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A Thesis for the Degree of Master of Science

Quality Evaluation and Hygienic State of Korean Kokjas and Mejus

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한국 곡자와 메주의 품질 평가 및 위생학적 상태

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of Korean Kokjas and Mejus

A dissertation

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Abstract

Kokjas and mejus are known traditional starters in Korea. They were used in the production of traditional Korean sauces and alcoholic beverages. This study was to investigate the quality and hygienic state of kokjas and mejus. The samples were collected from different regions and a total of 4 kokjas and 4 mejus were purchased. They were Gwangju kokja (GJk), Andong kokja (ADk), Busan Sanseong kokja (BSk), Busan Gijang al-kokja (BGk), Andong meju (ADm), Busan Daeyeon meju (BDm), Busan Gijang meju (BGm), and Kyeongnam Hapcheon meju (KHm), respectively.

The samples were evaluated on their color, moisture content, pH and enzyme activity. This study showed that the redness and yellowness values of mejus were larger than those of kokjas. All color parameters displayed no significant difference

($P<0.05$), with the exception of GJk and KHm on the lightness. The highest moisture contents were obtained from BSk as 8.1%, and were followed by ADk as 6.9%. GJk and KHm showed the lowest moisture contents as 3.1%. The range of pH values was shown to be 5.3-6.6. In addition, the glucoamylase and α-amylase activities of the GJk were the highest among kokjas and mejus, 1.70 U/g and 13.20 U/g, respectively. BDm showed relatively high enzyme activity among kokjas and mejus, and the activities of α-amylase, glucoamylase and protease of the BDm were 7.70 U/g, 0.45 U/g and 0.69 mg/min, respectively. The total number of aerobic bacteria was counted in the range of 4.5-7.2 log CFU/g. The lactic acid bacteria were detected only in ADm and BGm, that the total numbers of bacteria were 6.9 log CFU/g and 4.9 log CFU/g, respectively. The results of *Bacillus cereus* were 2.2-5.7 log CFU/g. Total coliforms and *Staphylococcus aureus* were in the range of 2.8-6.3 log CFU/g and 1.8-5.1 log CFU/g, respectively. The total number of yeasts and molds were in the range of 3.4-7.4 log CFU/g and 3.9-6.6 log CFU/g, respectively.

한국 곡자와 메주의 품질 평가 및 위생학적 상태

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요 약

곡자와 메주는 한국의 전통적인 발효 스타터로 알려져 있다. 이들은 한국의 장과 술의 제조에 사용되어 진다. 이 연구에서는 이러한 곡자와 메주의 품질과 위생학적 상태에 대하여 연구하였다. 곡자와 메주의 샘플은 각기 다른 지역에서 구매되었으며, 곡자는 광주곡자(GJk), 안동곡자(ADk), 부산산성곡자(BSk), 부산기장알곡자 (BGk) 4종류이며, 메주는 안동메주(ADm), 부산대연메주(BDm), 부산기장메주(BGm), 경남합천메주(KHm)이었다.

곡자와 메주시료는 색, 수분함량, pH와 효소활성도를 측정하였다. 적색도, 황색도는 메주가 곡자에 비하여 높은 값을 나타내었다. GJk, KHm를 제외한 모든 시료에서의 색차는 유의적인 차이를 보이지 않았다. 수분함량은 BSk가 8.1%로 가장 높았고, 그 다음으로는 ADk가 6.9%인 반면에, GJk와 KHm이 3.1%로 가장 낮았다. pH는 모든 시료가 5.3~6.6의 범위에 존재하였다. 그리고 알파아밀라아제와 글루코아밀라아제의 활성도는 GJk가 각각 13.20 U/g, 1.70 U/g으로 가장 높은 값을 나타내었다. BDm이 알파아밀라아제, 글루코아밀라아제, 프로테아제의 활성도가 각각 7.70 U/g, 0.45 U/g, 0.69 mg/min으로 전반적으로 높은 효소 활성도를 나타내었다. 생균수의 경우

4.5~7.2 log CFU/g의 값을 나타내었고, 유산균은 ADm과 BGm에서만 6.9 log CFU/g과 4.9 log CFU/g으로 발견되었다. *Bacillus cereus*는 2.2~5.7 log CFU/g의 값을 나타내었고, 대장균군은 2.8~6.3 log CFU/g, 황색포도상구균은 1.8~5.1 log CFU/g의 범위를 나타내었다. 효모와 곰팡이의 수는 각각 3.4~7.4 log CFU/g와 3.9~6.6 log CFU/g을 나타내었다.

Ⅰ. Introduction

Kokjas and mejus are two different types of traditional Korean starters. They are widely used in traditional fermented food. Kokja is generally made from wheat, barley, oats millet, rice, and one or two of these grains. Kokjas were used to produce Makgeolli, Cheongju and Soju (Jang JH., 1989). These alcoholic beverages accounted for a large proportion of beverages consumed in Korea. It has been reported that kokja was used for sponge and dough breads production (Choi I et al., 2010). Meju is usually made from soybean and rice, the meju was the main ingredient of fermented sauces such as Doenjang, Ganjang and Kochujang (Kim GT., 2000; Park KY., 2005; Jang SJ., 2014). These fermented sauces are an important part in Korean diet. In general, they were often added to rice to make bibimbap, served as barbecue sauce and used as seasoning in daily cooking to provide taste and nutrient substances (Jeong DW., 2017). Nowadays, traditional fermented beverages and sauces are becoming increasingly popular all over the world because of their high nutritional value and unique flavor (Shin D et al., 2015; Nile SH., 2015). For example, Makgeolli has been found to have various functional benefits such as anti-diabetes, anti-hypertensive and antioxidant effects (Nile SH., 2015; Jeong JW., 2011; Kim HR et al., 2010). However, Kochujang has anticancer, antimutagenic and analgesic effects (Park KY et al., 2003; Kwon DY., 2015). And some of the studies found that capsaicin in Kochujang have anti-obesity effect (Ahn IS et al., 2006; Shin HC et al., 2012).

