J Food Sci Nutr
Vol 12, p 51～57 (2007)

J Food Science and Nutrition

Changes in Microorganisms, Enzyme Activities and Taste Components of *Kochujang* Added with *Maesil* Extract during Fermentation

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Abstract

The effects of *maesil* extract addition on the palatability and quality of *kochujang*, a traditional Korean condiment, were investigated in terms of the microbial characteristics, enzyme activities, and taste components during 100 days of fermentation. Viable cell counts of bacteria and yeast in *maesil* extract-added *kochujang* (*maesil kochujang*) were increased in proportion to added *maesil* extract from 1 to 5% during fermentation, up to 80 and 20 days, respectively and maintained $5.0\sim14.5\times10^6$ CFU/g. Activities of α-amylase, β-amylase, and protease were also increased in proportion to added *maesil* extract up to 20, 20, and 60 days, respectively and were higher than those of control during the aging process. The major organic acids in *maesil kochujang* were citric and malic acid. Composition and content of free sugar were not changed remarkably in general by the addition of *maesil* extract except maltose. The major free sugars of *maesil kochujang* were in the order of glucose>sucrose>maltose, and glucose content decreased significantly as the ratio of *maesil* extract increased, while maltose content increased significantly ($p<0.05$).

Key words: *maesil kochujang*, microflora, enzyme activities, taste components

INTRODUCTION

Kochujang, a fermented hot pepper-soybean paste, is a popular traditional condiment in Korea and used for a long time along with soybean paste and soy sauce to provide hot, sweet, and savory tastes in foods. The various tastes of *kochujang* originate from raw materials, free sugars and free amino acids produced by microorganism and enzyme hydrolysis of raw materials during fermentation. In addition, alcohols and organic acids produced by yeasts and *Lactobacillus* enhance flavor and tastes of *kochujang* (1-4). *Kochujang* can be classified into two types. One is the traditional *kochujang* using *meju* and the other is commercial *kochujang* using *koji* or bacterial enzymes. Generally, *meju kochujang* is made of glutinous rice, *meju*, red pepper powder, and others, which is fermented by enzymatic reactions of bacteria and yeasts and requires a long fermentation and aging period. This process often generates an off-flavor and unacceptable taste because of various bacteria and fungi. In *koji kochujang*, *koji* which is glutinous rice inoculated with *Aspergillus oryzae*, is used instead of *meju* and fermented for one to three months.

A growing interest has been drawn to *kochujang*, due to its effects on weight and blood-pressure reductions and high poly-γ-glutamate content. It also contains more vitamin B_1, B_2, C, and folic acid than *doenjang* (soybean paste) or *ganjang* (soy sauce) (3). The quality of *kochujang* usually depends on the microorganisms which control the aging process, ratio of raw materials, and fermentation time. Recent studies are mainly focused on intervening the aging process by adding chitosan (1), sake cake (5), natural preservatives (6), alcohol (7). In addition, to improve the functionality of *kochujang*, various attempts were made by adding kiwifruit (8), *Lycium chinense* fruit (9), sea tangle chitosan (10), and apple and persimmon (11).

Prunus mume is a species of Asian plum in the family of Rasaceae. The tree originates from China, but it has also been grown in Korea, Taiwan, and Japan since ancient times (12). The tree is cultivated for its fruit and flowers and the fruit has been commonly used in preserved fruits, wine, herbs, juice, and jam processing. In traditional Chinese medicine, the smoked fruits are used for medicinal purpose. They are generally black in color and are believed to be effective against parasite, as well as in stopping ulcers and promoting a strong digestive system and heart (13-15). *Prunus mume* extracts showed remarkable antimicrobial effects against the wide spectrum of putrefactive and food spoilage microorganisms and also an anticancer activity (16-18).

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The purpose of this study was to examine the quality changes of *maesil kochujang* during 100 days of fermentation in terms of microbiological and enzyme activities and taste components. The results would provide a valuable information for the production of functional *kochujang*.

