Properties of *Doenjang* (Soybean Paste) Fermented with Multiple Starters

Min Jeong Cho¹ · Jae Yong Lee² · Kang Wook Lee¹ · Kye Man Cho¹ · Chang Kwon Lee⁴ · Gyeong Min Kim³ · Jeong Hee Shin³ · Jong Sang Kim³ · Hyun-Jin Kim³ · Jeong Hwan Kim¹,²

¹Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Korea
²Division of Applied Life Science (BK21 Plus), Graduate School, Gyeongsang National University, Jinju 660-701, Korea
³Department of Food Science, Gyeongnam National University of Science and Technology, Jinju 660-758, Korea
⁴Mong-Go Foods Co., Ltd., Changwon 641-465, Korea
⁵Garlic Research Institute, Namhae 668-812, Korea
⁶School of Applied Biosciences and Food Science and Biotechnology, Kyungpook National University, Daegu 702-701, Korea

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ABSTRACT

Multiple starters consisting of two *Bacillus amyloylqefaciens* strains (MJ1-4 and EMD17), *Pichia farinosa* SY80, and *Rhizopus oryzae* were used for *Doenjang* making. *Bacillus* strains were selected based on their abilities to inhibit toxigenic fungi and *Bacillus cereus*, fibrinolytic activity, and their ability to confer good flavor to *Cheonggukjang*. *P. farinosa* SY80 and *R. oryzae*, previously isolated from soy sauce, were selected because they were not inhibited by two bacilli. *Doenjang* was prepared by inoculation of multiple starters (A1 *Doenjang*). Control *Doenjang* was prepared by inoculation of *B. subtilis* KACC 16750 (Natto strain) and *Aspergillus oryzae* KCCM 60166 (A2 *Doenjang*). Another control (A3 *Doenjang*) was prepared by inoculation of microorganisms present in rice straw. *Doenjang* samples were fermented for 70 days at 20°C. pH of 3 samples decreased from the initial value of 6.4 to 5.8~6.0 and titratable acidity (TA) increased from 0.6 to 1.1~1.3. The amount of amino-type nitrogen increased during fermentation. There were slight differences in moisture, crude-protein, and crude-fat contents after 70 days. Contamination of fungi was observed only in A3 *Doenjang* and *B. cereus* was not detected from all 3 samples. A1 *Doenjang* showed the highest fibrinolytic activity and A2 *Doenjang* the second. These results indicate that *Doenjang* made with carefully selected starters was functionally improved and microbially more safe.

Key words - *Doenjang*, Fermentation, Multiple starters, Food safety

¹Corresponding author: Jeong Hwan Kim
Tel: +82-55-772-1904
Fax: +82-55-772-1909
E-mail: jeonghkm@gnu.ac.kr
I. Introduction

Soybean is a major protein sources for the Asian people. In particular, fermented soybean foods such as Cheonggukjang, Doenjang (soybean paste), and Ganjang (soy sauce) have served as side dishes, providing proteins for Korean (Choi et al., 2002). In general, Doenjang, and Ganjang are made by soaking Meju, fermented soybeans with a brick shape and serving as a source for proteases and amylases, in brine. Traditionally Meju is produced from soybean. Soybean soaked in water overnight is boiled and cooled, and the soybeans are molded into a brick shape. Then obtained Meju is overhang in the air for 3-4 months (Chang & Chang 2007). During this period, various microorganisms proliferate on the surface and inside of Meju. Among the various microorganisms, fungi such as Aspergillus oryzae and bacilli such as B. subtilis are the most important ones and contribute to the degradation of soy proteins and carbohydrates, generating flavor and taste (Ko et al., 2010).