The quality of fermented foods was affected by raw materials, microflora and fermentation conditions (Park HK., 2003; Jung ST et al., 2008). This showed that kokjas and mejus as raw materials were closely related to the quality of fermented foods (Woo KS et al., 2010; Shin D., 2015). The production of kokjas and mejus was a process of various microbial growth (Hong Y et al., 1997). The distribution of the main microbes during the fermentation of kokjas and mejus has been studied (Song SH., 2013), such as *Aspergillus oryzae*, *Rhizopus oryzae* and *Bacillus subtilis* (Jang SJ et al., 2011; Bal J et al., 2016). Studies showed that there were an existence of various yeasts and fungus in traditional kokjas and mejus, such as *Debaryomyces hansenii*, *Aspergillus tritici* and *Lichtheimia crymbifera* (Bal J et al., 2014). Besides, *Enterococcus durans* and *Bacillus licheniform* accounted for certain proportion in mejus (Kim YS et al., 2011). The growth of these various microorganisms affects the flavor of kokjas and mejus during the fermentation (Ray RR., 2014; Lee TS et al., 2005). A recent study showed that *Bacillus licheniformis* and *Staphylococcus succinus* in meju produced the volatile compounds during soybean fermentation (Jeong DW., 2017). And the enzyme produced by microorganisms also plays an important role in the formation of volatile compounds. For instance, the proteins in soybean were hydrolyzed into amino acids and small peptides by various proteases. The amino acids can be used by microorganisms and further converted into high alcohols, esters, organic acids and some volatile substances (Lee S et al., 2016; Yang S et al., 2013). Similarly, the glucoamylase and amylase were produced by various microorganisms in kokja, these enzymes can transform starch into small molecular substances such as glucose, maltose and dextrin during the fermentation (Ao Z et al., 2012; Tester RF., 1990; Lee SJ et al., 2007).

The production of kokja and meju differs in methods from different geographical environment (Lee JE., 2017). There is still a need for more research on common pathogenic bacteria distribution in there produce (Chen CH., 2001). In recent years, Korean fermented sauces were contaminated by pathogenic bacteria, especially made of meju and it has become a major problem for the safety of fermented sauces (Kim CW et al., 2015). Besides, the common food poisoning was associated with *Bacillus cereus* in contaminated grains and fermented sauces and beverages (Kim YS et al., 2008; Eom JS., 2016; Wilson JW et al., 2002).

This study aimed to investigate the quality and hygienic state of kokjas and mejus. The quality of kokjas and mejus was analyzed by measuring the pH, color, moisture content and enzyme activity. The hygienic state of kokjas and mejus was analyzed by counting the number of various microorganisms.

Ⅱ. Materials and Methods

1. Samples

Kokjas and mejus were collected from the market and factory in different cities in Korea. The detail information of samples were summarized in Table 1.

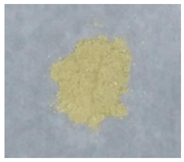
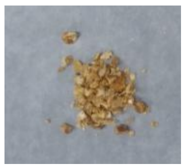
2. Reagents and medium

Trichloroacetic acid, 3,5-dinitrosalicylic acid (DNS), Folin-Ciocalteu's phenol reagent (Sigma A, St. Louis, MO, USA), L-tyrosine, dextrose anhydrous and soluble starch were used for enzyme activity assay. Mannitol Egg Yolk Polymyxin (MYP) medium (Oxoid Ltd, Basingstoke, England) with egg yolk emulsion (Oxoid Ltd, Basingstoke, England) and antimicrobic vial polymyxin (BD, Sparks, NV, USA) were used for the detected of the total number of *Bacillus cereus*. Bromocresol Purple (BCP) agar medium (Eiken C, Nogi, Japan) was used for counting the number of the lactic acid bacteria.

3. Color

The color of each kokja and meju was represented by lightness, redness and

Table 1. Experimental samples

Samples	Manufacture date	Expiration date	Composition	Type
GJk (Gwangju kokja)	-	-	-	
ADk (Andong kokja)	-	2019.03.06	Wheat 100%	
BSk (Busan Sanseong kokja)	2018.07.31	2019.07.31	Wheat 100%	
BGk (Busan Gijang kokja)	-	-	-	
ADm (Andong meju)	-	2018.12.30	Soybean 79.8% Wheat flour 20% Hwanggok 0.2%	
BDm (Busan Daeyeon meju)	-	-	-	
BGm (Busan Gijang meju)	-	-	-	
KHm (Kyeongnam Hapcheon meju)	2017.04.17	2018.04.17	Soybean 100%	

yellowness (Kim YS., 2008). The values of lightness, redness and yellowness were obtained by reflectance tintometer (SP60, Lavibond, Amesbury, UK). Eight petri dishes (90x15 mm) were filled with the powdered samples, and then covered with a clean petri dish lid. The color comparison among samples was based on color values (L-value, a-value and b-value). The tintometer was calibrated with a white standard plate (L= 94.57, a= -1.01, b= +0.01).

4. Moisture contents

Moisture contents of kokjas and mejus were determined by moisture analyzer (MX-50, A&D, Tokyo, Japan). Each sample was weighed 0.5-0.6 g to determine the moisture contents of kokjas and mejus.

5. pH

The pH of kokjas and mejus was measured using pH meter (Thermo, Beverly, LA, USA). Three gram of sample was mixed with 27 ml of distilled water at 25 .

