J. Microbiol. Biotechnol. (2016), 26(9), 1533–1541
http://dx.doi.org/10.4014/jmb.1605.05019

jmb

Properties of Doenjang (Soybean Paste) Prepared with Different Types of Salts

Jae Min Shim[1], Kang Wook Lee[1], Zhuang Yao[1], Hyun-Jin Kim[1,2], and Jeong Hwan Kim[1,2*]

[1]*Division of Applied Life Sci. (BK21 plus), Graduate School, Gyeongsang National University, Jinju 52828, Republic of Korea*
[2]*Institute of Agriculture and Life Sci., Gyeongsang National University, Jinju 52828, Republic of Korea*

Received: May 9, 2016
Revised: June 1, 2016
Accepted: June 5, 2016

First published online
June 10, 2016

*Corresponding author
Phone: +82-55-772-1904;
Fax: +82-55-772-1909;
E-mail: jeonghkm@gnu.ac.kr

pISSN 1017-7825, eISSN 1738-8872

Samples of doenjang (a fermented soybean paste) were prepared with different types of salts; purified salt (PS), 3-year-aged solar salt (SS3), 1-year-aged solar salt (SS1), and bamboo salt (BS, 3rd processing product). For starter doenjang samples, selected starters comprising two bacilli, one yeast, and one fungus were inoculated, whereas for non-starter doenjang samples, microorganisms present in rice straw were inoculated after enrichment. The doenjang samples were fermented for 13 weeks at 25°C. During the fermentation period, SS and BS doenjang samples showed higher bacilli counts as well as much lower yeast counts than PS doenjang. At 13 weeks, yeast counts of starter doenjang samples were 7.75, 5.69, 6.08, and 4.74 log CFU/g for PS, SS3, SS1, and BS doenjang, respectively. For non-starter doenjang samples, counts were 7.17, 5.05, 5.92, and 4.54 log CFU/g for PS, SS3, SS1, and BS doenjang, respectively. SS and BS promoted growth of bacilli but inhibited growth of yeasts compared with PS. *Debaryomyces hansenii* was the dominant yeast in PS doenjang, whereas *Candida guilliermondii* and *Pichia sorbitophila* were dominant in SS and BS doenjang. In the sensory evaluation, SS and BS doenjang scored better than PS doenjang. In conclusion, SS and BS seem better than PS for production of high-quality doenjang.

Keywords: Doenjang, solar salt, bamboo salt, purified salt, yeasts

Introduction

Salt is an essential seasoning used for making traditional Korean fermented foods such as kimchi (fermented vegetables), doenjang (fermented soybean paste), ganjang (a soy sauce made with fermented soybean), and jeotgals (fermented seafoods). Salt inhibits growth of spoilage microorganisms, allows selective growth of salt-tolerant organisms, and contributes to the development of flavors and extension of edible food periods [5, 16]. According to the Korean Food Standards Codex, solar salt is defined as salt consisting of mainly NaCl crystals produced after evaporation of sea water on a salt pond. Crystals in ground, washed, dehydrated, or dried forms are also classified as solar salt [8]. Solar salt contains significant amounts of minerals such as Mg, K, Ca, Fe, Zn, and Cu in addition to Na and Cl, and these minerals are essential for both humans and the growth of microorganisms such as lactic acid bacteria in foods, affecting the quality of fermented foods [11]. Bamboo salt is a special kind of salt obtained by recrystallization of solar salt melted at high temperature. In this process, a bamboo trunk is filled with solar salt, and its top and bottom are sealed with clay, and it is heated in a furnace. Solar salt is melted at 800–1,500°C and then recrystallized as the temperature decreases [13, 19]. The obtained bamboo salt is called 1st bamboo salt and is repeatedly subjected to the same melting-recrystallization process, eventually resulting in 9th bamboo salt [19]. Owing to the lengthy and difficult production procedure, bamboo salt, especially 9th bamboo salt, is believed to possess biofunctionality [19]. Bamboo salt contains a high amount of K, Mg, Fe, and other minerals. The K content of bamboo salt is three times higher than that of solar salt. Brine from bamboo salt is alkaline owing to the presence of minerals, and the pH is near 10 [13].

Solar salt has been widely used for making various fermented foods such as kimchi and doenjang in Korea,

due to its presumed improved functionality compared with pure salt. However, not many studies have been carried out on the qualities of fermented foods prepared with different types of salts. Specifically, the effects of solar salt or bamboo salt on growth of microorganisms have been seldom studied. In this work, doenjang was prepared with four different salts: pure salt (PS), 1-year-aged solar salt (SS1), 3-year-aged solar salt (SS3), and bamboo salt (BS, 3rd processing product). The doenjang samples were fermented for 13 weeks at 25°C. Growth of bacilli and yeasts were examined together with other properties of the doenjang samples during fermentation.

Materials and Methods

Preparation of Whole-Soybean Meju

Locally grown soybeans (Backtae, 2014 crop year) were purchased from Hamyang Nonghyup (Hamyang, Korea). The soybeans were washed with distilled water, soaked in distilled water for 18 h at room temperature, and then autoclaved at 121°C for 15 min. Starter meju was prepared by inoculating selected starter organisms into the cooled soybeans. *Bacillus amyloliquefaciens* MJ1-4 and *B. amyloliquefaciens* EMD17 were separately cultivated for 12 h in Luria-Bertani (LB, tryptone 10 g, yeast extract 5 g, NaCl 10 g, per liter, pH 7.0) broth with shaking (150 rpm) at 37°C, and cells were recovered by centrifugation at 12,000 ×*g* for 10 min. After washing with sterile water two times, cells from each strain were inoculated separately into cooked soybeans (200 g) at 1×10^6 CFU/g. *Pichia farinosa* SY80 was cultivated in yeast mold broth (YM broth; yeast extract 3 g, malt extract 3 g, peptone 5 g, dextrose 10 g, per liter, pH 6.2) with shaking at 30°C. Cells were recovered by centrifugation, washed twice with distilled water, and inoculated into 400 g of soybeans at 1×10^6 CFU/g. *Rhizopus oryzae* was grown on potato dextrose agar (PDA; 4 g infusion from 200 g of potato, dextrose 20 g, agar 15 g, per liter, pH 5.6) plates for 5 days at 25°C. Mycelia and spores were collected using a wooden stick and resuspended in sterile water. After gauze filteration, a spore suspension was obtained, and the number of spores was counted using a hemocytometer (Marienfeld-Superior, Germany). Spores were inoculated into 400 g of soybeans at 1×10^6 spores/g. Inoculated soybeans were incubated for 48 h at 37°C (*Bacillus* strains), 30°C (*P. farinosa*), or 25°C (*R. oryzae*). Whole-soybean meju was prepared by combining soybeans together after fermentation, followed by drying for 48 h at 55°C. The starter strains and whole-soybean meju preparation procedure were described in detail in our previous report [4].