MATERIALS AND METHODS

Materials

Kochujang premixture was obtained from Poorun Foods Co., Ltd., which was prepared by blending wheat powder (22%), wheat grain (20%), salt (10.5%), and purified water (47.5%). Wheat flour was first steamed with pressure after spraying the warm water and blended with ground wheat grain (inoculated with 0.05% spore suspension of *Aspergillus oryzae* starter and incubated at 35～40°C for 48～52 hr) in uniform sizes and salt, then stored in a fermentation tank for 1 month. *Maesil* extract was purchased from Saehan Maesil Farm (Miryang, Gyeongnam, Korea) and corn syrup (100% corn starch, TS Co., Ltd., Incheon, Korea), red pepper powder, mixed condiments (contained 38% red pepper powder, 15% salt, 7% garlic, and 4% onion), and spirits (Haitai Company, Seoul, Korea) were also obtained from Poorun Foods Co., Ltd. The soluble solid content and pH of *maesil* extract were 68.3°Brix and pH 2.8, respectively.

Kochujang preparation

Kochujang was prepared using the commercially manufacturing practice by Poorun Foods Co., Ltd. Aged *kochujang* premixture and 30% corn syrup were pasteurized at 70°C while blending 8% mixed condiments, 8.6% red pepper powder, 3% spirits, and different amount of *maesil* extract (0, 1, 2, and 5%). The mixtures (designated as 1% MK, 2% MK, and 5% MK, respectively) were then cooled down to 40～45°C, placed in a pot, and aged for 100 days at room temperature (23～24°C) during the periods of August through November of 2005 prior to measurements.

Viable cell counts

Viable cell counts were measured using the modified methods of Oh et al. (19). One gram of *maesil kochujang* was serially diluted tenfold with 0.1% peptone water, and was dispersed on solidified tryptic soy broth agar (Merck KGaA, Darmstadt, Germany) for bacteria and yeast malt extract agar (Becton, Dikinson Company, Sparks, MD, USA) for yeast, respectively. Bacteria were incubated at 30°C for 2～3 days, and yeast at 25°C for 5～6 days, and the colony was counted.

Enzyme activities

The extract of *maesil kochujang* (5 g each) was prepared by shaking in 100 mL distilled water for 4 hr at room temperature and filtered using a Whatman No. 2 filter. The filtrate was used to measure amylase and protease activities (20). For α-amylase activity measurement, 1 mL of filtrate was added to a mixture of 2 mL of 1% soluble starch and 1 mL of 0.02 M phosphoric acid buffer (pH 6.9), and heated at 40°C for 30 min. Ten mL of 1 M acetic acid was then added to stop the reaction, after which 10 mL of 3.33×10^{-4} N iodine solution was added. The activity of the reaction mixture was measured at 660 nm using a UV-spectrophotometer (UV-1201, Shimadzu Co, Ltd., Kyoto, Japan) and expressed as per mL filtrate.

β-Amylase activity was determined by the dinitrosalicylic acid method. A mixture of 1 mL of 1% soluble starch and 1 mL of acetate buffer (pH 4.8) was added to 1 mL of filtrate and heated at 30°C for 10 min. After addition of 3 mL of dinitrosalicylic acid reagent, the absorbance was measured at 660 nm using a UV-spectrophotometer (UV-1201, Shimadzu Co, Ltd., Kyoto, Japan). One unit of β-amylase activity was expressed as the liberation of 1 mg maltose per mL filtrate (21).

One mL of 1% casein dissolved in 0.1 M lactic acid butter (pH 3.0) and 1 mL of distilled water was blended in a test tube and heated at 30°C for 30 min. The reaction was stopped by adding 3 mL of 0.4 M trichloroacetic acid, and filtered. To 2 mL of reaction mixture, 5 mL of 0.55 M Na_2CO_3 and 3 mL of thrice diluted Folin reagent were added. The protease activity was measured at 660 nm by a UV-spectrophotometer (UV-1201, Shimadzu Co, Ltd., Kyoto, Japan), and one unit of the activity was expressed as the liberation of 1 μg tyrosine per g *maesil kochujang* (22).

