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Microbial Communities and Physicochemical Properties of Myeolchi Jeotgal (Anchovy Jeotgal) Prepared with Different Types of Salts S

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Myeolchi jeotgals (MJs) were prepared with purified salt (PS), solar salt aged for 1 year (SS), and bamboo salt (BS) melted 3 times at 10% and 20% (w/w) concentrations, and fermented for 28 weeks at 15°C. BS MJ showed higher pH and lower titratable acidities than the other samples because of the alkalinity of bamboo salt. Lactic acid bacteria counts increased until 4–6 weeks and then decreased gradually, and were not detected after 20 weeks from MJs with 10% salt. Yeast counts of PS MJs were higher than those of BS and SS MJs. Bacilli were detected in relatively higher numbers throughout the 28 weeks, like marine bacteria, but archae were detected in lower numbers during the first 10 weeks. When 16S rRNA genes were amplified from total DNA from PS MJ (10% salt) at 12 weeks, *Tetragenococcus halophilus* was the major species. However, *Staphylococcus epidermidis* was the dominant species for BS MJ at the same time point. In SS MJ, *T. halophilus* was the dominant species and *S. epidermidis* was the next dominant species. BS and SS MJs showed higher amino-type nitrogen, ammonia-type nitrogen, and volatile basic nitrogen contents than PS MJs. SS and BS were better than PS for the production of high-quality MJs.

Keywords: Myeolchi jeotgal, solar salt, bamboo salt, purified salt

Introduction

Jeotgals are salted and fermented Korean traditional seafoods prepared from various fishes, shrimps, oysters, fish eggs, and fish intestines [1]. Many different types of jeotgals are produced depending upon the raw materials, regions, and personal preferences, but the basic procedures are the same. Raw materials are mixed with salt in a container and fermentation starts. Final salt concentrations are 10–30% (w/w). Fermentation continues for several months, and several years for some jeotgals. During fermentation, the unique texture and flavor of each jeotgal is developed through hydrolysis of proteins by proteases originated from the raw materials and microorganisms [2]. Salt is an essential ingredient for jeotgals, and only salt and raw material are used for preparation of some jeotgals. The main role of salt is growth inhibition of spoilage microorganisms during fermentation. Under high salt concentrations, salt-tolerant microorganisms can grow and contribute to development of the unique flavor, taste, and texture of each jeotgal [3]. Solar salt (SS) 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 growth of many microorganisms, affecting the qualities of fermented foods [4]. Bamboo salt (BS) is obtained by recrystallization of SS after it is melted at high temperature. SS is filled in a bamboo trunk, and the top and bottom are sealed with clay, and then heated in a furnace. SS is melted at 800–1,500°C and then recrystallized as the temperature decreases [5, 6].

SS has been widely used for fermented foods such as kimchi (Korean fermented vegetable) and doenjang (Korean fermented soybean), and use of BS for fermented foods is also in the rise because of its functionalities [6]. However, few studies have been done on the effects of different types of salts on the qualities of fermented foods. In particular, the effects of SS and BS on the growth of microorganisms during fermentation are rarely studied. Growth of micro-

organisms affects the types of metabolites produced in fermented foods, and eventually determines the quality of foods. Therefore, understanding the effects of different salt types on the growth of microorganisms is important for the production of high-quality and functional fermented foods. In this work, myeolchi (anchovy, *Engraulis japonica*) jeotgals (MJs) were prepared with three different types of salts: purified salt (PS), 1-year aged SS, and BS. Jeotgal samples were fermented for 28 weeks at 15°C, and growth of microorganisms was examined together with other properties of jeotgal samples during fermentation.

Materials and Methods

Preparation of Myeolchi Jeotgal

Myeolchi (anchovy) was purchased from a local fish market (Tongyeong Suhyup, Korea) in December 2015. Immediately after purchase, the myeolchi was washed three times under running tap water, and stood for 10 min to remove excess water. Each 20 kg of myeolchi was then mixed with salt. The NaCl concentration of the MJs was adjusted to 10% and 20% (w/w), respectively, by adding different amount of each salt: 2,017 g (10%) and 4,033 g (20%) for PS (Hanju, Ulsan, Korea, 2015; NaCl 99.18%); 2,505 g (10%) and 5,010 g (20%) for SS (Taepong salt farm, Sinan, Jeonnam, Korea; aged for 1 year, NaCl 79.84%); and 2,116 g (10%) and 4,232 g (20%) for BS (Insanga, Hamyang, Gyeongnam, Korea; melted and recrystallized 3 times, NaCl 94.54%). The MJs were fermented for 28 weeks at 15°C and analyzed every 2 weeks during fermentation.