6. α-Amylase activity of Kokjas and Mejus

Each α-amylase extract was made from 2 g of samples and 20 ml of distilled water, and then the mixture was centrifuged at 9,000 rpm for 15 minutes. The supernatant was used as enzyme extract solution. The enzyme activities of kokjas and mejus were measured using the spectrophotometric method. The α-amylase activity assay method was used according to DNS method with a few modifications (Bal J., 2016; Yun SH et al., 2016; Miller GL., 1959). Briefly, the mixture of 0.25 ml of 1% (w/v) soluble starch solution, 0.65 ml of buffer (0.1 N disodium hydrogen phosphate-citric acid, pH 7.0) and 0.05 ml 0.1% of $CaCl_2$ solution was preheated at 37 for 10 minutes. 0.05 ml of enzyme solution was added to the mixture, and then the mixture was incubated at 37 for 20 minutes. And the enzyme mixture solution was incubated at 100 during 10 minutes and then centrifuged at 10,000 rpm for 10 minutes. Subsequently, the mixture of DNS solution (1.8 ml) and supernatant (0.6 ml) was reacted in water bath at 100 for 5 minutes, and then 2.6 ml of distilled water was added to the mixture for the reaction termination. The absorbance at 540 nm was measured with a microplate reader (EPOCH12, Bio Tek, Winooski, VT, USA). One unit of α-amylase activity was expressed as the number of gram of glucose by crude enzyme solution liquefied in 20 minutes.

7. Glucoamylase activity of Kokjas and Mejus

The glucoamylase extract was made of 1 g of sample mixed with 50 ml distilled water, and then the mixture was centrifuged at 9,000 rpm for 15 minutes. Measurement of glucoamylase activity also used DNS method (Lee SH., 2015). The reaction solution contained 1 ml of 2% soluble starch solution, 0.2 ml of buffer (200 mM sodium acetate, pH 5.0) and 100 μl of sample enzyme solution, which was mixed and reacted at 40 for 20 minutes. The reaction solution was stopped by adding 0.2 ml of 1 N NaOH. Then 0.6 ml of DNS solution was added to 200 μl of reaction solution, and the mixture was reacted at 100 for 3 minutes followed by adding 0.4 ml of distilled water to the mixture. Absorbance was measured at 540 nm. The unit of glucoamylase activity was defined as the amount of glucoamylase releasing 1 ml of enzyme extract from soluble starch per minute at 40 .

8. Protease activity of Kokjas and Mejus

The protease extract of GJk, KHm, BGm and BDm was prepared from 1 g of sample and 20 ml of distilled water. The protease extract of BGk, BSk, ADm and ADk was prepared from 1 g of sample and 5 ml of distilled water. And then each mixture was centrifuged at 9,000 rpm for 20 minutes. Protease activity assay used Folin-Ciocalteu method with some modifications (Lee SH., 2015). Briefly, 0.2 ml of substrate (0.6% casein solution was dissolved in 0.1 M phosphate buffer, pH 7.0) was preheated at 37 for 10 minutes. Then, 0.2 ml of enzyme extract was added to the preheated solution. The GJk, KHm, BGm and BDm mixture solution was reacted at 37 for 10 minutes. The BGk, BSk and ADm mixture solution was reacted at 37 for 60 minutes, except for 80 minutes in ADk mixture. All the reaction mixture was terminated by added 0.4 ml of 0.4 M TCA solution. After termination, the reaction mixture was incubated at 37 for 25 minutes followed by centrifugation (12,000 rpm for 15 minutes). Then, the mixture of 0.2 ml of supernatant, 1 ml of 0.4 M Na_2CO_3 and 1 ml of Folin-Ciocalteu's phenol reagent was incubated at 37 for 20 minutes. The absorbance was measured at 660 nm. The standard curve was plotted by using L-tyrosine solution (0-160 μg/ml). One unit of protease activity was expressed as the amount of tyrosine (mg) produced by

1 ml of enzyme at 37 per minute.

9. Microbial detection in Kokjas and Mejus

The total number of aerobic bacteria, *Staphylococcus aureus*, coliforms, yeast and mold in kokjas and mejus was detected by 3M petrifilm (Blackburn CW., 1996). 2 g of kokjas was suspended with 18 ml of phosphate buffer saline (PBS, pH 7.2) as sample suspensions. The sample suspensions were diluted with PBS (pH 7.2) to a serial diluent of 10^{-1} to 10^{-6}. For microbial detection, 1 ml of diluted solution of each sample was sequentially inoculated to the AC (Aerobic bacteria), STX (*Staphylococcus aureus*), EC (Coliform) and Y&M (Yeast and Mold) 3M petrifilm plates (3M Health care, St. Paul, MN, USA). The bacteria colonies were cultured at 35 for 24-48 hours. For yeast and mold, the plates were cultured at 25 for 3 days. *Bacillus cereus* was detected by MYP agar containing 11% egg yolk emulsion and 2% antimicrobic vial polymyxin and cultured at 35 for 24 hours (Drobniewski FA., 1993). Lactic acid bacteria were cultured on BCP agar for 3 days at 36 (Song SH et al., 2013). The total number of bacteria for each sample was expressed as log colony-forming units (log CFU/g).

10. Statistical analysis

The data were analyzed by a SAS statistical software (SAS version 9.4, 2014), and a significant difference of samples were determined by Duncan’s multiple range test at the level of $P < 0.05$.

Ⅲ. Results and Discussion

1. Color of Kokjas and Mejus

The color value of kokjas and mejus was showed in Table 2. The lightness value of BGk, ADk and BSk was significantly higher than that of GJk. Besides, the lightness value of the GJk was different among kokjas. The a value of GJk was the smallest one among kokjas and mejus, and it was indicated that the color of GJk was more dark and greenish than that of other kokjas and mejus. However, the redness values of mejus were higher than those of the kokjas, especially KHm sample. The yellowness values of ADk and BSk were lower than those of other kokjas and mejus, and ADk was very similar to BSk in color. The yellowness values of mejus were over 20, and there was a tiny difference in yellowness among mejus. Similarly, the yellowness values of mejus were higher than those of kokjas. As a whole, the redness and yellowness values of mejus were higher than those of kokjas. Whether it was kokja or meju, the chromatic values between kokjas or mejus were very similar. These results could be explained by the color difference associated with the raw materials of the fermentation starter. The raw materials of kokjas were mainly wheat, while mejus were made of soybean. For instance, the composition of KHm was only soybeans, and BSk was made with wheats. The redness and yellowness values of KHm were larger than BSk because of the composition of raw materials. KHm were more yellowish in color than ADm, which were made of 79.8%