Non-starter meju was prepared by inoculating natural microflora present in rice straw without further isolation and identification of microorganisms. Rice straws were collected at a rice field (Jinju, Gyeongnam, Korea) in April 2015 and cut into 4–5 cm pieces. The rice straw cuts (5 g) were added into LB broth and YM broth (chloramphenicol, 10 μg/ml) and incubated with shaking for 3 days at 37°C and 30°C, respectively. Presumed bacilli and yeast cells were recovered and inoculated into soybeans (400 g each) as described above. Fungi in rice straw were first grown in potato dextrose broth (PDB, pH 5.6) for 4 days at 25°C, after which the culture was spread on PDA plates. The plates were incubated at 25°C until covered by mycelia, and spores were recovered from the mycelia, counted, and inoculated into soybeans (400 g). The inoculated soybeans were fermented for 48 h and non-starter meju was prepared as described above.

Preparation of Doenjang

Doenjang was prepared by mixing dried whole-soybean meju with freshly cooked soybeans (1,520 g), salt, and water (800 g). The final NaCl concentration of the doenjang samples was adjusted to 12% (w/w) by adding different amounts of each salt: 480 g of PS (Hanju, Ulsan, 2015, NaCl 99.18%), 576.68 g of SS3 (Taepong, Sinan, Jeonnam, 2012, NaCl 82.55%), 596.22 g of SS1 (Taepong, 2014, NaCl 79.84%), and 503.56 g of BS (Insanga, Hamyang, Gyeongnam, 2014, NaCl 94.54%, 3rd BS). The moisture contents of PS and BS were 0.1 ± 0.0%, whereas those of SS3 and SS1 were 11.2 ± 0.2% and 14.3 ± 0.2%, respectively. The amounts of Ca (mg/kg) in PS, SS3, SS1, and BS were 58.01 ± 3.65, 262.64 ± 15.01, 193.11 ± 4.17, and 161.72 ± 11.95, respectively. The amounts of Mg (mg/kg) in PS, SS3, SS1, and BS were 9.26 ± 0.86, 955.09 ± 55.91, 756.80 ± 22.11, and 702.95 ± 17.13, respectively. The above data were obtained at the Food Metabolomics Laboratory of Gyeongsang National University.

The weights of the freshly prepared doenjang samples were 4,000, 4,096.68, 4,116.22, and 4,023.56 g for PS, SS3, SS1, and BS doenjang, respectively. The doenjang samples were fermented for 13 weeks at 25°C and analyzed every week.

Viable Cell Counting

Ten grams of each doenjang sample was mixed with 90 ml of peptone water (0.1% (w/v)) and homogenized using a stomacher (Stomacher 80; Seward, USA). The homogenate was filtered through a bag filter (Interscience, France) and serially diluted with peptone water. Diluted samples (0.1 ml) were spread on LB and YM (chloramphenicol, 10 μg/ml) agar plates, and incubated for 24 h at 37°C, and 48 h at 30°C, respectively. Total bacilli counts were calculated after typical bacilli colonies on LB plates were counted and multiplied by a dilution factor. Total yeast counts were calculated by counting colonies on YM plates.

Identification of Yeasts

Ten yeast colonies were randomly selected from YM (chloramphenicol, 10 μg/ml) agar plates inoculated with each doenjang sample (non-starter) after 13 weeks of fermentation. Selected colonies were cultivated in YM broth (chloramphenicol, 10 μg/ml) for 48 h with shaking (150 rpm) at 30°C. The culture (1 ml) was centrifuged at 12,000 ×*g* for 20 min to obtain cells. Genomic DNA was prepared from cells by using a HiGene Genomic DNA Prep kit (Biofact, Korea). The ITS1-5.8S rDNA–

ITS2 region was amplified by using the following primer set: ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCC GCTTATTCATATGC-3′) [17]. A MJ Mini personal thermal cycler (BioRad, USA) was used, and the reaction mixture consisted of 2 µl of genomic DNA, 1 µl of each primer, 0.5 µl of *Ex Taq* polymerase (5 unit/µl; Takara, Japan), 5 µl of 10× buffer, 5 µl of dNTP mixture (2.5 mM each), and 36.5 µl of distilled water. Initial denaturation was carried out at 95°C for 10 min followed by 35 cycles of 95°C for 1 min, 60°C for 30 sec, and 72°C for 1 min. Final extension was carried out at 72°C for 10 min. The PCR product was sequenced at Cosmogenetech (Korea) and the nucleotide sequences were analyzed by BLAST (NCBI, USA).

pH, Titratable Acidity, and Amino-Type Nitrogen Measurements

Doenjang (10 g) was mixed with 40 ml of distilled water, and shaken in a water bath (150 rpm, 30°C) for 1 h, after which the supernatant was obtained by centrifugation (4,000 ×*g*, 20 min). The pH level of the supernatant was measured using a pH meter (DMS, Korea) and titratable acidity (TA) was calculated by titrating the supernatant with 0.1 N NaOH until pH 8.4. The amount of NaOH was used to calculate the amount of lactic acid (%).

Amino-type nitrogen was measured by the formol titration method [14]. Doenjang sample (5 g) was mixed with 100 ml of distilled water, after which the mixture was shaken in a water bath (150 rpm, 30°C) for 1 h. The supernatant from centrifugation (10 ml) was titrated with 0.1 N NaOH until pH 8.4. The sample was mixed with distilled water (10 ml) and formaldehyde solution (pH 8.4, 10 ml) and left to stand for 10 min at room temperature. Titration with 0.1 N NaOH was repeated until pH 8.4 and the amount of NaOH was used to calculate the amino-type nitrogen content as shown below.

Amino-type nitrogen (mg%)
= [sample titration (ml) - blank test (ml)] × 1.4 × F × D × 100/S

where 1.4 corresponds to the amino-type nitrogen (in mg) equivalent to 1 ml of 0.1 N NaOH. F is the factor of 0.1 N NaOH, D is the dilution fold, and S is the amount of sample (5 g).