Doenjang is prepared by adding brine to Meju and fermentation continues for several months in an earthenware pot. Fermented soybean foods including Doenjang and Ganjang prepared by traditional ways have always been exposed to a danger, contamination by toxinogenic microorganisms because the fermentation processes are carried out in open environments (Gálvez et al., 2008). One of the methods to reduce the contamination is the use of starters for Meju preparation. Starters producing antifungal and/or antibacterial substances are useful. Especially bacteriocins from lactic acid bacteria (LAB) are promising as possible food preservatives because most LAB are considered as safe organisms (GRAS class) (Gálvez et al., 2007). Not only LAB but also some bacilli such as B. subtilis, B. licheniformis, and B. amyloliquefaciens are GRAS and some strains produce diverse antimicrobial substances (Stein 2005). Therefore it is reasonable to select Bacillus strains with antimicrobial activities and use them as starters for fermented soy foods. In addition to improved microbial safety, another expected advantage of using starter(s) is the improvement in functionality of the fermented foods if starter strains or other strains co-cultured with starters confer some specific functionality. One example is the fibrinolytic activity of some bacilli. In this report, multiple starters consisting of two B. amyloliquefaciens strains (MJ1-4 and EMD17), Pichia farinosa SY80, and Rhizopus oryzae were used for Doenjang preparation. These strains were selected based on their abilities to inhibit toxinogenic microorganism (B. amyloliquefaciens strains), and strong fibrinolytic activity. A fungus and a yeast strain were selected based on their compatibilities with 2 bacilli strains. B. amyloliquefaciens MJ1-4 and EMD17 have strong antifungal activities and inhibit an ochratoxin producing Penicillium species (Lee & Kim 2012). B. amyloliquefaciens EMD17 also inhibits B. cereus strongly (data are not shown). Pichia farinosa SY80 and R. oryzae, both isolated from soy sauce (data are not shown), were not inhibited significantly when grown with two bacilli. Meju was prepared using four organisms and Doenjang was made. Some properties of Doenjang fermented with multiple starters are described and the improvement in functionality and safety of Doenjang were reported.

II. Materials and Methods

2.1 Preparation of Doenjang

Soybean (crop year, 2012) was purchased from
Hamyang Nonghyup (Hamyang-gun, Gyeongnam, Korea). A flow chart for Doenjang making is shown (Fig.1). Soybeans (600 g) were washed, soaked in distilled water for 15 h at room temperature, and then autoclaved for 50 min at 121°C. Cooled soybeans were inoculated with Bacillus strains, Pichia farinosa SY80, and fungi, separately. For A1 whole soybean Meju, B. amylobioferaciens EMD17 and MJ1-4 were grown in LB broth (tryptone 10 g, yeast extract 5 g, NaCl 5 g per liter, pH 7.0) separately until the absorbance value at 600 nm (OD₆₀₀) reached 1.5. The culture was centrifuged at 12,000 x g for 10 min at 4°C. Cell pellet was washed with 2 times using sterile water and resuspended in the same volume of sterile water. Soybeans (100 g each) were inoculated with EMD17 and MJ1-4 cells (1%, v/w, dry soybean weight), separately, and the 2 batch of soybeans were incubated at 37°C for 45 h. P. farinosa SY80 was grown in YM broth (yeast extract 3.0 g, malt extract 3.0 g, peptone 5.0 g, dextrose 10.0 g, per liter, pH 6.2) until the OD₆₀₀ reached 1.5 and cells were recovered. Soybeans (200 g) were inoculated with yeast cells as described above (1%, v/w, dry soybean weight) and incubated at 30°C for 45 h. Soybeans (200 g) were inoculated with Rhizopus oryzae spore suspension (1x10⁸ spores/ml) (1%, v/w, dry soybean weight) and incubated at 25°C for 45 h. After 45 h of fermentation, soybeans were combined (600 g total) and dried at 55°C for 2 days. Dried whole soybean Meju (600 g) were mixed with autoclaved soybeans (600 g) and brine. Prepared Doenjang (Doenjang A1) was 2.9 kg and the final NaCl concentration was 12% (w/v). Control Doenjang (Doenjang A2) was prepared by inoculating 2 well-known starters with high proteolytic activities, B. subtilis KACC16750, a strain isolated from Natto, and Aspergillus oryzae KCCM60166, a Koji fungus. B. subtilis KACC16750 was grown in LB broth until the OD₆₀₀ reached 1.5. Then soybeans (300 g) were inoculated (1%, v/w, dry soybean weight) and incubated at 37°C for 45 h. Soybeans (300 g) were inoculated with A. oryzae spore suspension (1.0 x 10⁸/ml) (1%, v/w, dry soybean weight) and incubated at 25°C for 45 h. Both fermented soybeans were combined and dried at 55°C for 2 days and mixed with cooked soybeans (600 g) and brine, resulting in A2 Doenjang. Another control Doenjang (A3) was prepared as followings. Soybeans (600 g) were covered with rice straw and incubated at 30°C for 45 h. After dried at 55°C for 2 days, soybeans were mixed with cooked soybeans (600 g) and brine. Fermentation of A1 and two control (A2 and A3) Doenjang was proceeded for 70 days at 20°C and Doenjang samples were taken for analyses at