Table 2. Hunter L, a and b values of kokjas and mejus

	L	a	b
GJk	68.64±0[g]	+0.64±0[g]	+17.92±0[e]
ADk	78.59±0.1[b]	+2.39±0[e]	+13.22±0[g]
BSk	76.94±0.1[c]	+1.94±0[f]	+11.95±0[h]
BGk	79.67±0.1[a]	+1.97±0[f]	+16.81±0.1[f]
ADm	72.66±0.1[f]	+6.03±0[c]	+21.06±0.1[c]
BGm	76.35±0.1[d]	+5.69±0[d]	+22.13±0.1[a]
BDm	73.60±0.1[e]	+6.31±0[b]	+20.08±0[d]
KHm	68.82±0.1[g]	+7.13±0[a]	+21.52±0.1[b]

L: lightness, a: redness, b: yellowness

soybeans, 20% wheat flours and 0.2% yellow grains. The color of kokjas and mejus was used as a basis for quality evaluation (Kim YS., 2008). In general, the fermented alcohol beverages with high lightness and low yellowness values were required for extending shelf life. Moreover, the color of kokjas and mejus was changed by the growth of various microorganisms during fermentation. And the chromaticity of mejus was affected by soybean fermentation in the presence or absence of light (Choi UK., 2010). Thus, the color difference of kokjas and mejus in this study may be also affected by different fermentation conditions such as light intensity and fermentation time.

2. Moisture contents of Kokjas and Mejus

The moisture contents of kokjas and mejus were shown in Fig. 1. The moisture contents of ADk and BSk were higher than those of other samples. The moisture contents of GJk, ADk, BSk, BGk, ADm, BGm, BDm and KHm were 3.1%, 6.9%, 8.1%, 4.7%, 3.7%, 4.1%, 3.9% and 3.1%, respectively. In general, the moisture contents of mejus were lower than those of kokjas. The difference in moisture contents between samples was probably due to the raw materials ratio and drying methods. The moisture contents were relatively low compared to other research. The moisture contents of kokjas and mejus were an important microbial growth index, and research suggested that the moisture contents must be kept low level to prevent bacterial contamination (Yu KW et al., 2012). Besides, the moisture contents were influenced by enzyme activities and rheological properties (Choi JY., 2000; Kim YS et al., 2008). It was explained that high moisture contents in kokjas

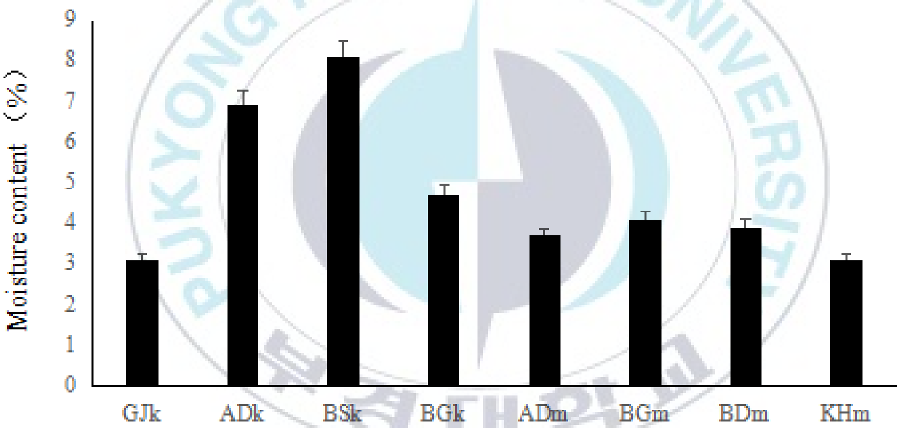


Fig. 1. Moisture content of kokjas and mejus.

and mejus decreased protease production. And there was a correlation between protease activity and moisture contents. Overall, the suitable moisture contents were necessary for the production of high quality kokjas and mejus.

3. pH of Kokjas and Mejus

The pH in kokjas and mejus was determined by pH meter as the range of 5.30-6.52, and the pH value of distilled water was 5.60. There was no difference in pH value among mejus. The results were shown in Fig. 2. The pH values of kokjas and mejus were higher than those of the traditional kokjas and mejus. Similarly, the pH values of all samples were in the range of 4-7 (Bal J et al., 2014). Some studies suggested that the pH values may be an important indicator of fermentation metabolites and microbial growth (Lee SH., 2015; Rousk J., 2009). The pH of kokjas and mejus was changed by metabolites produced by various microbial during fermentation. These molecules can contribute to the flavor of kokjas and mejus. In addition, the growth of pathogenic bacteria were inhibited at low pH level. And fermentative molds had high liquefaction and saccharification ability in a low pH environment (Lee JE et al., 2017). GJk had the lowest pH values among kokjas and mejus, accordingly the GJk had high enzyme activity in kokjas and mejus. Conversely, lactic acid bacteria growth had occurred under an acidic environment that can inhibit the growth of some harmful bacteria, thus extending shelf life for fermented products. And the low pH level were indicated by organic acid produced during fermentation. Hence, the level of pH affects the growth of various microorganisms during fermentation, and further microorganisms had effect on

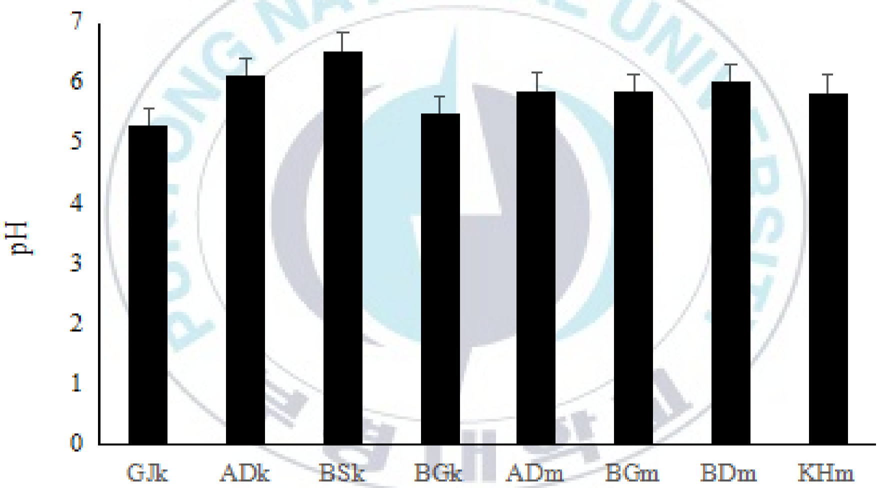


Fig. 2. Comparison in pH in kokjas and mejus.

enzyme activity and flavor of kokjas and mejus.