Moisture Content, Salinity, and Crude Fat and Crude Protein Contents of Doenjang Samples

The moisture contents of the doenjang samples were measured by using an infrared moisture analyzer (MX-50; AND, Japan). For salinity measurements, 10 g of doenjang was mixed with 40 ml of distilled water. The supernatant was obtained after shaking in a water bath and centrifugation as stated above. The salinity of the supernatant was measured by using a saltmeter (PAL-SALT; Atago, Japan). Measurements were repeated three times, and the average values are shown. AOAC methods were used to measure the crude fat and protein contents [1]. Powdered samples after freeze-drying were used. The Soxhlet extraction method was used for crude fat determinations and the Kjeldahl nitrogen titration method for crude protein determinations.

Sensory Evaluation

After fermentation for 13 weeks, doenjang samples were evaluated for their sensory properties. Commercial doenjang (Chungjungone, Korea; salinity 11.65%), prepared in a traditional manner, was included as a control. The color and flavor (delicate flavor, salty, sweet, and sour) were evaluated. Fifty grams of doenjang was mixed with 500 ml of spring water (Samdasoo, Korea), boiled for 10 min, and then provided to panels. The color, flavor, and taste were evaluated based on a 7-point scale, and the overall acceptabilities were rated. Samples were randomly marked using three-digit numbers. Panels consisted of 30 graduate and undergraduate students (male:female = 1:1, average age, 24.8 years old).

Statistical Analyses

All measurements were repeated three times, and the results are shown as the mean ± standard deviation. Sensory data were analyzed by one-way ANOVA and Duncan's multiple range test was done using the SPSS ver. 18 (SPSS Inc., USA) package ($p < 0.05$).

Results and Discussion

Changes in Viable Counts of Bacilli and Yeasts during Fermentation

Viable counts of bacilli and yeasts from doenjang samples were counted every week during fermentation, and data from selected weeks are shown in Table 1. Immediately after doenjang preparation (day 0), BS doenjang had the highest bacilli count (9.36 ± 0.58 log CFU/g) among the four starter doenjang samples. PS doenjang showed the highest bacilli count (9.91 ± 0.07 log CFU/g) at 3 weeks of fermentation, after which the counts decreased to 8.99 ± 0.06 log CFU/g at 5 weeks. After 5 weeks, bacilli counts were maintained until 13 weeks. For SS3 doenjang, the bacilli count was 8.86 ± 0.10 log CFU/g on day 0, increased to 9.55 ± 0.08 log CFU/g at 5 weeks, and reached a maximum of 10.14 ± 0.06 log CFU/g at 11 weeks, followed by a slow decrease to 9.61 ± 0.10 log CFU/g at 13 weeks. The same patterns were observed for the non-starter doenjang samples. Throughout the fermentation period, PS doenjang had lower bacilli counts than SS and BS doenjang. This was probably caused by the higher mineral contents of SS and BS, and these minerals are required for growth of bacilli. No bacilli were detected from PS and BS, and low numbers were detected from the SS samples (2.17 ± 0.08 log CFU/g for SS3, 2.15 ± 0.06 log CFU/g for SS1). The results indicate that the numbers of inherent bacilli in salts were very low, and their presence most likely did not affect the growth of inoculated bacilli. Yeasts were not detected from any of the four salts (results not shown).

Table 1. Changes in the viable cell numbers of bacilli and yeasts during fermentation.

	Sample	Fermentation period (week)							
		0	1	3	5	7	9	11	13
Bacilli (log CFU/g)	A-1[a]	8.79 ± 0.05	8.87 ± 0.07	9.91 ± 0.08	8.99 ± 0.07	9.02 ± 0.04	9.12 ± 0.09	9.37 ± 0.05	8.76 ± 0.11
	B-1[b]	8.86 ± 0.10	8.85 ± 0.04	8.82 ± 0.15	9.55 ± 0.08	9.55 ± 0.07	9.73 ± 0.10	10.14 ± 0.06	9.61 ± 0.10
	C-1[c]	9.14 ± 0.01	9.00 ± 0.08	9.19 ± 0.15	9.25 ± 0.08	9.37 ± 0.05	9.43 ± 0.03	10.04 ± 0.06	9.33 ± 0.03
	D-1[d]	9.36 ± 0.59	9.04 ± 0.05	9.06 ± 0.08	9.24 ± 0.04	9.12 ± 0.07	9.45 ± 0.03	9.42 ± 0.03	9.10 ± 0.09
	A-2	7.81 ± 0.14	8.26 ± 0.22	9.03 ± 0.17	7.85 ± 0.04	8.56 ± 0.05	8.93 ± 0.05	9.19 ± 0.09	8.81 ± 0.08
	B-2	7.69 ± 0.04	7.84 ± 0.05	9.62 ± 0.11	8.19 ± 0.02	8.94 ± 0.08	8.54 ± 0.06	9.44 ± 0.02	8.73 ± 0.08
	C-2	7.72 ± 0.03	7.92 ± 0.04	9.08 ± 0.06	7.98 ± 0.03	8.56 ± 0.08	8.75 ± 0.60	8.12 ± 0.07	8.69 ± 0.22
	D-2	7.59 ± 0.10	7.40 ± 0.53	9.00 ± 0.15	7.87 ± 0.06	8.60 ± 0.13	8.53 ± 0.05	8.53 ± 0.05	8.16 ± 0.06
Yeasts (log CFU/g)	A-1	4.78 ± 0.07	4.89 ± 0.09	7.30 ± 0.03	8.64 ± 0.08	8.39 ± 0.10	8.69 ± 0.09	7.99 ± 0.14	7.75 ± 0.11
	B-1	4.53 ± 0.04	4.69 ± 0.05	4.54 ± 0.07	4.05 ± 0.06	5.36 ± 0.02	4.63 ± 0.12	5.49 ± 0.08	5.69 ± 0.16
	C-1	5.30 ± 0.03	5.55 ± 0.09	4.71 ± 0.16	5.00 ± 0.05	5.34 ± 0.35	4.93 ± 0.06	5.80 ± 0.08	6.08 ± 0.08
	D-1	4.84 ± 0.12	4.92 ± 0.06	4.60 ± 0.04	4.71 ± 0.06	4.57 ± 0.10	4.51 ± 0.18	5.31 ± 0.03	4.74 ± 0.13
	A-2	5.55 ± 0.05	5.39 ± 0.04	6.36 ± 0.04	8.29 ± 0.01	8.06 ± 0.07	8.14 ± 0.05	7.05 ± 0.06	7.17 ± 0.07
	B-2	4.92 ± 0.07	4.87 ± 0.15	4.48 ± 0.05	3.80 ± 0.05	4.80 ± 0.06	4.47 ± 0.07	4.46 ± 0.02	5.05 ± 0.04
	C-2	5.46 ± 0.13	5.61 ± 0.12	4.73 ± 0.06	4.44 ± 0.06	5.02 ± 0.55	4.56 ± 0.05	5.09 ± 0.07	5.92 ± 0.07
	D-2	5.33 ± 0.07	5.45 ± 0.03	5.13 ± 0.02	5.14 ± 0.05	4.72 ± 0.21	5.25 ± 0.08	5.06 ± 0.03	4.54 ± 0.09

[a]A denotes PS doenjang, [b]B denotes SS3 doenjang, [c]C denotes SS1 doenjang, and [d]D denotes BS doenjang. 1 indicates starter doenjang and 2 indicates non-starter doenjang.