Organic acids

Ten grams of the sample was diluted with 90 mL distilled water. The mixture was agitated for 30 min at 25°C and filtered twice using a Whatman No. 2 filter and 0.45-μm membrane filter. The filtrate was passed through Sep-pak C_{18} cartridge, and the eluates were analysed using an HPLC (Lab Alliance HPLC System, Science Park Road, PA, USA). The C_{18} analytical column was used and the column temperature was 25°C. The mobile phase used was 0.05 M KH_2PO_4 at 0.7 mL/min. UV-VIS detector was used at 215 nm.

Free sugars

One gram of the sample was diluted with 10 mL distilled water. The mixture was agitated for 10 min and then centrifuged at $10,000\times g$ for 10 min. The supernatant was filtered twice using a Whatman No. 2 filter and 0.45-μm membrane filter. The filtrate was passed through Sep-pak C_{18} cartridge, and the eluates were analyzed using an HPLC (WPP 1506, Waters, Milford, MA, USA). The column used was μ-Bondpak NH_2 (300 mm × 3.9 mm), the eluent was the mixture of acetonitrile and water (85:15) at 1.5 mL/min. Differential refractometer RI detector was used.

RESULTS AND DISCUSSION

Changes in microbial populations

Changes in populations of bacteria and yeasts in *maesil kochujang* during fermentation are shown in Table 1. Bacterial counts of all treatments increased slightly up to 60 days in control, up to 80 days in *maesil kochujang*, then decreased slightly and maintained a level of $8.1\sim12.5\times10^6$ CFU/g. This difference in reaching the maximum viable cell count of bacteria in samples was also shown by Kim et al. (20) with irradiated traditional *kochujang*, Choo and Shin (23) with pumpkin-added *kochujang*, and Ahn et al. (24) with mushrooms. Viable cell counts of bacteria in *maesil kochujang* were increased with increasing *maesil* extract from 1 to 5% during fermentation, showing the highest viable cell count of bacteria, 14.5×10^6 CFU/g in 5% MK. Bacterial counts are still in the range of $10^6\sim10^7$ CFU/g and the similar results were reported with sea tangle chitosan (10) and pumpkin (23) red ginseng (25). In contrast, Choo and Shin (23) reported that the addition of pumpkin did not influence bacterial counts at the end of fermentation. Shin et al. (25) also reported that viable cell count of bacteria in *kochujang* was not affected by the addition of red ginseng and Ahn et al. (24) reported similar results with mushrooms.

The yeast population increased slightly from $6.0\sim8.0\times10^6$ CFU/g at the initial fermentation stage to $7.0\sim9.0\times10^6$ CFU/g after 20 days of fermentation, then decreased to $5.0\sim6.9\times10^6$ CFU/g after 100 days of fermentation. The decrease in yeast count was due to reduction in pH (26). Kim et al. (9) and Shin et al. (27) showed the similar degree of yeast count of 10^6 CFU/g, however, Choo and Shin (23) showed 10^3 CFU/g of viable cell count of yeast. Viable cell counts of yeast in *maesil kochujang* were also increased with increasing *maesil* extract from 1 to 5% during fermentation, showing the highest viable cell count of yeast, 9.0×10^6 CFU/g in 5% MK.