Viable Cell Counting

Ten gram of each MJ 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 with a bag filter (Interscience, France) and diluted serially with peptone water. Diluted samples were spread on de Man-Rogosa-Sharpe (MRS) agar (Acumedia, USA) plates for lactic acid bacteria (LAB) counting, marine agar (BD Difco, USA) plates for marine bacteria counting, LB (Acumedia) plates for bacilli counting, YM agar (BD Difco) plates for yeasts counting, and DSMZ954 agar (casamino acids 5 g, yeast extract 5 g, Tris 12.1 g, KCl 2 g, $MgCl_2 \cdot 6H_2O$ 20 g, $CaCl_2 \cdot 2H_2O$ 0.2 g, NaCl 200 g, agar 20 g, per liter, pH 7.4) plates for archaea counting. The plates were incubated for 24 h at 37°C (marine, LB, and DSMZ954 agar plates), and 48 h at 30°C (MRS and YM agar plates), respectively.

Identification of Bacterial Species by a Culture-Independent Method

MJs with 10% NaCl were collected at 12 weeks of fermentation and total DNA was extracted from each sample by using an EZ-10 spin column soil DNA mini-prep kit (Bio Basic Inc., Canada). 16S rRNA genes were amplified from the extracted DNA samples by using the primer pair F1 (5′-TGACGGTACCTAACCAGAAAGCCACGGCT-3′) and R1 (5′-GTTTGTCACCGGCAGTCACCTTAGAGTGC-3′). PCR was done under the following conditions: denaturation at 94°C for 5 min and then 30 cycles of 30 sec at 94°C, 30 sec at 62°C, and 1 min at 72°C. The amplified fragments were purified from an agarose gel using a PCR purification kit (FavorPrep PCR purification kit; Favorgen, Taiwan) and then ligated with the pGEM-T easy vector (Promega, USA). *E. coli* DH5α (Invitrogen, USA) competent cells were transformed with the ligation mixture and colonies were selected on LB plates with ampicillin (100 μg/ml), isopropyl β-D-1-thiogalactopyranoside (500 μg/ml), and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (80 μg/ml). Thirty well isolated colonies were selected from each MJ and their plasmids were prepared for DNA sequencing. DNA sequences were determined at Cosmogenetech (Korea), and the BLAST program provided by the National Center for Biotechnology Information was used to find homologous sequences in the data library (http://www.ncbi.nlm.nih.gov/ BLAST).

pH and Titratable Acidity Measurements

Ten gram of each homogenized MJ was mixed with 40 ml of distilled water, and shaken for 1 h in a water bath (150 rpm, 30°C). The supernatant was obtained after centrifugation (4,000 ×*g*, 20 min). The pH of the supernatant was measured using a pH meter (DP-215M; DMS, Korea) and titratable acidity (TA) was calculated by titrating the supernatant with 0.1 N NaOH to pH 8.4. The amount of NaOH was used to calculate the amount of lactic acid (%).

Amino-Type Nitrogen (ANN), Ammonia-Type Nitrogen (AMN) and Volatile Basic Nitrogen (VBN) Measurements

Five gram of each homogenized MJ was mixed with 95 ml of distilled water, and the mixture was shaken for 1 h in a water bath (150 rpm, 30°C). The ANN contents were measured by the formol titration method [7]. The AMN contents were measured by an indophenol blue method [8]. Five gram of each homogenized MJ was mixed with 95 ml of distilled water, and the mixture was shaken for 1 h in a water bath (150 rpm, 30°C). The mixture was centrifuged, and 1 ml of supernatant was mixed with 3 ml of solution A (phenol 10 g, sodium nitroprusside dehydrate 1 g, EDTA 4 g, per liter). After standing for 5 min at room temperature, 5 ml of solution B ($Na_2HPO_4 \cdot 12H_2O$ 9 g, NaOH 6 g, and NaOCl 10 ml, per 1 liter) was added and stood for 20 min at 37°C. Changes in absorbance were measured at 665 nm with a spectrophotometer (UV-1601, Shimadzu, Japan), and the AMN content was determined on the basis of the standard curve of $(NH_4)_2SO_4$. For measurement of VBN, 10 g of each homogenized MJ was mixed with 30 ml of distilled water and 20 ml of 20% trichloroacetic acid solution. Mixed samples were centrifuged (4°C, 8,000 ×*g*, 10 min) and filtered with a membrane filter (0.45 μm; Advatec, Japan). The VBN contents of the filtered samples were determined by Conway's method using Conway units [9].

Salinity and Neutral Protease Activity Measurements

For salinity measurements, 10 g of each homogenized MJ was mixed with 40 ml of distilled water, and shaken in a water bath for 1 h. The supernatant was obtained by centrifugation as stated above, and salinity was measured by a saltmeter (PAL-SALT; Atago, Japan). Measurements were repeated three times and the average values are shown. The neutral protease activities of the MJs were determined using the modified methods of Cho *et al.* [10] with 1.0% casein (w/v) as the substrate.