![Flowchart of preparation method of Doenjang](image-url)
time points (0, 15, 30, 45, and 70 day).

2.2 Analyses of Doenjang during fermentation

Moisture contents of Doenjang samples were measured by using a moisture analyzer (MX-50, AND, Tokyo, Japan). Ten gram of Doenjang sample was mixed with 10 ml of distilled water and the pH of Doenjang sample was measured by using a pH meter (DMS, Seoul, Korea). Titratable acidity (TA), crude-protein, and crude-fat contents of Doenjang samples were determined by the methods of AOAC (2000). Doenjang sample (10 g) was mixed with 40 ml of distilled water and then TA was determined using 0.1 N NaOH solution and the result was expressed as % lactic acid.

2.3 Amino-type nitrogen contents of Doenjang samples

Amino-type nitrogen contents were measured using the modified method of formol titration method (Ko et al., 2010). Doenjang samples were first freeze-dried by using a freeze-dryer (FDU-1200, Eyela, Tokyo, Japan). Five gram of freeze dried sample was mixed with distilled water (95 ml) and centrifuged at 11,000 x g for 30 min. Supernatant was adjusted to pH 8.4 by adding 0.1 N NaOH. Subsequently, distilled water (10 ml) and neutral formalin (10 ml) was added to the solution. The solution was titrated with 0.1 N NaOH to reach pH 8.4. The amount of amino-type nitrogen was calculated using the following equation:

\[
\text{Amino-type nitrogen (mg\%)} = \frac{\text{[sample titration (ml)} - \text{blank test (ml)]}}{1.4 \times F \times D \times 100 / S}
\]

Where the constant, 1.4, is the amount (mg) of amino-type nitrogen equal to 1 ml of 0.1 N NaOH, \( F \) is the factor of 0.1N NaOH, \( D \) is the dilution factor, and \( S \) is the sample amount (g).

2.4 Reducing sugar contents of Doenjang samples

The amount of reducing sugar was determined according to the method of Miller (Miller 1959). One gram of freeze dried Doenjang sample was mixed with distilled water (200 ml) and centrifuged at 11,000 x g for 30 min. Supernatant was filtered through a Whatman No.2 filter paper (Waters, Milford, MA, USA). Two milliliter of filtrate was mixed with 2 ml of dinitrosalicylic acid (DNS) reagent, boiled for 5 min, and the \( \text{OD}_{575} \) was measured.

2.5 Fibrinolytic activities of Doenjang samples

Fibrinolytic activities (FA) of Doenjang samples were determined by the fibrin plate method (Jeong et al., 2007; Chang & Chang 2007). Freeze-dried Doenjang sample (1 gram) was first resuspended in 10 milliliter of Tris-HCl buffer (pH 8.0) and then centrifuged and filtered. Each 20 \( \mu \)l of homogenized Doenjang sample was spotted on a fibrin plate. One mU of plasmin (P1867, Sigma, St. Louis, MO, USA) was spotted together on the same plate as a positive control. The plate was incubated for 16 h at 37\(^\circ\)C and the size of clear zone was measured. FA of Doenjang sample was calculated using the following equation:

\[
\text{Fibrinolytic activity (\%)} = \frac{\text{[the area of clear zone of sample} / \text{the area of clear zone of plasmin]}}{100}
\]

2.6 Sensory evaluation

A consumer survey was performed to investigate the acceptance of A1, A2, and A3 Doenjang and the panel consisted of 6 men and 6 women (mean age 26.6 years). Doenjang (40 g) and water (400 ml) were mixed and boiled for 10 min. After
cooling, *Doenjang* was served in 20 ml white paper cups coded with three-digit numbers. Water was provided for mouth rinsing between sample tasting. A standard nine-point scale was used for evaluation of acceptance of samples (1, strongly dislike; 9, strongly like).