4. Enzyme activities of Kokjas and Mejus

The enzyme activities of 8 different kinds of kokjas and mejus were shown in Table 3. The glucoamylase activity was the highest in GJk, followed by BDm and ADk. The range of protease activity values of kokjas and mejus was 0.02-0.69 tyrosine mg/min. The range of glucoamylase activities was 0.11-1.70 U/g. Kokjas and mejus with the highest α-amylase activity were shown in GJk (13.20 U/g) and BDm (7.70 U/g), respectively. Besides, BDm had the highest protease activity followed by BGm, the lowest was ADk. The protease activity of kokjas was lower than that of mejus. However, the protease activity of ADm was lower than that of GJk, BGk and BSk because the ADm sample only contained 79.8% soybeans, and close to the deadline. KHm was also an expired sample, but KHm still showed high protease activity among kokjas and mejus because it contained 100% soybeans. The activities of α-amylase and glucoamylase of kokjas were overall higher than those of mejus, whereas the protease activity of kokjas was lower than that of mejus. This showed that the relationship between the raw material composition and enzyme activity of kokja and meju. The activities of glucoamylase, α-amylase and protease were relatively high in BDm and GJk. BDm and GJk had higher enzyme activity in kokjas and mejus. And ADk had the lowest enzyme activity among kokjas and mejus. Therefore, expired or nearly expired samples had a large effect on enzyme activities. In addition, BSk showed high moisture contents among kokjas and mejus, and BGk showed relatively low moisture contents among kokjas and mejus.

Table 3. Enzyme activities of kokjas and mejus

	Enzyme activities		
	α-Amylase (U/g)	Glucoamylase (U/g)	Protease (tyrosine mg/min)
GJk	13.20±2.6[a]	1.70±0.1[a]	0.23±0[c]
ADk	3.40±1.2[c]	0.40±0[b]	0.02±0[d]
BSk	6.00±1.2[b]	0.21±0[d]	0.05±0[d]
BGk	6.14±1.0[b]	0.38±0[bc]	0.09±0[d]
ADm	2.62±0.5[c]	0.11±0[e]	0.03±0[d]
BGm	3.14±0.2[c]	0.31±0[c]	0.57±0[ab]
BDm	7.70±1.3[b]	0.45±0[b]	0.69±0.2[a]
KHm	0.28±0.3[d]	0.15±0[de]	0.52±0[b]

However, the enzyme activities of BSk and BGk were not significantly different among kokjas and mejus. The moisture contents of kokjas and mejus had little effect on enzyme activity. There were great differences in glucoamylase activity of fermentation starters at different pH value (So MH., 1999). Moreover, there were many types of microorganisms in kokjas and mejus, and enzymes were produced by these different microorganisms. The glucoamylase, amylolytic and proteolytic enzymes were produced by *Aspergillus oryzae* (Yang S., 2013). Some molds were found in kokjas and mejus, such as *Absidia* sp., *Rhizopus* sp., and *Mucor* sp., and these molds also can make glucoamylases and α-amylases (Lee JE., 2017). The various microorganisms could be denoted by enzyme activities during the fermentation (Jeong DW et al., 2014). And enzyme activity was an important indicator of quality evaluation. GJk and BDm had high enzyme activity among kokjas and mejus, and the total number of yeast and mold was relatively high proportion in GJk and BDm. Besides, amylases and proteases were involved in saccharification and protein digestion (Yang S., 2011). According to the other researches, the range of α-amylase activity and glucoamylase activity values of kokjas was 0.69-65.53 U/g and 0.01-1.94 U/g, respectively (Lee J., 2017). The range of the protease activity values of kokjas and mejus was 0-1 tyrosine mg/min (So MH., 1999). The α-amylase activity value of kokjas was slightly lower than the data of other research. As a whole, the enzyme activity of kokjas and mejus was affected by pH, raw materials, moisture contents, storage time and microbial distribution.

5. Microbial distribution in Kokjas and Mejus

The range of total number of aerobic bacteria, coliforms, *Bacillus cereus*, *Staphylococcus aureus,* yeasts and molds was 4.5-7.2 log CFU/g, 2.8-6.3 log CFU/g, 2.2-5.7 log CFU/g, 1.8-5.1 log CFU/g, 3.4-7.4 log CFU/g and 3.9-6.6 log CFU/g, respectively. Lactic acid bacteria were not detected in the GJk, ADk, BSk, BGk, BDm and KHm. However, the total number of lactic acid bacteria of ADm and BGm was 6.9 log CFU/g and 4.9 log CFU/g, respectively. The results was shown in Table 4. Besides, the highest total number of aerobic bacteria in kokjas and mejus was ADm (7.2 log CFU/g) and the lowest the number of aerobic bacteria in kokjas and mejus was BSk (4.5 log CFU/g). For the pathogenic bacteria in samples, the total number of *Bacillus cereus* of GJk was significantly higher than the other samples, while there were no significant difference in ADk, BGk, ADm, BGm, BDm and KHm. In addition, the Korean regulations formulated that *Bacillus cereus* content should not exceed 4 log CFU/g in fermented food (Kim CW., 2015). The number of *Bacillus cereus* was 5.7 log CFU/g in GJk perhaps because it was contaminated by unwanted microbes during production. And the total number of *Staphylococcus aureus* in GJk was significantly higher than other kokjas and mejus. The total number of coliforms was relatively difference from each other. The total coliform bacteria number of GJk was only 2.8 log CFU/g, and the total number of BGm was 6.3 log CFU/g. Overall, GJk and BGm were more likely than other samples to have been contaminated. However, the total number of pathogenic bacteria of BSk was below 8 log CFU/g. BSk had a good hygiene state among kokjas and mejus. For yeast and mold, the highest total number of yeasts was GJk (7.4 log CFU/g), and the total number of molds of ADk was the highest among all