Changes in enzyme activities

Kochujang has sweet taste from a starch hydrolyzate and savory taste from the soybean protein hydrolyzate and nucleic acids (10). Activities of saccharifying amylase was very active up to 20 days of fermentation. This corresponded to the increase of reducing sugar at that time. Rapid decrease of total sugar and reducing sugar indicated that these sugars after 60 days of fermentation might be utilized for alcohol fermentation by yeast or metabolism of microorganism. In general, pattern of decrease in total sugar was very correlated with formation of ethyl alcohol. Activities of α-amylase in *maesil kochujang* were higher than those of control and were increased in proportion to added *maesil* extract from 1 to 5% during fermentation (Fig. 1). Similar results were shown by Oh et al. (28) who reported that α-amylase activity in horseradish powder-added *kochujang* was higher than that of control group and also by Park and Oh (29) who reported that α-amylase activity in traditional *kochujang* increased slightly up to 40 days of *meju* fermentation and then stabilized. In contrast, Choo and

Table 1. Changes in viable cell counts of microorganism of *maesil kochujang* during fermentation (unit: CFU/g)

Microbe	Fermentation (days)	*Kochujang*			
		Control	1% MK[1)]	2% MK	5% MK
Bacteria ($\times10^6$)	0	6.0 ± 0.43^c	7.0 ± 0.78^{bc}	7.5 ± 0.81^b	8.5 ± 0.31^a
	20	6.0 ± 0.51^c	7.0 ± 0.69^{cb}	7.5 ± 0.64^b	8.5 ± 0.93^a
	40	8.0 ± 0.21^d	9.0 ± 0.21^c	10.0 ± 0.26^b	11.5 ± 0.48^a
	60	10.0 ± 0.34^c	11.0 ± 0.33^b	12.0 ± 0.19^{ab}	12.5 ± 0.88^a
	80	8.5 ± 0.21^c	12.5 ± 0.24^b	14.0 ± 0.24^{ab}	14.5 ± 0.72^a
	100	8.1 ± 0.61^b	12.3 ± 0.18^a	12.5 ± 0.43^a	12.4 ± 0.63^a
Yeast ($\times10^6$)	0	6.0 ± 0.38^c	6.5 ± 0.41^{ab}	7.5 ± 0.28^b	8.0 ± 0.81^a
	20	7.0 ± 0.94^d	7.5 ± 0.21^c	8.0 ± 0.36^b	9.0 ± 0.21^a
	40	6.4 ± 0.21^c	7.0 ± 0.88^{bc}	7.5 ± 0.66^b	8.5 ± 0.41^a
	60	5.8 ± 0.46^d	6.5 ± 0.75^c	7.0 ± 0.23^b	7.8 ± 0.58^a
	80	5.3 ± 0.28^c	6.2 ± 0.69^b	6.7 ± 0.93^{ab}	7.3 ± 1.03^a
	100	5.0 ± 0.31^c	5.9 ± 0.58^b	6.3 ± 1.01^{ab}	6.9 ± 0.87^a

[1)]MK: *Maesil kochujang*.
[a-d]Means with different letters in the same row are significantly different according to Duncan's multiple range test ($p<0.05$).

Fig. 1. Changes in α-amylase activity of *maesil kochujang* during fermentation.

Fig. 2. Changes in β-amylase activity of *maesil kochujang* during fermentation (same legend as in Fig. 1).

Shin (23) reported that activities of amylase were not affected by the addition of pumpkin and Kim (30) reported that amylase activities increased at the late stage of fermentation in garlic and onion-added *kochujang*.

β-Amylase activities of *maesil kochujang* increased rapidly up to 20 days of fermentation and slightly decreased until 40 days of fermentation and slowly regained their activities at the end of fermentation (Fig. 2). Activities of β-amylase in *maesil kochujang* were also higher than those of control and were increased in proportion to added *maesil* extract from 1 to 5% during fermentation. Oh et al. also reported with horseradish powder (28) and mustard powder (19) that *kochujang* added with respective condiments had high enzyme activity than control. However, Kim (30) reported that β-amylase activities of *kochujang* added with alcohol, mustard powder, or chitosan increased up to mid fermentation, but at slightly lower level than those of the control group.