Statistical Analyses

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

Results and Discussion

Changes in Viable Counts of LAB, Marine Bacteria, Archae, Bacilli, and Yeasts

Viable cells of LAB, marine bacteria, archae, bacilli, and yeasts were counted at every 2 weeks during fermentation. Bacilli counts were 3.04–3.51 log CFU/g for MJs with 10% salt (Table 1) and 2.18–2.85 log CFU/g for MJs with 20% salt immediately after preparation (0 week, Table 2). The

Table 1. Changes in the viable cell numbers of marine bacteria, bacilli, yeast, LAB, and archae during fermentation of myeolchi jeotgals (10%).

Type of salt	Microorganism	Storage (weeks)														
		0	2	4	6	8	10	12	14	16	18	20	22	24	26	28
PS	Marine bacteria	3.38 ± 0.03^{a}	3.99 ± 0.18^{ab}	4.40 ± 0.12^{b}	4.26 ± 0.07^{a}	3.99 ± 0.13^{a}	3.79 ± 0.09^{a}	3.46 ± 0.11^{a}	3.52 ± 0.12^{a}	4.71 ± 0.31^{b}	4.81 ± 0.04^{a}	4.90 ± 0.09^{a}	3.20 ± 0.06^{a}	3.30 ± 0.08^{a}	3.15 ± 0.06^{ab}	4.34 ± 0.11^{c}
	Bacilli	3.51 ± 0.25^{b}	4.30 ± 0.23^{a}	4.63 ± 0.04^{a}	3.83 ± 0.13^{a}	3.94 ± 0.27^{a}	3.51 ± 0.04^{a}	3.38 ± 0.12^{a}	3.36 ± 0.29^{a}	4.75 ± 0.50^{b}	4.73 ± 0.03^{a}	3.56 ± 0.34^{a}	2.92 ± 0.08^{a}	2.98 ± 0.10^{a}	2.82 ± 0.09^{ab}	2.72 ± 0.27^{a}
	Yeast	-	-	2.61 ± 0.04^{c}	2.63 ± 0.13^{c}	2.57 ± 0.35^{b}	2.48 ± 0.08^{b}	2.57 ± 0.17^{b}	2.56 ± 0.28^{b}	2.84 ± 0.18^{c}	2.91 ± 0.26^{a}	2.15 ± 0.05^{b}	1.51 ± 0.37^{a}	1.08 ± 0.10^{a}	1.23 ± 0.23^{a}	1.15 ± 0.07^{a}
	LAB	2.49 ± 0.27^{ab}	3.40 ± 0.04^{b}	5.30 ± 0.30^{c}	4.68 ± 0.13^{c}	3.87 ± 0.19^{b}	3.08 ± 0.16^{a}	2.76 ± 0.16^{a}	2.51 ± 0.04^{a}	3.26 ± 0.26^{b}	3.28 ± 0.06^{b}	3.91 ± 0.11^{c}	-	-	-	-
	Archaea	3.15 ± 0.15^{b}	3.32 ± 0.17^{b}	2.34 ± 0.09^{a}	2.15 ± 0.05^{a}	2.04 ± 0.10^{a}	-	-	-	-	-	-	-	-	-	-
SS	Marine bacteria	3.20 ± 0.20^{a}	4.11 ± 0.13^{b}	4.68 ± 0.03^{c}	4.45 ± 0.11^{a}	3.77 ± 0.22^{a}	4.26 ± 0.08^{b}	3.94 ± 0.06^{b}	4.64 ± 0.09^{b}	3.85 ± 0.15^{a}	4.79 ± 0.19^{a}	4.70 ± 0.23^{a}	3.32 ± 0.08^{a}	3.89 ± 0.10^{c}	3.48 ± 0.16^{b}	3.40 ± 0.10^{b}