### 2.7 Statistical analyses

Results are presented as mean value ± standard deviation (SD). Statistical comparisons were made by analysis of variance (ANOVA) (version 9.2; SAS Institute, Inc., Cary, NC, USA) procedure followed by Duncan’s multiple range tests. A $p < 0.05$ was considered significantly different.

### III. Results and Discussion

#### 3.1 Changes in pH and TA of *Doenjang* during fermentation

During the 70 days of fermentation, pH of *Doenjang* samples decreased and TA increased (Fig. 2). The initial pH of 6.4 decreased to 6.0, 5.9, and 5.8, for *Doenjang* A1, A2, and A3, respectively, at 70 days. TA of *Doenjang* A1, A2, and A3 increased from the initial value of 0.6 to 1.1, 1.1, and 1.3, respectively at 70 days. During *Doenjang* fermentation, pH decreases because microorganisms produce acids, including lactic acid and acetic acid (Kim & Rhyu 2000). Probably, some acid producing microorganisms present in rice straw may proliferate in *Doenjang* A3 and caused the increase in TA (pH decrease).

#### 3.2 Changes in chemical components of *Doenjang* during fermentation

Table 1 shows the changes in moisture, crude fat, and crude protein of *Doenjang* samples. Moisture contents of all 3 samples decreased gradually from the initial 58% to 56% at 70 days. Decrease in the moisture content was caused by natural evaporation during fermentation as reported by other researchers (Park et al., 2009). Crude fat contents of *Doenjang* samples increased from the initial value of 9.8-9.9%
Table 1. Changes in chemical components of Doenjang during fermentation.

<table>
<thead>
<tr>
<th>Doenjang</th>
<th>Fermentation period (day)</th>
<th>Moisture (%)</th>
<th>Crude protein (%)</th>
<th>Crude fat (%)</th>
<th>Amino nitrogen (mg%)</th>
<th>Reducing sugar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0</td>
<td>58.9±0.8</td>
<td>14.2±0.6</td>
<td>9.8±0.4</td>
<td>163.3±8.1</td>
<td>1.30±0.0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>58.1±0.2</td>
<td>14.4±0.3</td>
<td>11.5±1.0</td>
<td>259.0±7.0</td>
<td>4.33±0.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>57.3±0.2</td>
<td>14.3±0.6</td>
<td>12.9±1.0</td>
<td>424.7±8.1</td>
<td>11.49±0.0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>55.8±0.9</td>
<td>14.7±0.5</td>
<td>12.5±0.8</td>
<td>479.7±9.0</td>
<td>6.99±0.1</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>55.9±1.1</td>
<td>14.6±0.4</td>
<td>12.0±0.3</td>
<td>513.3±8.1</td>
<td>6.98±0.4</td>
</tr>
<tr>
<td>A2</td>
<td>0</td>
<td>58.7±0.9</td>
<td>14.3±0.6</td>
<td>9.9±0.2</td>
<td>275.3±4.0</td>
<td>1.42±0.0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>58.3±0.7</td>
<td>14.3±0.4</td>
<td>12.6±1.2</td>
<td>408.3±4.0</td>
<td>4.22±0.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>57.0±0.5</td>
<td>14.5±0.4</td>
<td>12.8±0.7</td>
<td>664.5±7.1</td>
<td>7.87±0.0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>55.6±0.4</td>
<td>14.7±0.2</td>
<td>12.6±0.1</td>
<td>690.7±8.1</td>
<td>7.64±0.1</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>55.6±1.2</td>
<td>14.6±0.4</td>
<td>12.2±0.1</td>
<td>707.8±6.9</td>
<td>7.58±0.8</td>
</tr>
<tr>
<td>A3</td>
<td>0</td>
<td>58.2±0.2</td>
<td>14.3±0.3</td>
<td>9.9±1.5</td>
<td>245.9±5.7</td>
<td>1.37±0.0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>58.1±0.7</td>
<td>14.3±0.8</td>
<td>12.2±0.1</td>
<td>338.3±4.0</td>
<td>3.32±0.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>57.8±0.3</td>
<td>14.2±0.4</td>
<td>12.4±0.6</td>
<td>526.4±7.4</td>
<td>7.36±0.2</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>56.3±0.6</td>
<td>14.7±0.4</td>
<td>12.4±0.8</td>
<td>636.5±7.1</td>
<td>5.67±0.2</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>56.0±1.1</td>
<td>14.6±0.6</td>
<td>11.9±0.2</td>
<td>656.1±3.2</td>
<td>5.50±0.7</td>
</tr>
</tbody>
</table>