Yeast counts showed the most significant differences among the doenjang samples. PS doenjang (starter and non-starter) had much higher yeast counts than the other doenjang samples. Yeast counts were similar among all the doenjang samples on day 0 and the 1st week of fermentation, whereas yeast counts of PS doenjang increased rapidly after the 1st week and those of the other doenjang were maintained or decreased slightly (Table 1). For PS doenjang, the highest yeast counts were observed at 5 weeks for non-starter and 6 weeks for starter doenjang. After 6 weeks of fermentation, yeast counts of PS doenjang were 8.69 (starter, not shown) and 8.14 (non-starter, not shown) log CFU/g. At the same time, those of SS3 were 4.63 (starter, not shown) and 4.47 (non-starter, not shown) log CFU/g, those of SS1 were 4.93 (starter, not shown) and 4.56 (non-starter, not shown) log CFU/g, and those of BS were 4.51 (starter, not shown) and 5.25 (non-starter, not shown) log CFU/g. The yeast counts of PS doenjang were 1,000–10,000 times higher than those of the other doenjang samples. The yeast counts of PS doenjang were maintained until 9 weeks and then gradually decreased. After 13 weeks, the yeast counts of PS doenjang were 7.75 (starter) and 7.17 (non-starter) log CFU/g, those of SS3 doenjang were 5.69 (starter) and 5.05 (non-starter) log CFU/g, those of SS1 were 6.08 (starter) and 5.92 (non-starter) log CFU/g, and those of BS were 4.74 (starter) and 4.54 (non-starter) log CFU/g. Although the differences in yeast counts between PS doenjang and the other doenjang samples decreased at 13 weeks compared with those at 6 weeks, PS doenjang still showed 100–1,000 times higher yeast counts than the other doenjang samples.

Yeasts produce alcohol and other metabolites, which confer some desirable flavors to doenjang. However, overgrowth of yeasts is considered to be undesirable for the quality of doenjang. Other metabolites are responsible for the unpleasant flavor of doenjang and produce CO_2, which causes swelling of doenjang during storage [15]. In this respect, SS and BS have an advantage over PS for doenjang preparation since they prevent excessive growth of yeasts during doenjang fermentation. It is suspected that some compound(s) present in SS and BS, but absent from PS, discouraged growth of yeasts. Many minerals are present in SS and BS, and one or more compounds in combination might be responsible.

A similar result was reported for kimchi fermentation. Chang *et al.* [2] prepared kimchi with 4-year-aged SS (SS4), 1-year-aged SS (SS1), and purified salt (PS), followed by storage for 5 months at −1°C. From PS kimchi, yeasts were detected (2.1 log CFU/ml) at 1 month and the counts increased to 5.1 log CFU/ml at 5 months. For SS1 kimchi, yeast counts were 1.1 log CFU/ml at 1 month and 4.7 log

CFU/ml at 5 months. For SS4 kimchi, yeasts were not detected until 4 months and the count was 1.3 log CFU/ml at 5 months [2]. No studies have yet investigated the effect of salts on yeast counts of doenjang until this work. Considering both the previous reports and our results, it is clear that SS and BS promote growth of some microorganisms while inhibiting growth of others. Growth stimulation of bacilli by SS and BS is generally beneficial for the quality of doenjang, since bacilli secrete enzymes hydrolyzing the proteins and starch of soybeans, thereby accelerating ripening of and conferring favorable tastes to doenjang. Use of SS and BS for doenjang fermentation has two advantages: growth stimulation of bacilli, and prevention of yeast overgrowth. Further studies on the metabolomics of doenjang prepared with different salts are necessary to correlate different microflora with different metabolite profiles.

Identification of Yeasts

A total of 40 yeast colonies were selected from non-starter doenjang samples fermented for 13 weeks at 25°C. Four yeast species were identified: *Debaryomyces hansenii*, *Candida guilliermondii*, *Pichia sorbitophila*, and *Wickerhamomyces anomalus* (Table 2). Among the 10 colonies from PS doenjang (A-2), nine were identified as *D. hansenii*, whereas one was not identified since its PCR product was not amplified.

For SS3 doenjang (B-2), six were *C. guilliermondii*, two were *P. sorbitophila*, one was *D. hansenii*, and one was unidentified. For SS1 doenjang (C-2), six were *C. guilliermondii*, two were *P. sorbitophila*, one was *D. hansenii*, and one was *W. anomalus*.

Table 2. Identification results for yeast from non-starter doenjang at 13 weeks.

Doenjang (non-starter)	Most matched yeast (% identity) [a]	Matched colonies / total colonies (%)
PS	*Debaryomyces hansenii* (100.0%)	9/10 (90%)
	Unidentified	1/10 (10%)
SS3	*Debaryomyces hansenii* (99.0%)	1/10 (10%)
	Candida guilliermondii (100.0%)	6/10 (60%)
	Pichia sorbitophila (99.0%)	2/10 (20%)
	Unidentified	1/10(10%)
SS1	*Debaryomyces hansenii* (99.0%)	1/10 (10%)
	Candida guilliermondii (100.0%)	6/10 (60%)
	Pichia sorbitophila (99.0%)	2/10 (20%)
	Wickerhamomyces anomalus (100.0%)	1/10 (10%)
BS	*Candida guilliermondii* (100.0%)	5/10 (50%)
	Pichia sorbitophila (99.0%)	5/10 (50%)

[a]ITS (550 bp) was amplified and sequenced. BLAST was used to find the species.

For BS doenjang (D-2), five were *C. guilliermondii* and five were *P. sorbitophila.* Although the total sample numbers (10 colonies from each sample) were not large enough to make any conclusion, the identification results still indicate that salt type might determine the dominant yeast species in doenjang. For example, nine out of 10 colonies were identified as *D. hansenii* from PS doenjang. However, *D. hansenii* was detected just once from SS3 and SS1 doenjang, and not at all from BS doenjnag. Thus, *D. hansenii* might be a major constituent of PS doenjang, whereas *C. guilliermondii* together with *P. sorbitophila* were the dominant species in SS and BS doenjang.