	Bacilli	3.26 ± 0.13^{ab}	3.96 ± 0.20^{a}	4.81 ± 0.09^{b}	3.90 ± 0.19^{a}	3.49 ± 0.31^{a}	4.15 ± 0.08^{c}	3.62 ± 0.06^{b}	4.65 ± 0.13^{b}	3.78 ± 0.33^{a}	4.71 ± 0.24^{a}	4.15 ± 0.09^{b}	2.86 ± 0.30^{a}	2.92 ± 0.22^{a}	2.95 ± 0.04^{b}	2.49 ± 0.16^{a}
	Yeast	-	-	1.83 ± 0.11^{b}	1.83 ± 0.07^{b}	1.08 ± 0.22^{a}	1.34 ± 0.17^{a}	2.11 ± 0.11^{a}	2.00 ± 0.10^{a}	2.08 ± 0.08^{a}	2.38 ± 0.16^{a}	2.18 ± 0.04^{b}	2.75 ± 0.49^{b}	1.65 ± 0.33^{b}	1.90 ± 0.52^{ab}	1.70 ± 0.19^{b}
	LAB	2.74 ± 0.25^{b}	3.57 ± 0.07^{c}	4.23 ± 0.03^{b}	3.81 ± 0.19^{a}	3.57 ± 0.23^{ab}	3.92 ± 0.06^{b}	3.18 ± 0.12^{b}	4.23 ± 0.10^{b}	2.98 ± 0.12^{ab}	2.49 ± 0.19^{a}	2.81 ± 0.19^{b}	-	-	-	-
	Archaea	2.54 ± 0.16^{a}	2.71 ± 0.30^{a}	2.62 ± 0.12^{b}	2.15 ± 0.13^{a}	1.81 ± 0.19^{a}	1.32 ± 0.12^{a}	-	-	-	-	-	-	-	-	-
BS	Marine bacteria	3.15 ± 0.04^{a}	3.78 ± 0.02^{a}	4.18 ± 0.04^{a}	4.43 ± 0.11^{a}	3.88 ± 0.26^{a}	3.81 ± 0.05^{a}	3.87 ± 0.07^{b}	3.48 ± 0.31^{a}	4.04 ± 0.27^{a}	4.71 ± 0.13^{a}	4.76 ± 0.13^{a}	3.18 ± 0.15^{a}	3.72 ± 0.06^{b}	2.85 ± 0.25^{a}	2.65 ± 0.44^{a}
	Bacilli	3.04 ± 0.27^{a}	4.08 ± 0.11^{a}	4.62 ± 0.12^{a}	4.45 ± 0.23^{b}	3.68 ± 0.17^{a}	3.76 ± 0.10^{b}	3.88 ± 0.12^{c}	3.45 ± 0.10^{a}	3.99 ± 0.23^{a}	4.40 ± 0.44^{a}	3.11 ± 0.23^{a}	3.23 ± 0.18^{a}	2.83 ± 0.04^{a}	2.76 ± 0.12^{a}	2.38 ± 0.05^{a}
	Yeast	-	-	1.11 ± 0.09^{a}	1.04 ± 0.06^{a}	1.18 ± 0.12^{a}	1.30 ± 0.15^{a}	2.72 ± 0.07^{b}	1.98 ± 0.08^{a}	2.49 ± 0.09^{b}	4.15 ± 0.37^{b}	2.04 ± 0.04^{a}	3.18 ± 0.18^{b}	2.46 ± 0.34^{c}	2.32 ± 0.26^{b}	2.18 ± 0.10^{c}
	LAB	2.18 ± 0.12^{a}	3.11 ± 0.11^{a}	3.58 ± 0.18^{b}	4.26 ± 0.16^{b}	3.34 ± 0.04^{a}	3.28 ± 0.10^{a}	3.38 ± 0.08^{b}	2.52 ± 0.08^{a}	2.54 ± 0.28^{a}	2.32 ± 0.10^{a}	2.43 ± 0.03^{a}	-	-	-	-
	Archaea	2.36 ± 0.06^{a}	2.49 ± 0.19^{a}	2.52 ± 0.11^{ab}	2.15 ± 0.15^{a}	1.79 ± 0.05^{a}	1.34 ± 0.06^{a}	-	-	-	-	-	-	-	-	-