to 12.0% (A1), 12.2% (A2), and 11.9% (A3), respectively, at 70 days. No significant changes in crude protein content was observed from all three Doenjang samples during fermentation as reported by Chang & Chang (2007).

Changes in the amino-type nitrogen and reducing sugar contents of Doenjang samples are shown in Table 1. The amino-type nitrogen content is one of the important indicators reflecting the successful fermentation of foods including Doenjang (Kim & Rhyu 2000; Kim et al., 1999). Amino-type nitrogen increased in all three Doenjang samples. During Doenjang fermentation, proteins are degraded into peptides and amino acids, causing the increase in amino-type nitrogen. Amino-type nitrogen content of a fermented food is often used as an indicator for the degree of fermentation. Aspergillus oryzae and Bacillus species are known to secrete various proteolytic enzymes, causing the hydrolysis of soy proteins (Kim & Rhyu 2000; Kim et al., 1999). Sugars such as glucose, fructose, and maltose are responsible for the sweet taste of Doenjang and the amount of total reducing sugars is also an index showing the quality of Doenjang (Kim & Rhyu 2000). In all three Doenjang samples, reducing sugars increased until 30 days and then decreased gradually. Doenjang A1 showed the highest reducing sugar content (11.49%) at 30 days, and Doenjang
Table 2. Changes in fibrinolytic activity of Doenjang during fermentation.

<table>
<thead>
<tr>
<th>Fermentation period (day)</th>
<th>Doenjang A1 (Plasmin (1 nU = 100.0))</th>
<th>Doenjang A2</th>
<th>Doenjang A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>164.0±20.1</td>
<td>136.0±18.3</td>
<td>100.0±6.3</td>
</tr>
<tr>
<td>16</td>
<td>285.0±21.2</td>
<td>169.0±4.1</td>
<td>121.0±3.5</td>
</tr>
<tr>
<td>30</td>
<td>297.0±16.2</td>
<td>172.0±8.2</td>
<td>124.0±7.0</td>
</tr>
<tr>
<td>45</td>
<td>320.0±5.6</td>
<td>181.0±4.2</td>
<td>120.0±5.2</td>
</tr>
<tr>
<td>70</td>
<td>312.0±5.6</td>
<td>175.0±4.2</td>
<td>122.0±1.7</td>
</tr>
</tbody>
</table>

A2 showed the highest value (7.58%) at 70 days. The increase in the reducing sugars is closely related to amylase activities during fermentation. Reducing sugars released from carbohydrates in soybeans are used as nutrients for microorganisms, substrates for the production of alcohol and organic acids. Thus reducing sugars decreased in the late stage of fermentation (Kim et al., 1999).

3.3 Fibrinolytic activity and antimicrobial activity of Doenjang

Doenjang A1 showed the highest FA during the 70 days of fermentation period (Table 2). The highest FA (320%) was observed at 45 days in Doenjang A1. Doenjang A2 showed 181% and Doenjang A3 120% at the same period. B. amyloliquifaciens MJ1-4 with strong antifungal activity (Lee & Kim 2012) also possesses very high FA. This is responsible for the high FA of Doenjang A1. The FA of B. amyloliquifaciens MJ1-4 is impressive when compared with Doenjang A2. B. subtilis KACC16750 was used for fermentation of Doenjang A2 and this strain was isolated from Natto. Doenjang A2 was used as a positive control because it was fermented with two well-known starters, B. subtilis and Aspergillus oryzae. Therefore, Doenjang A2 was expected to possess high FA and indeed it showed higher FA than Doenjang A3, a naturally fermented Doenjang. FA of Doenjang can be increased significantly by using carefully selected strains as starters. One of the advantages of using starter(s) is the enhancement of a specific functionality of a food, i.e. FA of Doenjang. Another more important advantage is the reduction of contamination of a food by spoilage or toxinogenic microorganisms such as B. cereus or toxinogenic fungi.