Yeasts such as *D. hansenii*, *C. mogii*, *P. anomala*, *Zygosaccharomyces rouxi*, *Absidia corymbifera*, and *Sterigmatomyces halophilus* were detected from homemade and commercial doenjang samples using a culture-independent method [12]. Certainly, the quality of doenjang is affected by both the species and number of yeasts to some extent. *D. hansenii* is a moderate halophile that grows optimally at 3–5% (w/v) salt content but can tolerate up to 25% [18]. *D. hansenii* grows better than *Saccharomyces cerevisiae* at high K^+ and Na^+ concentrations and produces a high amount of lipids [18]. A high proportion of *D. hansenii* in PS doenjang is likely to affect metabolite profiles and contribute to some properties of PS doenjang.

pH, Titratable Acidity, and Amino-Type Nitrogen Measurements

Immediately after doenjang preparation, the pH levels of BS doenjang were 8.33 (starter) and 7.8 (non-starter), which were higher than those of the other doenjang (pH 6.0–6.8). This can be attributed to the high mineral content of BS, which contains large amounts of K, Si, Fe, and PO_4 and has an alkaline aqueous solution (pH 10) [13]. The high pH values of BS doenjang were observed throughout the fermentation period (Fig. 1). For starter doenjang, the pH values were maintained until 7 weeks, and then gradually decreased. At the end of fermentation, the pH of starter doenjang was in the range of 5.90–6.47, whereas those of non-starter doenjang were 5.83–6.42. Starter doenjang showed higher pH values than non-starter doenjang for most of the fermentation period. It was reported that the pH of doenjang decreases during fermentation, since acids such as propionic acid, lactic acid, and acetic acid are produced by lactic acid bacteria and yeasts [3, 11]. For all doenjang samples, the pH values decreased gradually, while the titratable acidity (TA) increased gradually, which is in agreement with previous reports [3, 11]. At 13 weeks, PS doenjang showed lower pH values and higher TA values than those of SS and BS doenjang. BS doenjang

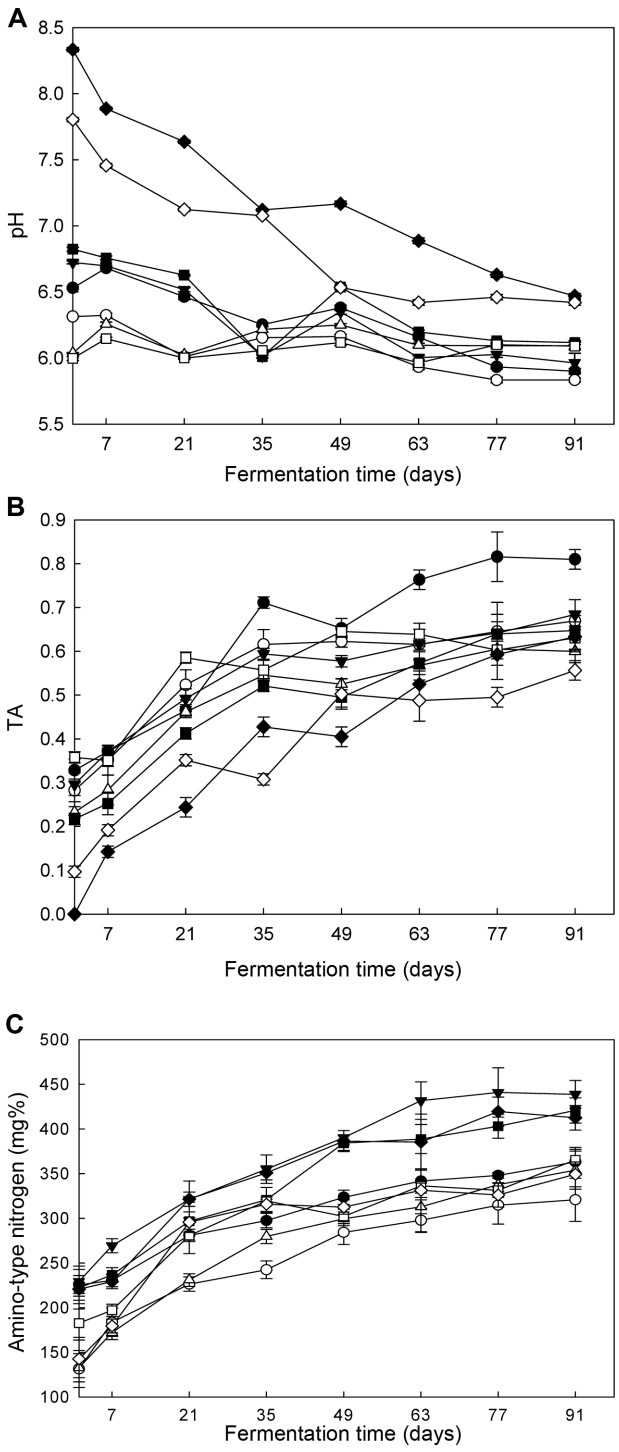

Fig. 1. Changes in pH (**A**), titratable acidity (**B**), and amino-type nitrogen contents (**C**) of doenjang samples.
●, PS doenjang (starter); ○, PS doenjang (non-starter); ▼, SS3 doenjang (starter); △, SS3 doenjang (non-starter); ■, SS1 doenjang (starter); □, SS1 doenjang (non-starter); ◆, BS doenjang (starter); ◇, BS doenjang (non-starter).

showed the highest pH and lowest TA values, whereas SS doenjang showed intermediate values. The results indicate that different salt types affected the pH and TA values of doenjang.