Values are the mean ± SD from triplicate determinations. Significant differences (a-c) in the same tested counts are tested ($p < 0.05$, Duncan's multiple range test).

Table 2. Changes in the viable cell numbers of marine bacteria, bacilli, yeast, LAB, and archaea during fermentation of myeolchi jeotgals (20%).

Type of salt	Microorganism	Storage (weeks)														
		0	2	4	6	8	10	12	14	16	18	20	22	24	26	28
PS	Marine bacteria	3.38 ± 0.03^{a}	3.99 ± 0.18^{ab}	4.40 ± 0.12^{b}	4.26 ± 0.07^{a}	3.99 ± 0.13^{a}	3.79 ± 0.09^{a}	3.46 ± 0.11^{a}	3.52 ± 0.12^{a}	4.71 ± 0.31^{b}	4.81 ± 0.04^{a}	4.90 ± 0.09^{a}	3.20 ± 0.06^{a}	3.30 ± 0.08^{a}	3.15 ± 0.06^{ab}	4.34 ± 0.11^{c}
	Bacilli	3.51 ± 0.25^{b}	4.30 ± 0.23^{a}	4.63 ± 0.04^{a}	3.83 ± 0.13^{a}	3.94 ± 0.27^{a}	3.51 ± 0.04^{a}	3.38 ± 0.12^{a}	3.36 ± 0.29^{a}	4.75 ± 0.50^{b}	4.73 ± 0.03^{a}	3.56 ± 0.34^{a}	2.92 ± 0.08^{a}	2.98 ± 0.10^{a}	2.82 ± 0.09^{ab}	2.72 ± 0.27^{a}
	Yeast	-	-	2.61 ± 0.04^{c}	2.63 ± 0.13^{c}	2.57 ± 0.35^{b}	2.48 ± 0.08^{b}	2.57 ± 0.17^{b}	2.56 ± 0.28^{b}	2.84 ± 0.18^{c}	2.91 ± 0.26^{a}	2.15 ± 0.05^{b}	1.51 ± 0.37^{a}	1.08 ± 0.10^{a}	1.23 ± 0.23^{a}	1.15 ± 0.07^{a}
	LAB	2.49 ± 0.27^{ab}	3.40 ± 0.04^{b}	5.30 ± 0.30^{c}	4.68 ± 0.13^{c}	3.87 ± 0.19^{b}	3.08 ± 0.16^{a}	2.76 ± 0.16^{a}	2.51 ± 0.04^{a}	3.26 ± 0.26^{b}	3.28 ± 0.06^{b}	3.91 ± 0.11^{c}	-	-	-	-
	Archaea	3.15 ± 0.15^{b}	3.32 ± 0.17^{b}	2.34 ± 0.09^{a}	2.15 ± 0.05^{a}	2.04 ± 0.10^{a}	-	-	-	-	-	-	-	-	-	-
SS	Marine bacteria	3.20 ± 0.20^{a}	4.11 ± 0.13^{b}	4.68 ± 0.03^{c}	4.45 ± 0.11^{a}	3.77 ± 0.22^{a}	4.26 ± 0.08^{b}	3.94 ± 0.06^{b}	4.64 ± 0.09^{b}	3.85 ± 0.15^{a}	4.79 ± 0.19^{a}	4.70 ± 0.23^{a}	3.32 ± 0.08^{a}	3.89 ± 0.10^{c}	3.48 ± 0.16^{b}	3.40 ± 0.10^{b}
	Bacilli	3.26 ± 0.13^{ab}	3.96 ± 0.20^{a}	4.81 ± 0.09^{b}	3.90 ± 0.19^{a}	3.49 ± 0.31^{a}	4.15 ± 0.08^{c}	3.62 ± 0.06^{b}	4.65 ± 0.13^{b}	3.78 ± 0.33^{a}	4.71 ± 0.24^{a}	4.15 ± 0.09^{b}	2.86 ± 0.30^{a}	2.92 ± 0.22^{a}	2.95 ± 0.04^{b}	2.49 ± 0.16^{a}
	Yeast	-	-	1.83 ± 0.11^{b}	1.83 ± 0.07^{b}	1.08 ± 0.22^{a}	1.34 ± 0.17^{a}	2.11 ± 0.11^{a}	2.00 ± 0.10^{a}	2.08 ± 0.08^{a}	2.38 ± 0.16^{a}	2.18 ± 0.04^{b}	2.75 ± 0.49^{b}	1.65 ± 0.33^{b}	1.90 ± 0.52^{ab}	1.70 ± 0.19^{b}
	LAB	2.74 ± 0.25^{b}	3.57 ± 0.07^{c}	4.23 ± 0.03^{b}	3.81 ± 0.19^{a}	3.57 ± 0.23^{ab}	3.92 ± 0.06^{b}	3.18 ± 0.12^{b}	4.23 ± 0.10^{b}	2.98 ± 0.12^{ab}	2.49 ± 0.19^{a}	2.81 ± 0.19^{b}	-	-	-	-
	Archaea	2.54 ± 0.16^{a}	2.71 ± 0.30^{a}	2.62 ± 0.12^{b}	2.15 ± 0.13^{a}	1.81 ± 0.19^{a}	1.32 ± 0.12^{a}	-	-	-	-	-	-	-	-	-
BS	Marine bacteria	3.15 ± 0.04^{a}	3.78 ± 0.02^{a}	4.18 ± 0.04^{a}	4.43 ± 0.11^{a}	3.88 ± 0.26^{a}	3.81 ± 0.05^{a}	3.87 ± 0.07^{b}	3.48 ± 0.31^{a}	4.04 ± 0.27^{a}	4.71 ± 0.13^{a}	4.76 ± 0.13^{a}	3.18 ± 0.15^{a}	3.72 ± 0.06^{b}	2.85 ± 0.25^{a}	2.65 ± 0.44^{a}
	Bacilli	3.04 ± 0.27^{a}	4.08 ± 0.11^{a}	4.62 ± 0.12^{a}	4.45 ± 0.23^{b}	3.68 ± 0.17^{a}	3.76 ± 0.10^{b}	3.88 ± 0.12^{c}	3.45 ± 0.10^{a}	3.99 ± 0.23^{a}	4.40 ± 0.44^{a}	3.11 ± 0.23^{a}	3.23 ± 0.18^{a}	2.83 ± 0.04^{a}	2.76 ± 0.12^{a}	2.38 ± 0.05^{a}
	Yeast	-	-	1.11 ± 0.09^{a}	1.04 ± 0.06^{a}	1.18 ± 0.12^{a}	1.30 ± 0.15^{a}	2.72 ± 0.07^{b}	1.98 ± 0.08^{a}	2.49 ± 0.09^{b}	4.15 ± 0.37^{b}	2.04 ± 0.04^{a}	3.18 ± 0.18^{b}	2.46 ± 0.34^{c}	2.32 ± 0.26^{b}	2.18 ± 0.10^{c}
	LAB	2.18 ± 0.12^{a}	3.11 ± 0.11^{a}	3.58 ± 0.18^{b}	4.26 ± 0.16^{b}	3.34 ± 0.04^{a}	3.28 ± 0.10^{a}	3.38 ± 0.08^{b}	2.52 ± 0.08^{a}	2.54 ± 0.28^{a}	2.32 ± 0.10^{a}	2.43 ± 0.03^{a}	-	-	-	-
	Archaea	2.36 ± 0.06^{a}	2.49 ± 0.19^{a}	2.52 ± 0.11^{ab}	2.15 ± 0.15^{a}	1.79 ± 0.05^{a}	1.34 ± 0.06^{a}	-	-	-	-	-	-	-	-	-