Fig. 3. Changes in protease activity of *maesil kochujang* during fermentation (same legend as in Fig. 1).

Protease activity gradually increased during fermentation up to 60 days of fermentation with highest activities at 60 days fermentation in all treatments and rather rapidly decreased at the end of fermentation (Fig. 3). Kim et al. (31) also reported similar findings. Throughout the fermentation period, protease activities of *maesil kochujang* were higher that those of control group. These results were similar to that of previous findings of Kim (30) who reported that neutral protease activity of *kochujang* increased up to mid fermentation and decreased thereafter. Oh et al. (19) also reported that activities of neutral and acidic proteases in *kochujang* added with mustard powder were slightly higher than those of the control group during fermentation.

Changes in organic acids

Organic acids in *kochujang* are mainly produced by microorganisms during fermentations and some are liberated from raw materials. The content and composition of organic acids are also another important factors in the quality of *kochujang*. *Maesil* extract had 0.47 mg% of citric acid, 0.43 mg% of malic acid, 0.25 mg% of oxalic acid, and 0.06 mg% of fumaric acid (32,33). Organic acids in *maesil kochujang* appeared in the order of tartaric acid>succinic acid>citric acid>lactic acid at the early stage of fermentation. After 100 days of fermentation, the order had been changed to citric acid>malic acid>tartaric acid>succinic acid (Table 2). Kim

Table 2. Changes in organic acids of *maesil kochujang* during fermentation (unit: %)

Organic acid	Days	Control	1% MK[1)]	2% MK	5% MK
Succinic acid	0	18.40±0.51[a]	16.04±0.49[b]	17.66±0.63[ab]	15.68±0.21[c]
	60	3.66±0.21[a]	1.83±0.43[b]	1.58±0.18[c]	1.92±0.36[b]
	100	3.50±0.23[a]	1.69±0.40[b]	1.31±0.24[c]	1.70±0.31[ab]
Citric acid	0	14.90±0.12[a]	13.42±0.11[c]	14.68±0.08[ab]	14.30±0.30[b]
	60	19.95±0.23[a]	19.67±0.16[b]	19.53±0.21[b]	18.26±0.32[c]
	100	18.09±0.6[b]	18.56±0.11[a]	18.31±0.23[ab]	17.87±0.56[c]
Tartaric acid	0	17.17±0.33[c]	21.50±0.58[b]	18.78±0.23[c]	23.01±0.45[a]
	60	5.15±0.39[c]	5.20±0.42[c]	5.45±0.32[b]	5.65±0.32[a]
	100	3.50±0.62[a]	3.31±0.25[b]	3.42±0.39[b]	3.45±0.49[b]
Lactic acid	0	6.77±0.26[b]	6.54±0.36[c]	7.03±0.62[ab]	7.68±0.33[a]
	60	[2)]			
	100				
Malic acid	0				
	60	13.09±0.42[c]	13.50±0.91[c]	14.47±0.29[b]	15.52±0.26[a]
	100	12.21±0.53[c]	12.10±0.61[c]	13.13±0.33[b]	14.20±0.86[a]
	Total (day 100)	37.30	35.66	36.15	37.22

[1)]See footnotes in Table 1. [2)]Not detected.
[a-c]Means with different letters in the same row are significantly different according to Duncan's multiple range test ($p<0.05$).

and Song (8) showed that the major organic acids of kiwifruit added traditional *kochujang* were in the order of malic acid>citric acid>succinic acid>acetic acid >lactic acid. Shin et al. (34) also reported that organic acids of traditional *kochujang* produced in Jeonbuk region were in the order of succinic acid>citric acid>lactic acid.

