traditional Korean fermented soyfood. Food Sci. Biotechnol. 15: 643-646 (2006)
4. Kim TJ, Sung CH, Kim YJ, Jung BM, Kim ER, Choi WS, Jung HK, Chun HN, Kim WJ, Yoo SH. Effects of a soaking-fermentation-drying process on the isoflavone and γ-aminobutyric acid contents of soybean. Food Sci. Biotechnol. 16: 83-89 (2007)
5. Lee EJ, Lee HJ, Kim JK. Antimutagenic effects on aflatoxin B1 of soybean pastes fermented by *Bacillus* strains. Food Sci. Biotechnol. 14: 878-880 (2005)
6. Lim SM, Lee GJ, Park SM, Ahn DH, Im DS. Characterization of *Lactobacillus cellobiosus* D37 isolated from soybean paste as a probiotic with anti-cancer and antimicrobial properties. Food Sci. Biotechnol. 15: 792-798 (2006)
7. Park HK, Kim JK. Fermentation microorganisms producing flavors of *kochujang*. pp. 85-98. In: 2005 International Symposium on Functionalities of Hot Red Peppers and Hot Sauce (Microorganisms and Human Well-being). July 1, Korea University, Seoul, Korea. Sunchang County, Sunchang *Kochujang* Research Association, Research Center for Industrial Development of Biofood Materials, Chonbuk National University, Jeonju, Korea (2005)
8. Park HK. Development of novel method for the fermentation and isolation of molds strains that decompose butyric acid, abnormal odor compound, in Korean traditional soy sauce and soybean paste. PhD thesis, Yeungnam University, Gyeongbuk, Korea (2006)
9. Kim JK, Jang SK, Kim SY, Park SY, Kim KS. Distribution of volatile organic acids in traditional Korean soy sauce and micro-organisms producing the organic acids. J. Resource Development, Yeungnam University, Korea 9: 63-69 (1990)
10. Park HK, Lee EJ, Kim JK. Standardization of taste and aroma of traditional Korean soybean paste. J. Resource Development, Yeungnam University, Korea 24: 82-101 (2005)
11. Lee EJ, Kim JK. Characteristics of taste components of *cheonggukjang* fermented with *B. subtilis*. Food Sci. Biotechnol. 13: 572-575 (2004)
12. Park KJ, Kim KJ. Studies on manufactured of the Korean soy sauce. The Report of NIRI 20: 89-98. Seoul, Korea (1970)
13. Tochikuratatz RR. Science and Technics of Soy Sauce. Japan Brewing Association, Tokyo, Japan. p. 112 (1995)
14. Kageyama H, Mori S, Sato O. The simultaneous measurement of volatile fatty acid and lactic acid in the sludge gas chromatography. Anim. Sci. Technol. 44: 465-469 (1972)
15. Johnston MR. Sensory Evaluation Method for the Practicing Food Technologist. IFT Short Course, Boston, MA, USA (1979)