Values are the mean ± SD from triplicate determinations. Significant differences (a-c) in the same tested counts are tested ($p < 0.05$, Duncan's multiple range test).

counts increased and reached the highest numbers at 4 weeks; 4.62–4.81 log CFU/g for MJs with 10% salt and 4.65–4.71 log CFU/g for MJs with 20% salt. Thereafter, the counts increased and decreased repeatedly until 18 weeks, and then decreased. At 28 weeks, bacilli counts were 2.38–2.72 log CFU/g for MJs with 10% salt and 2.11–2.38 log CFU/g for MJs with 20% salt. Different salt types did not significantly affect bacilli counts of MJs, but MJs with 10% salt had higher numbers than MJs with 20% salt. Our results agreed with an early report, where the viable counts of MJ (20% salt, 20–25°C) increased until 50 days and then decreased gradually until 150 days [11]. Bacteria such as *Pediococcus*, *Pseudomonas*, *Sarcina*, *Micrococcus*, and *Halobacteriun* were isolated [11]. However, identification was based on morphologies and biochemical properties in early reports, and thus the accuracy of identification is in doubt. One recent study reported that *Bacillus* and related genera were dominant organisms in MJ, and their proteolytic enzymes were believed to contribute to the development of flavor of MJ [12]. *Virgibacillus halodenitrificans* was pointed out as the most important species for MJ fermentation and suggested to be used as a starter for jeotgal [12].

Yeasts were maintained in lower numbers, being less than bacilli, LAB, and marine bacteria. For MJs with 10%

salt, PS MJs showed higher yeast counts than SS and BS MJs until 18 weeks of fermentation, with two exceptions (BS MJ at 12 and 18 weeks), but yeast counts of PS MJ were less than those of SS and BS MJ samples after 20 weeks. The yeast counts were 1.15, 1.70, and 2.18 log CFU/g for PS, SS, and BS MJ (10% salt), respectively, at 28 weeks. It is unclear why yeast counts of PS MJ decreased more rapidly than other MJs after 20 weeks. Among MJs with 20% salt, yeasts were detected from PS MJ at 4 weeks, earlier than BS MJ, and the numbers were higher than those of SS and BS MJs most times during the fermentation period (Table 2). Yeast counts of MJs with 20% salt were 2.15, 1.51, and 0.00 log CFU/g for PS, SS, and BS MJ, respectively, at 28 weeks.

Higher yeast counts in fermented foods with PS were also observed for doenjang and kimchi. Doenjang with PS (12%, the same batch used for this work) showed significantly higher yeast counts than doenjang with SS or BS [3]. Yeast counts of PS doenjang (non-starter) were 5.39, 8.29, 8.14, and 7.17 log CFU/g at 1, 5, 9, and 13 weeks of fermentation, respectively [3]. Those of SS (aged for 1 year, product from the same salt farm but different production year) doenjang were 5.61, 4.44, 4.56, and 5.92 log CFU/g, and those of BS (from the same batch used for this work) doenjang were 5.45, 5.14, 5.25, and 4.54 log CFU/g at the same time points [3]. Significant differences in yeast counts were also observed in kimchi prepared with different types of salts. Yeast counts of SS and BS kimchi were lower than those of PS kimchi. The yeast counts were 5.91, 2.90, and 1.40 log CFU/g for PS, SS, and BS kimchi, respectively after 20 weeks of fermentation at −1°C (results not shown).