The addition of *maesil* led to significant increase in tartaric, lactic, and malic acid content and decrease in succinic acid ($p<0.05$). During the course of fermentation, the content of succinic acid and tartaric acid were decreased dramatically regardless of sample preparations. These results were different from findings of Kim and Song (8) who showed succinic acid level increased significantly during fermentation in kiwifruit-added traditional *kochujang*. However, Shin et al. (35) showed succinic acid content of traditional *kochujang* prepared with various raw materials decreased during fermentation. In the case of lactic acid, after 60 days of fermentation it disappeared in all samples. In contrast, the content of citric acid increased during the fermentation and peaked after 60 days. On the other hand, malic acid appeared after 60 days of fermentation, adding *maesil* significantly increased the content of malic acid ($p<0.05$). Jung et al. (11) also reported that *kochujang* prepared with apple and persimmon showed that the contents of citric acid and malic acid were higher than that of lactic acid.

Changes in free sugars

Free sugars in *kochujang* are derived from amyloytic enzymes of microorganisms, and their main components are glucose, fructose, and maltose (5,36). Changes in free sugar composition of *maesil kochujang* during fermentation are presented in Table 3. The contents of glucose and sucrose increased about 4 times up to 60 days of fermentation except 5% MK showing about 2 times com-

Table 3. Changes in free sugars of *maesil kochujang* during fermentation (unit: %)

Free sugar	Days	Control	1% MK[1)]	2% MK	5% MK
Glucose	0	3.47±0.23[a]	3.34±0.12[b]	3.23±0.21[b]	3.00±0.26[c]
	60	12.29±0.56[c]	13.91±0.17[a]	13.02±0.51[b]	12.33±0.75[c]
	100	3.07±0.30[c]	3.53±0.26[a]	3.32±0.40[ab]	3.29±0.23[b]
Sucrose	0	0.94±0.08[b]	1.00±0.04[a]	0.88±0.02[c]	1.02±0.06[a]
	60	3.65±0.23[b]	4.00±0.36[a]	3.73±0.46[b]	2.31±0.61[c]
	100	0.96±0.03[c]	1.03±0.02[a]	0.98±0.01[b]	0.96±0.03[c]
Maltose	0	0.03±0.01[c]	0.13±0.02[b]	0.11±0.04[b]	0.45±0.01[a]
	60	0.40±0.05[ab]	0.43±0.05[a]	0.35±0.02[b]	0.26±0.03[c]
	100	0.05±0.02[c]	0.12±0.03[a]	0.12±0.01[a]	0.10±0.02[b]

[1)]See footnotes in Table 1.
[a-c]Means with different letters in the same row are significantly different according to Duncan's multiple range test ($p<0.05$).

pared to the starting value. Afterwards those contents decreases gradually to their original values. Oh and Park (37) also reported increase in sucrose during the fermentation with traditional *kochujang*. Decrease in glucose can be explained by the fact that free sugars consumed by microorganisms as energy source during fermentation. Composition and content of free sugar in *maesil kochujang* were not changed remarkably in general by the addition of *maesil* extract except maltose. Addition of *maesil* extract significantly increased maltose content especially in 5% MK ($p<0.05$). In the case of glucose content, it changed from 3.47% to 3.00% of 5% MK. Addition of 1% *maesil* extract, especially after 60 days of fermentation led to the highest free sugar content in glucose and sucrose.

Quality characteristics of *kochujang* prepared with apple and persimmon indicated the major free sugar during fermentation was maltose (11). Kim and Song (8) showed that the free sugars of kiwifruit-added traditional *kochujang* were mainly glucose (4.01～6.10%), fructose (0.52～0.67%) and maltose (3.43～4.35%) after 60 days of fermentation. Kim (30) also reported that condiments like garlic and onion added *kochujang* have glucose (12.29～15.81%) and fructose (1.25～3.29%). These differences mainly came from variations in fermentation periods, conditions and ingredients. Kim and Song (8) also reported that the contents and composition of free sugar in traditional *kochujang* depended upon the raw materials added.

ACKNOWLEDGEMENT

This research was supported by RIC program of MCIE.

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(Received February 9, 2007; Accepted March 8, 2007)