Starter doenjang samples showed higher amino-type nitrogen contents than non-starter doenjang throughout the fermentation period (Fig. 1). Among the starter doenjang samples, SS3 doenjang showed higher values than the other doenjang samples. For non-starter doenjang samples, SS1 and BS doenjang showed higher values. The values increased gradually from day 0 until the end of fermentation. After 13 weeks, the amino-type nitrogen contents of starter doenjang were 363.27 ± 13.97 (PS), 438.90 ± 15.51 (SS3), 420.84 ± 14.03 (SS1), and 412.51 ± 13.75 mg% (BS). The values for non-starter doenjang were 320.72 ± 24.15 (PS), 353.96 ± 21.34 (SS3), 365.46 ± 14.56 (SS1), and 349.30 ± 13.97 mg% (BS). Starter doenjang samples showed higher amino-type nitrogen contents than non-starter doenjang samples owing to the high enzymatic activities of the selected starter strains. In this work, non-starter doenjang samples were inoculated with pre-enriched microorganisms initially present in rice straw without further identification of species. In this respect, non-starter doenjang in this work is different from so-called "traditional doenjang," which is naturally inoculated with organisms present in rice straw without pre-enrichment. Although the same numbers of organisms were inoculated, the enzymatic activities of non-starter organisms were lower than those of selected starters. PS doenjang showed the lowest values possibly due to the lower bacilli counts of PS doenjang. Amino-type nitrogen content acts as an indicator of the degree of doenjang fermentation, since amino-type nitrogen is released from soy proteins by proteases secreted by bacilli and fungi, and the value reflects the degree of protein hydrolysis [11].

Moisture Content, Salinity, and Crude Fat and Crude Protein Contents of Doenjang Samples

Immediately after preparation (day 0), the moisture contents of doenjang samples were 64–66%. The moisture contents decreased rapidly until 3 weeks, and then decreased gradually, reaching 53–62% at 13 weeks (Table 3). BS doenjang showed the lowest moisture contents at 13 weeks, 53.27% for starter and 52.91% for non-starter doenjang. For BS doenjang, the lower moisture content was due to the lower water adsorption capacity of BS as a result of melting and recrystallization at high temperature [6]. PS doenjang showed the highest values (59.62% for starter and 61.76% for non-starter) at 13 weeks, whereas SS doenjang samples

Table 3. Changes in the moisture content and salinity during fermentation.

	Sample	Fermentation period (weeks)							
		0	1	3	5	7	9	11	13
Moisture content (%)	A-1[a]	65.56	65.14	61.42	59.71	60.62	57.02	59.93	59.62
	B-1[b]	65.66	65.26	59.87	58.89	59.74	59.65	57.13	56.12
	C-1[c]	64.18	61.49	59.52	57.87	58.22	55	55.72	56.13
	D-1[d]	64.71	63.13	56.96	55.84	57	55.23	54.74	53.27
	A-2	65.69	64.55	60.13	59.79	61.02	60.54	62.1	61.76
	B-2	65.58	64.46	59.75	60.21	59.77	60.11	61.35	61.12
	C-2	63.69	62.79	58.03	57.19	58.01	58.09	58.46	57.23
	D-2	65.09	64.83	57.21	57.01	59.03	54.68	53.81	52.91
Salinity (%)	A-1	10.44 ± 0.29	9.75 ± 0.02	10.45 ± 0.06	10.79 ± 0.06	10.25 ± 0.06	10.07 ± 0.58	11.36 ± 0.04	11.84 ± 0.04
	B-1	10.59 ± 0.30	9.43 ± 0.02	10.07 ± 0.08	10.19 ± 0.06	9.60 ± 0.08	10.68 ± 0.11	10.79 ± 0.06	10.95 ± 0.08
	C-1	11.04 ± 0.25	8.08 ± 0.04	10.05 ± 0.05	11.47 ± 0.08	10.83 ± 0.05	10.77 ± 0.06	11.07 ± 0.10	10.84 ± 0.11
	D-1	11.61 ± 0.68	10.53 ± 0.02	10.80 ± 0.07	10.17 ± 0.06	10.27 ± 0.17	10.08 ± 0.14	10.17 ± 0.06	10.56 ± 0.08
	A-2	9.73 ± 0.06	8.71 ± 0.02	9.37 ± 0.05	9.04 ± 0.08	9.67 ± 0.08	10.67 ± 0.06	10.13 ± 0.08	10.47 ± 0.06
	B-2	10.45 ± 0.30	10.27 ± 0.02	10.88 ± 0.08	10.60 ± 0.06	10.37 ± 0.08	10.23 ± 0.11	10.57 ± 0.06	10.72 ± 0.08
	C-2	12.04 ± 0.31	9.31 ± 0.02	11.89 ± 0.02	10.24 ± 0.04	10.71 ± 0.10	11.43 ± 0.08	11.43 ± 0.08	11.60 ± 0.04
	D-2	10.20 ± 0.14	9.31 ± 0.02	9.87 ± 0.06	9.45 ± 0.06	11.08 ± 0.11	11.28 ± 0.11	11.75 ± 0.10	11.44 ± 0.08

[a]A denotes PS doenjang, [b]B denotes SS3 doenjang, [c]C denotes SS1 doenjang, and [d]D denotes BS doenjang. 1 indicates starter doenjang and 2 indicates non-starter doenjang.

Table 4. Changes in the crude fat content (%) during fermentation. (%)

Ferm. periods (weeks) \ Sample	Starter doenjang				Non-starter doenjang			
	PS	SS3	SS1	BS	PS	SS3	SS1	BS
0	15.85 ± 0.14	14.44 ± 0.20	16.23 ± 0.14	16.26 ± 0.22	16.35 ± 0.17	15.52 ± 0.39	14.43 ± 0.31	16.45 ± 0.09
2	15.18 ± 0.31	14.67 ± 0.66	15.83 ± 0.09	15.26 ± 0.28	14.81 ± 0.16	14.43 ± 0.37	14.81 ± 0.20	15.26 ± 0.35
4	15.47 ± 0.28	15.46 ± 0.19	16.73 ± 0.17	16.91 ± 0.19	15.31 ± 0.34	15.09 ± 0.32	15.79 ± 0.20	15.75 ± 0.31
6	15.31 ± 0.16	15.54 ± 0.16	16.63 ± 0.19	16.41 ± 0.31	15.08 ± 0.23	15.74 ± 0.29	15.31 ± 0.22	15.53 ± 0.25
8	15.29 ± 0.20	15.60 ± 0.26	16.42 ± 0.21	16.26 ± 0.14	15.57 ± 0.25	15.55 ± 0.27	15.50 ± 0.26	16.56 ± 0.30
10	15.60 ± 0.20	16.32 ± 0.35	16.34 ± 0.18	16.62 ± 0.28	15.56 ± 0.36	16.43 ± 0.31	15.83 ± 0.20	17.13 ± 0.20
12	15.18 ± 0.10	16.33 ± 0.22	16.44 ± 0.20	16.48 ± 0.38	15.25 ± 0.08	16.16 ± 0.10	16.18 ± 0.22	17.00 ± 0.18

Table 5. Changes in the crude protein content (%) during fermentation.