These results show a possibility that some compound(s) in SS and BS might discourage growth of yeasts. Overgrowth of yeasts in fermented foods is generally considered undesirable, because yeasts deteriorate the texture and flavor of foods by secreting hydrolyzing enzymes such as pectinases and generating off-flavors [13]. In this respect, SS and BS have an advantage over PS.

LAB counts of MJs with 10% salt were 2.18–2.74 log CFU/g at 0 week and increased gradually, reaching the highest numbers at 4–6 weeks. Then, the counts decreased gradually and were not detected at 22 weeks and later. LAB were detected in lower numbers between 2 and 8 weeks from MJs with 20% salt. Marine bacteria were detected in higher numbers throughout the fermentation period. The maximum counts were observed at 18–20 weeks (4.76–4.90 log CFU/g) for MJs with 10% salt, and 4 weeks (4.52–5.54 log CFU/g) for MJs with 20% salt. Archae were detected in lower numbers during the early stage of fermentation and were not detected after 8 or 10 weeks.

Table 3. Identification results by a culture-independent method.

MJ	Species	Clone numbers out of 30 (%)
PS MJ	*Tetragenococcus halophilus*	29 (96.7%)
	No reaction[a]	1 (3.3%)
SS MJ	*Tetragenococcus halophilus*	22 (73.4%)
	Staphylococcus epidermidis	4 (13.4%)
	Staphylococcus caprae	1 (3.3%)
	Bacillus hormeckiae	1 (3.3%)
	Bacillus pumilus	1 (3.3%)
	Lactobacillus plantarum	1 (3.3%)
BS MJ	*Staphylococcus epidermidis*	17 (56.7%)
	Tetragenococcus halophilus	10 (33.3%)
	Uncultured bacterium	1 (3.3%)
	No reaction	2 (6.7%)

[a]Sequencing reaction failed.

Identification of Bacterial Species by a Culture-Independent Method

Total DNA was prepared from each MJ (10% salt, 12 weeks) and used as a template for amplification of 16S rRNA genes. Thirty colonies on agar plates were randomly selected from each sample and sequenced. For PS MJ, 29 clones (96.7%) had sequences identical to those of *Tetragenococcus halophilus* strains, and one clone was failed for sequencing (3.3%) (Tables 3 and S1). Among 30 clones from SS MJ, 22 were *T. halophilus* (73.3%), 5 were staphylococci (4 *S. epidermidis* and 1 *S. caprae*) (16.7%), 2 were bacilli (*B. horneckiae* and *B. pumilus*) (6.7%), and 1 was *Lactobacillus plantarum* (3.3%) (Tables 3 and S2). Among 30 clones from BS MJ, 17 were *S. epidermidis* (56.7%), 10 were *T. halophilus* (33.3%), 1 was an uncultured bacterium (3.3%), and 2 were failed for sequencing (6.7%) (Tables 3 and S3). The results were unexpected because the primer pairs were designed to specifically amplify 16S rRNA genes from bacilli, and BLAST of both primer sequences generated matching 16S rRNA genes from only bacilli and a few uncultured bacteria. No sequences from *T. halophilus* or *S. epidermidis* were matched (data not shown).

It was unclear why the primer pairs failed to amplify specifically bacilli genes, but the results were interesting. Although just 30 clones from each MJ were examined, the results still showed differences in bacterial species among MJs. *T. halophilus* and *S. epidermidis* were the two most dominant species in MJs with 10% salt, and different salt types seemingly affected the ratio of the two species. Staphylococci have been reported as a major group among

various jeotgals [14], which was confirmed in this study. *T. halophilus* was the major bacteria from two different jeotgals (26–33% salt) prepared in Jeju, Republic of Korea [15]. Considering our results and previous reports, it can be concluded that *T. halophilus* is a major species for jeotgals with various salt concentrations. This is the first report on the effect of different types of salts on the major bacterial species of MJs. Future studies on the roles of *T. halophilus* and *S. epidermidis* in the quality of jeotgals are necessary.

pH and Titratable Acidities of MJ Samples

Immediately after preparation, the pH of BS MJ was 7.20 ± 0.04 (10% salt) and 7.23 ± 0.03 (20% salt), higher than those of other MJs (pH 6.45–6.60) (Fig. 1). This was due to the high mineral contents of BS, which contains a large amount of K, Si, Fe, and PO_4 and its aqueous solution is strongly alkaline (pH 10) [5, 16]. BS MJs maintained higher pH values throughout the fermentation period, and BS MJ with 20% salt had the highest pH values.