Table 4. Total number of total aerobic bacteria, lactic acid bacteria, *Bacillus cereus*, coliforms, *Staphylococcus aureus*, yeast and mold in kokjas and mejus

(Unit: log CFU/g)

	Aerobic bacteria	Lactic acid bacteria	*Bacillus cereus*	Coliforms	*Staphylococcus aureus*	Yeast	Mold
GJk	6.1±0^{c}	ND	5.7±0.2^{b}	2.8±0.1^{f}	5.1±0^{a}	7.4±0^{a}	5.8±0.1^{c}
ADk	5.3±0.1^{e}	ND	3.2±0.1^{e}	5.2±0.1^{b}	1.8±0.1^{f}	5.9±0.1^{b}	6.6±0.1^{a}
BSk	4.5±0.1^{f}	ND	2.2±0.2^{g}	3.7±0^{d}	2.1±0.1^{e}	6.2±0^{c}	6.1±0.1^{b}
BGk	6.4±0.4^{b}	ND	3.4±0.1^{c}	4.3±0.1^{c}	3.0±0^{c}	6.1±0.1^{d}	4.9±0.1^{e}
ADm	7.2±0.1^{a}	6.9±0^{a}	3.4±0.1^{d}	3.6±0.2^{d}	4.0±0^{b}	3.4±0.1^{g}	3.9±0.1^{h}
BGm	6.2±0.1^{b}	4.9±0.1^{b}	3.3±0.1^{a}	6.3±0.1^{a}	3.0±0.1^{c}	4.7±0.1^{e}	4.3±0^{g}
BDm	5.2±0.1^{d}	ND	3.2±0.1^{c}	4.3±0.1^{c}	3.1±0.1^{c}	4.7±0.1^{e}	5.0±0.1^{d}
KHm	6.1±0.1^{c}	ND	3.4±0.1^{f}	3.0±0.1^{e}	2.6±0^{d}	4.5±0.1^{f}	4.4±0.1^{f}

ND: not detected in 1.0 g of sample.

kokjas and mejus. The total number of yeasts and molds in ADm was the lowest among kokjas and mejus, 3.4 log CFU/g and 3.9 log CFU/g, respectively. The aerobic bacteria, yeasts and molds had a large proportion in kokjas and mejus in Fig. 3. In particular, there was a large percentage of the number of aerobic bacteria in ADm and KHm, the proportion of yeast colonies in GJk was significant. And there were a large proportion of molds in ADk and BSk. In addition, it has been reported that fungi, yeast and bacteria were widely present in kokjas and mejus. And the bacterial microflora were dominated in traditional kokjas and mejus. These microorganisms in kokjas and mejus were very important for the quality of kokjas and mejus (Bal J et al., 2014). Moreover, the growth of various microbes has high correlation with moisture contents, enzyme activity, pH, color and temperature. A large number of pathogenic bacteria were detected in GJk. The moisture contents of GJk were lower among kokjas and mejus in Fig. 1. These results showed that the beneficial bacteria did not grow adequately because of low moisture content. Besides, some molds such as *Aspergillus flavus* produce more aflatoxins at acidic pH (Bal J et al., 2014). This suggested that fermentation conditions had an effected on the production of beneficial and harmful bacteria. The mashing, fermentation and ripening processes of kokjas and mejus were associated with various microorganisms. A study showed that molds were related with the saccharification process, and yeasts were associated with fermentation (Bal J., 2016). ADm had a relatively low proportion of yeasts and molds among kokjas and mejus, and ADm showed lower enzyme activity in Table 3. However, there were a high percentage of yeasts and molds and a low level of pathogenic bacteria in BSk. High L and low a value of BSk was showed in Table 2. It was indicated that BSk was the best raw

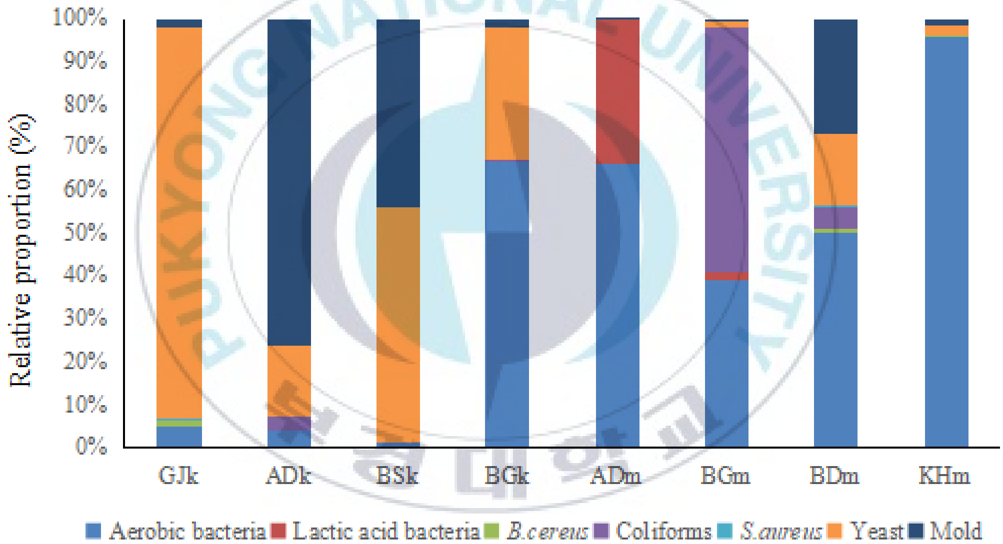


Fig. 3. Comparison of total number of bacteria in kokjas and mejus.

materials for producing high quality Korean traditional food among samples. Some studies showed that the range of the number of total bacteria and fungi was 5.0-12.0 log CFU/10g and 2.0-12.0 log CFU/10g, respectively (Song SH., 2013; Hong Y., 1997). The total number of bacteria in other researches was similar to present study. And the level of molds in kokjas and mejus was lower than the other studies of the data.