Weeks \	Starter doenjang				Non-starter doenjang			
	PS	SS3	SS1	BS	PS	SS3	SS1	BS
0	13.96 ± 0.16	15.56 ± 0.35	15.18 ± 0.12	14.44 ± 0.24	14.00 ± 0.40	14.55 ± 0.24	14.49 ± 0.20	14.34 ± 0.17
2	14.36 ± 0.16	15.84 ± 0.18	15.24 ± 0.21	14.33 ± 0.17	14.05 ± 0.18	14.45 ± 0.10	14.28 ± 0.08	14.20 ± 0.08
4	14.52 ± 0.08	15.66 ± 0.12	14.97 ± 0.12	14.21 ± 0.26	14.17 ± 0.12	14.45 ± 0.14	14.20 ± 0.12	14.27 ± 0.10
6	14.03 ± 0.13	15.56 ± 0.08	15.42 ± 0.12	14.96 ± 0.18	14.20 ± 0.14	14.92 ± 0.14	14.51 ± 0.10	14.08 ± 0.12
8	14.67 ± 0.10	15.52 ± 0.38	15.07 ± 0.26	14.47 ± 0.12	14.15 ± 0.51	14.60 ± 0.16	14.25 ± 0.15	14.49 ± 0.12
10	14.71 ± 0.12	15.98 ± 0.08	14.87 ± 0.20	15.03 ± 0.24	14.21 ± 0.14	15.30 ± 0.14	15.00 ± 0.14	14.60 ± 0.29
12	15.01 ± 0.14	16.02 ± 0.18	14.92 ± 0.08	14.92 ± 0.16	14.28 ± 0.16	15.21 ± 0.20	14.97 ± 0.18	14.61 ± 0.23

Table 6. Sensory evaluation of starter doenjang samples.

Item / Sample	Color	Flavor				Taste				Overall Acceptability
		Delicate flavor	Salty	Sweet	Sourness	Salty	Sweet	Sourness	Palatable taste	
PS	3.60 ± 0.93^{a}	5.03 ± 1.03^{a}	4.20 ± 1.03^{a}	3.73 ± 0.98^{a}	3.27 ± 1.17^{a}	5.83 ± 1.26^{a}	3.03 ± 1.07^{a}	3.23 ± 0.97^{b}	3.50 ± 1.01^{a}	3.90 ± 1.06^{a}
SS3	3.73 ± 1.08^{a}	4.80 ± 1.06^{a}	4.00 ± 1.08^{a}	3.33 ± 1.12^{a}	3.57 ± 1.25^{a}	5.43 ± 1.07^{a}	3.17 ± 1.18^{a}	2.53 ± 0.86^{a}	3.70 ± 1.15^{ab}	4.43 ± 1.04^{ab}
SS1	4.03 ± 1.13^{a}	4.83 ± 0.91^{a}	3.73 ± 0.94^{a}	3.50 ± 1.01^{a}	3.07 ± 0.94^{a}	5.60 ± 1.16^{a}	2.93 ± 1.05^{a}	3.50 ± 1.25^{b}	4.17 ± 0.99^{bc}	4.27 ± 1.05^{ab}
BS	4.97 ± 1.03^{b}	4.80 ± 1.27^{a}	4.17 ± 1.23^{a}	3.37 ± 1.19^{a}	3.13 ± 1.07^{a}	5.67 ± 1.03^{a}	3.30 ± 1.12^{a}	3.00 ± 0.91^{ab}	4.40 ± 1.10^{c}	4.63 ± 1.10^{b}
Control[1]	4.17 ± 1.09^{ab}	5.00 ± 1.17^{a}	5.67 ± 0.96^{c}	3.20 ± 1.16^{a}	3.57 ± 1.04^{a}	4.77 ± 0.97^{a}	3.07 ± 1.05^{a}	3.97 ± 1.03^{b}	5.03 ± 1.19^{c}	4.97 ± 1.10^{b}

Mean ± SD; Means with a different letter in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

[1]Control is a commercial product.

Table 7. Sensory evaluation of non-starter doenjang samples.

Item / Sample	Color	Flavor				Taste				Overall Acceptability
		Delicate flavor	Salty	Sweet	Sourness	Salty	Sweet	Sourness	Palatable taste	
PS	4.20 ± 1.00^{b}	4.30 ± 1.06^{a}	3.57 ± 0.97^{a}	3.30 ± 0.95^{a}	3.13 ± 1.20^{a}	4.10 ± 1.54^{a}	3.33 ± 1.09^{a}	2.80 ± 1.37^{a}	4.27 ± 1.26^{bc}	3.93 ± 0.90^{a}
SS3	3.33 ± 0.84^{a}	5.03 ± 1.10^{b}	3.77 ± 1.07^{a}	3.23 ± 1.01^{a}	2.83 ± 1.21^{a}	5.67 ± 1.30^{b}	3.50 ± 1.25^{a}	3.57 ± 1.68^{b}	3.43 ± 1.43^{a}	4.47 ± 1.04^{a}
SS1	4.33 ± 0.99^{b}	4.80 ± 0.92^{ab}	3.70 ± 1.09^{a}	3.53 ± 1.17^{ab}	3.30 ± 0.95^{a}	5.90 ± 0.96^{b}	3.13 ± 1.04^{a}	3.70 ± 1.56^{b}	3.70 ± 1.15^{ab}	4.43 ± 0.82^{a}
BS	5.30 ± 1.02^{c}	4.40 ± 1.25^{a}	4.03 ± 1.10^{a}	4.00 ± 0.98^{b}	3.17 ± 1.02^{a}	4.53 ± 1.41^{a}	3.23 ± 0.97^{a}	3.30 ± 0.88^{ab}	4.53 ± 1.11^{c}	4.23 ± 1.38^{a}
Control[1]	4.17 ± 1.09^{ab}	5.00 ± 1.17^{b}	5.67 ± 0.96^{c}	3.20 ± 1.16^{a}	3.57 ± 1.04^{a}	4.77 ± 0.97^{ab}	3.07 ± 1.05^{a}	3.97 ± 1.03^{b}	5.03 ± 1.19^{c}	4.97 ± 1.10^{b}

Mean ± SD; Means with a different letter in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

[1]Control is a commercial product.

had intermediate values. In a previous study, the moisture content of SS doenjang was higher than that of PS doenjang, suggesting that the minerals in SS conferred higher water holding capacity to SS doenjang [3]. However, our results were different, possibly due to the different doenjang preparation and fermentation conditions. In this work, the moisture contents of doenjang samples just after preparation were 64–66%, which are quite higher than the values (51–52%) of the previous report.