B. amyloliquifaciens MJ1-4 inhibits some toxinogenic fungi such as Aspergillus species producing aflatoxin B1 and Penicillium species producing ochratoxin (Lee & Kim 2012). B. amyloliquifaciens MJ1-4 also inhibits Listeria monocytogenes ATCC 19111. B. amyloliquifaciens EMD17 inhibits Penicillium species producing ochratoxin in addition to B. cereus ATCC 14579 and L. monocytogenes ATCC 19111 (data are not shown). Therefore, the combined use of 2 strains effectively inhibits growth of toxinogenic or spoilage organisms. During the fermentation period, no contamination of fungi was observed except Doenjang A3. Only inoculated fungi, R. oryzae for A1 and A. oryzae for A2, were observed when A1
and A2 samples were spreaded on agar plates. However, various unidentified fungi grew when Doenjang A3 sample was spreaded onto plates (data are not shown). B. cereus was not detected from all 3 Doenjang samples when samples were spreaded onto B. cereus selective plates (PEMBA, MB cell, Seoul, Korea) (results not shown). B. cereus contamination is one of the serious problems for various foods including fermented soyfoods (Stenfors Arnesen et al., 2004). Starter(s) capable of inhibiting B. cereus effectively has obvious advantage over other starters without inhibiting activities. In this experiment, Doenjang samples were fermented in a closed incubator and this is likely the reason why B. cereus was not detected. If Doenjang samples were fermented under more open environments or Meju was prepared by traditional way, contamination of B. cereus and fungi might happen. Two B. amylophilac crescens strains used in this work effectively inhibit B. cereus when they were used for Cheonggukjang where B. cereus were purposefully inoculated together with B. amylophilac crescent starters (data are not shown). It is possible to prepare Doenjang and other fermented soyfoods more microbiologically safe by using these strains. However, further studies should be performed to increase the antimicrobial capacities of these strains under the real production conditions, more open and uncontrolled environments.

### 3.4 Sensory evaluation of Doenjang

Sensory attributes of three types of Doenjang were evaluated (Table 3). Doenjang A1 was better than Doenjang A2 and A3 in terms of flavor. B. amylophilac crescent MJ1-4 and B. amylophilac crescent MJ1-4 were previously evaluated and selected as starters because Cheonggukjang fermented with each strain gave good flavor whereas other bacilli with antimicrobial activities conferred bad flavor on Cheonggukjang (data are not shown). Doenjang A2 was better than Doenjang A1 and A3 in terms of taste. The overall acceptance were 5.92, 5.33, and 5.25 for A2, A1, and A3, respectively. Although some differences in flavor, taste, and overall acceptance were noticed by panels, no significant differences were not detected among samples. More through studies are needed in the future.

Preparation of Doenjang with improved properties is important because Doenjang is consumed daily in Korean diet. Functionally improved and microbiologically more safe Doenjang is desirable. Use of selected starters can be an efficient method to achieve the goal. In this work, we showed the possibility to improve the functionality and safety of Doenjang by inoculating 2 Bacillus, 1 yeast, and 1 fungus strains isolated from fermented soyfoods.

### Table 3. Sensory evaluation of Doenjang samples.

<table>
<thead>
<tr>
<th>Doenjang</th>
<th>Color</th>
<th>Flavor</th>
<th>Taster</th>
<th>Overall acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6.58±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.17±1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.25±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.33±1.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A2</td>
<td>6.92±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.17±1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.75±1.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.92±1.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A3</td>
<td>6.42±1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.92±1.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.08±1.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.25±1.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are mean ± SD (n=12); Values in the same column with different superscripts are significantly different by Duncan’s multiple range test (p<0.05).
Future studies should be carried out in the area, development of more convenient way to inoculation, evaluation of qualities of Doenjang through long-term storage, and sensory evaluations.

IV. Acknowledgments

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