Ⅳ. Conclusion

As a traditional starter, kokjas and mejus were continuously improved and widely used in fermentation industry. This study analyzed the quality characteristics, microbial distribution and enzyme activities in kokjas and mejus with different areas of Korea. There were significant differences in the physiochemical properties of kokjas and mejus. BDm had higher enzyme activity than other kokjas and mejus. Besides, BSk had a higher level of hygienic state because of the lower proportions of *Bacillus cereus*, total coliforms and *Staphylococcus aureus*. Although this study investigated the quality and hygienic state of a few kokjas and mejus, the data showed the current hygienic state of fermented starters in Korea some regions. Further, it still need more attention about the pathogenic bacteria contamination problem of traditional fermented products. Only a good manufacture environment and starter can produce high quality fermented foods.

Ⅴ. References

Ahn I. S., Do M. S., Kim S. O., Jung H. S., Kim Y. I., Kim H. J., and Park K. Y., Antiobesity effect of Kochujang (Korean fermented red pepper paste) extract in 3T3-L1 adipocytes. Journal of Medicinal Food, 2006, 9: 15-21.

Ao Z., Quezada-Calvillo R., and Nichols Jr B. L., The nature of raw starch digestion. Journal of Pediatric Gastroenterology and Nutrition, 2012, 55: S42-S43.

Bal J., Yun S. H., Chun J., Kim B. T., and Kim D. H., Taxonomic characterization, evaluation of toxigenicity, and saccharification capability of *Aspergillus* section *Flavi* isolates from Korean traditional wheat-based fermentation starter nuruk. Mycobiology, 2016, 44: 155-161.

Bal J., Yun S. H., Song H. Y., Yeo S. H., Kim J. H., Kim J. M., and Kim D. H., Mycoflora dynamics analysis of Korean traditional wheat-based nuruk. Journal of Microbiology, 2014, 52: 1025-1029.

Blackburn C. W., Baylis C. L., and Petitt S. B., Evaluation of Petrifilm^{TM} methods for enumeration of aerobic flora and coliforms in a wide range of foods. Letters in Applied Microbiology, 1996, 22: 137-140.

Chen C. H., Ding H. C., and Chang T. C., Rapid identification of *Bacillus cereus* based on the detection of a 28.5-kilodalton cell surface antigen. Journal of Food Protection, 2001, 64: 348-354.

Choi I., and Choi W. S., Effects of Kokja as a fermentation starter on sponge-and-dough bread properties. Journal of the Korean Society for Applied Biological Chemistry, 2010, 53: 50-55.

Choi J. Y., Lee T. S., and Noh B. S., Quality characteristics of the kochujang prepared with mixture of meju and koji during fermentation. Korean Journal of Food Science and Technology, 2000, 32: 125-131.

Choi U. K., and Bajpai V. K., Comparative study of quality characteristics of meju, a Korean soybean fermentation starter, made by soybeans germinated under dark and light conditions. Food and Chemical Toxicology, 2010, 48: 356-362.

Drobniewski F. A., *Bacillus cereus* and related species. Clinical Microbiology Reviews, 1993, 6: 324-338.

Eom J. S., and Choi H. S., Inhibition of *Bacillus cereus* growth and toxin production by *Bacillus amyloliquefaciens* RD7-7 in fermented soybean products. Journal of Microbiology and Biotechnology, 2016, 26: 44-55.

Hong Y., Kim Y. B., Park S. O., and Choi E. H., Microflora and physiochemical characteristics of nuruk and main mashes during fermentation of a traditional andong soju. Food Science and Biotechnology, 1997, 6: 297-303.

Jang J. H., History of Korean traditional rice wine. Korean J Dietary Cult, 1989, 4: 271-274.

Jang S. J., Kim Y. J., Lee S. H., Park Y. S., Park J. M., and Bai D. H., *Oceanobacillus gochujangensis* sp. nov., isolated from gochujang a traditional Korean fermented food. Journal of Microbiology, 2014, 52: 1050-1055.

Jang S. J., Kim Y. J., Park J. M., and Park Y. S., Analysis of microflora in gochujang, Korean traditional fermented food. Food Science and Biotechnology, 2011, 20: 1435-1440.

Jeong D. W., Heo S., Lee B., Lee H., Jeong K., Her J. Y., Lee K. G., and Lee J. H., Effects of the predominant bacteria from meju and doenjang on the production of volatile compounds during soybean fermentation. International Journal of Food Microbiology, 2017, 262: 8-13.

Jeong D. W., Kim H. R., Jung G., Han S., Kim C. T., and Lee J. H., Bacterial community migration in the ripening of doenjang, a traditional Korean fermented soybean food. Journal of Microbiology and Biotechnology, 2014, 24: 648-660.

Jeong J. W., Nam P. W., and Lee S. J., Antioxidant activities of Korean rice wine concentrates. Journal of Agricultural and Food Chemistry, 2011, 59: 7039-7044.

Jung S. T., Kim M. H., Shin D. H., and Kim Y. S., Isolation and identification of *Bacillus* sp. with high protease and amylase activity from Sunchang traditional kochujang. Food Science and Biotechnology, 2008, 17: 519-526.

Kim C. W., Cho S. H., Kang S. H., Park Y. B., Yoon M. H., Lee J. B., No W. S., and Kim J. B., Prevalence, genetic diversity, and antibiotic resistance of *Bacillus cereus* isolated from Korean fermented soybean products. Journal of Food Science, 2015, 80: M123-M128.