The salinities of the doenjang samples were maintained between 10% and 12% during fermentation. After 13 weeks, PS doenjang (starter) showed the highest value of 11.84 ± 0.04%, whereas BS doenjang (starter) showed the lowest value of 10.56 ± 0.08% (Table 3). Among the non-starter doenjang samples at 13 weeks, SS1 doenjang showed the highest value of 11.60 ± 0.04%, whereas PS doenjang showed the lowest value of 10.47 ± 0.06%. Different salt types did not affect the salinities of doenjang samples.

The crude fat contents of doenjang samples were maintained at 14–16% during the fermentation period (Table 4). The highest value (17.13%) was observed in non-starter BS doenjang at 10 weeks (results not shown). The crude protein contents were maintained at 14–16% (Table 5). The highest value (16.02) was observed in SS3 doenjang (starter) at 10 weeks (results not shown). PS doenjang showed lower values during the fermentation period.

Sensory Evaluation

Among the starter doenjang samples, BS doenjang showed a higher score for color than the control (commercial product) (Table 6). There were no significant differences in flavor among doenjang samples except compared with the control, which had a higher score for salty flavor. PS doenjang showed a higher score for salty taste, but there was no significant difference from the others. For palatable taste, BS doenjang showed the highest score, followed by SS1, SS3, and PS doenjang. For overall acceptability, control doenjang scored the best, followed by BS > SS3 > SS1 > PS doenjang. Similar results were obtained from the non-starter doenjang samples (Table 7). BS doenjang scored the best for color. Overall acceptability was in the order of control > SS3 > SS1 > BS > PS, with no significant differences.

Commercial doenjang as the control scored higher than the other doenjang samples in this work, possibly due to the presence of additives such as ethanol, red pepper, and mustard powder used for commercial doenjang, which improve the flavor and taste of the product [7]. The overall acceptability scores for SS and BS doenjang were higher than those of PS doenjang, and the result agreed with a previous report [9, 10]. Doenjang prepared with SS or BS showed better sensory properties than PS doenjang owing to growth acceleration of the starter bacilli and growth inhibition of yeasts by SS and BS.

Acknowledgments

This work was supported by Grant No. 201300290 to the Solar Salt Research Center of Mokpo National University from the Ministry of Oceans and Fisheries of Korea. J.M. Shim, K.W. Lee, and Z. Yao were supported by the BK21 Plus program, MOE, Republic of Korea.

References

1. AOAC. 2000. *Official Method of Analysis.* 17th Ed. Association of Official Analytical Chemists. Gaithersburg, MD, USA.
2. Chang JY, Kim IC, Chang HC. 2014. Effect of solar salt on kimchi fermentation during long-term storage. *Kor. J. Food Sci. Technol*. **46:** 456-464.
3. Chang M, Kim IC, Chang HC. 2010. Effect of solar salt on the quality characteristics of doenjang. *J. Kor. Soc. Food Sci. Nutr.* **39:** 116-124.
4. Cho MJ, Lee JY, Lee KW, Cho KM, Lee CK, Kim GM, *et al.* 2014. Properties of doenjang (soybean paste) fermented with multiple starters. *J. Agric. Life Sci.* **48:** 291-300.
5. Heo OS, Oh SH, Shin HS, Kim MR. 2005. Mineral and heavy metal contents of salt and salted-fermented shrimp. *Kor. J. Food Sci. Technol*. **37:** 519-524.
6. Jeong MW, Jeong JK, Kim SJ, Park KY. 2013. Fermentation characteristics and increased functionality of doenjang prepared with bamboo salt. *J. Kor. Soc. Food Sci. Nutr*. **42:** 1915-1923.
7. Jo YJ, Cho IH, Song CK, Kim YS. 2011. Comparison of fermented soybean paste (doenjang) prepared by different method based on profiling of volatile compounds. *J. Food Sci*. **76:** C368-C379.
8. KFDA. 2011. *Korea Food and Drug Administration. Food standards Codex I*, pp. 200-203. Korean Food Industry Association, Seoul, Korea.
9. Kim HG, Hong JH, Song CK, Shin HW, Kim KO. 2010. Sensory characteristics and consumer acceptability of fermented soybean paste (doenjang). *J. Food Sci*. **75:** S375-S383.
10. Kim JW, Doo HS, Kwon TH, Kim YS, Shin DH. 2011. Quality characteristics of doenjang meju fermented with *Aspergillus* species and *Bacillus subtilis* during fermentation. *Kor. J. Food Preserv*. **18:** 397-406.
11. Kim SH, Kim SJ, Kim BH, Kang SG, Jung ST. 2000. Fermentation of doenjang prepared with sea salts. *Kor. J. Food Sci. Technol*. **32:** 1365-1370.
12. Kim TW, Lee JH, Kim SE, Park MH, Chang HC, Kim H-Y. 2009. Analysis of microbial communities in doenjang, a Korean fermented soybean paste, using nested PCR-denaturing gradient gel electrophoresis. *Int. J. Food Microbiol*. **131:** 265-271.
13. Kim YH, Ryu HI. 2003. Elements in a bamboo salt and comparison of its elemental contents with those in other salts. *Yakhak Hoeji* **47:** 135-141.
14. Ko BK, Kim KM, Hong YS, Lee CH. 2010. Metabolomic assessment of fermentative capability of soybean starter treated with high pressure. *J. Agric. Food Chem.* **58:** 8738-8747.
15. Mok CK, Song KT, Lee JY, Park YS, Lim SB. 2005. Change in microorganisms and enzyme activity of low salt soybean paste (doenjang) during fermentation. *Food Eng. Prog.* **9:** 112-117.
16. Park J-W, Kim S-J, Kim S-H, Kim B-H, Kang S-G, Nam S-H, Jung S-T. 2000. Determination of mineral and heavy metal contents of various salts. *Kor. J. Food Sci. Technol.* **32:** 1442-1445.
17. Petersen KM, Møller PL, Lespersen L. 2011. DNA typing methods for differentiation of *Debaryomyces hansenii* strains and other yeasts related to surface ripened cheeses. *Int. J. Food Microbiol*. **69:** 11-24.
18. Breuer U, Harms H. 2006. *Debaryomyces hansenii* – an extremophilic yeast with biotechnological potential. *Yeast* **23:** 415-437.
19. Zhao X, Song J-L, Jung O-S, Lim Y-I, Park K-Y. 2014. Chemical properties and in vivo gastric protective effects of bamboo salt. *Food Sci. Biotechnol*. **23:** 895-902.