Kim G. T., Hwang Y. I., and Lim S. I., Carbon dioxide production and quality changes in Korean fermented soybean paste and hot pepper-soybean paste. Journal of the Korean Society of Food Science and Nutrition, 2000, 29: 807-813.

Kim H. R., Kim J. H., and Bae D. H., Characterization of yakju brewed from glutinous rice and wild-type yeast strains isolated from nuruks. Journal of Microbiology and Biotechnology, 2010, 20: 1702-1710.

Kim Y. S., Ahn Y. S., Jeong D. Y., and Shin D. H., Isolation and identification of *Bacillus cereus* from fermented red pepper-soybean paste (kochujang), and its heat resistance characteristics. Food Science and Biotechnology, 2008, 17: 123-129.

Kim Y. S., Kim M. C., Kwon S. W., Kim S. J., Park I. C., Ka J. O., and Weon H. Y., Analyses of bacterial communities in meju, a Korean traditional fermented soybean bricks, by cultivation-based and pyrosequencing methods. The Journal of Microbiology, 2011, 49: 340-348.

Kim Y. S., Oh B. H., and Shin D. H., Quality characteristics of Kochujang prepared with different meju fermented with *Aspergillus* sp. and *Bacillus subtilis*. Food Science and Biotechnology, 2008, 17: 527-533.

Kwon D. Y., Chung K. R., Yang H. J., and Jang D. J., Gochujang (Korean red pepper paste): a Korean ethnic sauce, its role and history. Journal of Ethnic Foods, 2015, 2: 29-35.

Lee J. E., and Kim J. H., Concept of Nuruk on Brewing Technology. Brewing Technology. InTech, 2017: 123-134.

Lee J., Restoration of the traditional Korean Nuruk and the brewing characteristics analysis. Journal of Microbiology and Biotechnology, 2017: 896-908.

Lee S. H., Baek S. Y., Kang J. E., Jeon C. O., Kim D. H., Kim M. D., and Yeo S. H., Effects of temperature on the changes of enzymatic activities and metabolite during wheat nuruk fermentation. Microbiology Biotechnology Letters, 2015, 43: 378-384.

Lee S. J., Kwon Y. H., and Kim H. R., Chemical and sensory characterization of Korean commercial rice wines (yakju). Food Science and Biotechnology, 2007, 16: 374-380.

Lee S., Shin K. J., and Lee S. J., Exploring nuruk aroma; Identification of volatile compounds in commercial fermentation starters. Food Science and Biotechnology, 2016, 25: 393-399.

Lee T. S., and Choi J. Y., Volatile flavor components in mash of takju prepared by using *Aspergillus kawachii* nuruks. Korean Journal of Food Science and Technology, 2005, 37: 944-950.

Miller G. L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 1959, 31: 426-428.

Nile S. H., The nutritional, biochemical and health effects of makgeolli–a traditional Korean fermented cereal beverage. Journal of the Institute of Brewing, 2015, 121: 457-463.

Park H. K., Gil B., and Kim J. K., Characteristics of taste components of commercial kochujang. Food Science and Biotechnology, 2003, 12: 119-121.

Park K. Y., Jung K. O., Rhee S. H., and Choi Y. H., Antimutagenic effects of doenjang (Korean fermented soypaste) and its active compounds. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 2003, 523: 43-53.

Park K. Y., and Jung K. O., Fermented soybean products as functional foods: functional properties of doenjang (fermented soybean paste). Asian Functional Foods. CRC Press, 2005: 563-604.

Ray R. R., and Roy S., Tradition trend and prospect of fermented food products: A brief overview. World Journal of Pharmacy and Pharmaceutical Sciences, 2014, 3: 272-286.

Rousk J., and Brookes P. C., Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology, 2009, 75: 1589-1596.

Shin D., and Jeong D., Korean traditional fermented soybean products: Jang. Journal of Ethnic Foods, 2015, 2: 2-7.

Shin H. C., Cho J. Y., Lee K. H., Ma Y. K., Jeong J. H., Lee H. J., Park K. H., and Moon J. H., Diterpene glycosides from Korean fermented red pepper paste (gochujang) and their origin. Food Chemistry, 2012, 130: 1024-1030.

So M. H., Characteristics of a modified Nuruk made by inoculation of traditional Nuruk microorganisms. Korean Journal Food Nutrition, 1999, 12: 219-225.

Song S. H., Lee C., Lee S., Park J. M., Lee H. J., Bai D. H., Yoon S. S., Choi J. B., and Park Y. S., Analysis of microflora profile in Korean traditional nuruk. Journal of Microbiology and Biotechnology, 2013, 23: 40-46.

Tester R. F., and Morrison W R. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. Cereal Chemistry, 1990, 67: 551-557.

Wilson J. W., Schurr M. J., and LeBlanc C. L., Mechanisms of bacterial pathogenicity. Postgraduate Medical Journal, 2002, 78: 216-224.

Woo K. S., Ko J. Y., Song S. B., Lee J. S., Oh B. G., Kang J. R., Nam M. H., Ryu I. S., Jeong H. S and Seo M. C., Physicochemical characteristics of Korean traditional wines prepared by addition of sorghum (Sorghum bicolor L. Moench) using different Nuruks. Journal of the Korean Society of Food Science and Nutrition, 2010, 39: 548-553.

Yang S., Choi S. J., Kwak J., Kim K., Seo M., Moon T. W., and Lee Y. W., *Aspergillus oryzae* strains isolated from traditional Korean Nuruk: fermentation properties and influence on rice wine quality. Food Science and Biotechnology, 2013, 22: 425-432.

Yang S., Lee J., Kwak J., Kim K., Seo M., and Lee Y. W., Fungi associated with the traditional starter cultures used for rice wine in Korea. Journal of the Korean Society for Applied Biological Chemistry, 2011, 54: 933-943.

Yu K. W., Lee S. E., and Choi H. S., Optimization for rice koji preparation using *Aspergillus oryzae* CJCM-4 isolated from a Korean traditional meju. Food Science and Biotechnology, 2012, 21: 129-135.

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