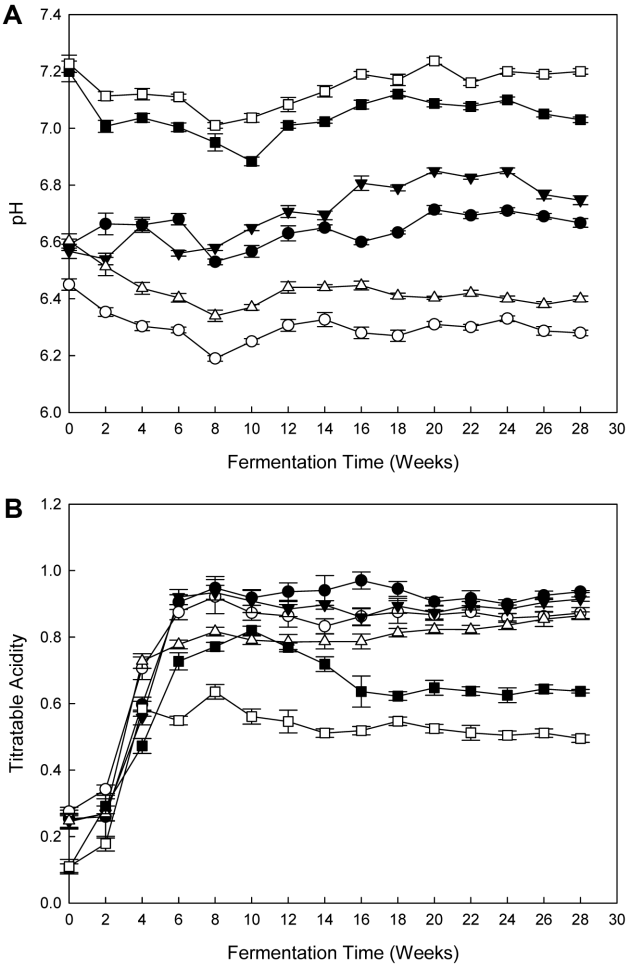

Fig. 1. Changes in pH (**A**) and titratable acidity (**B**) of myeolchi jeotgals prepared with different types of salts during fermentation.

●, Purified salt (10%); ○, purified salt (20%); ▼, solar salt (10%); △, solar salt (20%); ■, bamboo salt (10%); □, bamboo salt (20%).

For MJs with 20% salt, the pH decreased from initial values (6.45–7.23) until 8 weeks (6.19–7.00), and then increased gradually. The final pH values (6.28–7.20) were slightly lower than the initial values. BS MJ with 10% salt showed the same pattern. During the early stage of fermentation, LAB proliferated, and produced organic acids such as lactic acid and acetic acid, causing a decrease in pH [9, 17]. During the late stage of fermentation, bacilli and other bacteria produced amines, ammonia, and other basic compounds from proteins, causing an increase in pH. PS and SS MJs with 10% salt showed a different pattern, where the initial pH values (6.59 and 6.57) were maintained until 8–10 weeks, and then increased slowly, and the final values (6.67 and 6.75) were slightly higher than the initial values. The concentration and type of salt caused different microbial communities in MJs, which was responsible for the different pH values of MJs during fermentation. A similar result was reported for squid jeotgal [18]. When squid jeotgals (7% and 10% salt) were fermented at 10°C for 35 days, jeotgal with 7% salt showed a significant increase in pH compared with jeotgal with 10% salt at the end of fermentation.

TA values of MJs were changed following the same patterns as pH changes but in a reverse direction. TA values increased rapidly until 8–10 weeks, reached the highest points, and then maintained or decreased slightly from the highest values (Fig. 1B).

Amino-Type Nitrogen, Ammonia-Type Nitrogen, and Volatile Basic Nitrogen Contents of MJ Samples

ANN is used as an indicator for the degree of protein hydrolysis. Proteins in the raw materials are degraded to lower molecular weight nitrogen compounds such as peptides and amino acids by proteases during fermentations [19]. These changes confer unique physical properties and flavor to fermented foods. The ANN contents of the MJs immediately after preparation were 109.8–126.03 mg% (Fig. 2A). The AAN contents increased continuously during fermentation, especially between 2 and 4 weeks. MJs with 10% salt had higher AAN contents than MJs with 20% salt, and this was due to higher numbers of microorganisms of MJs with 10% salt. MJs with 10% salt had 706.51–729.75 mg% at 4 weeks whereas MJs with 20% salt had 367.70–602.00 mg% at the same time. In a previous study, the ANN content was higher in ascidian jeotgal with low salinity than in

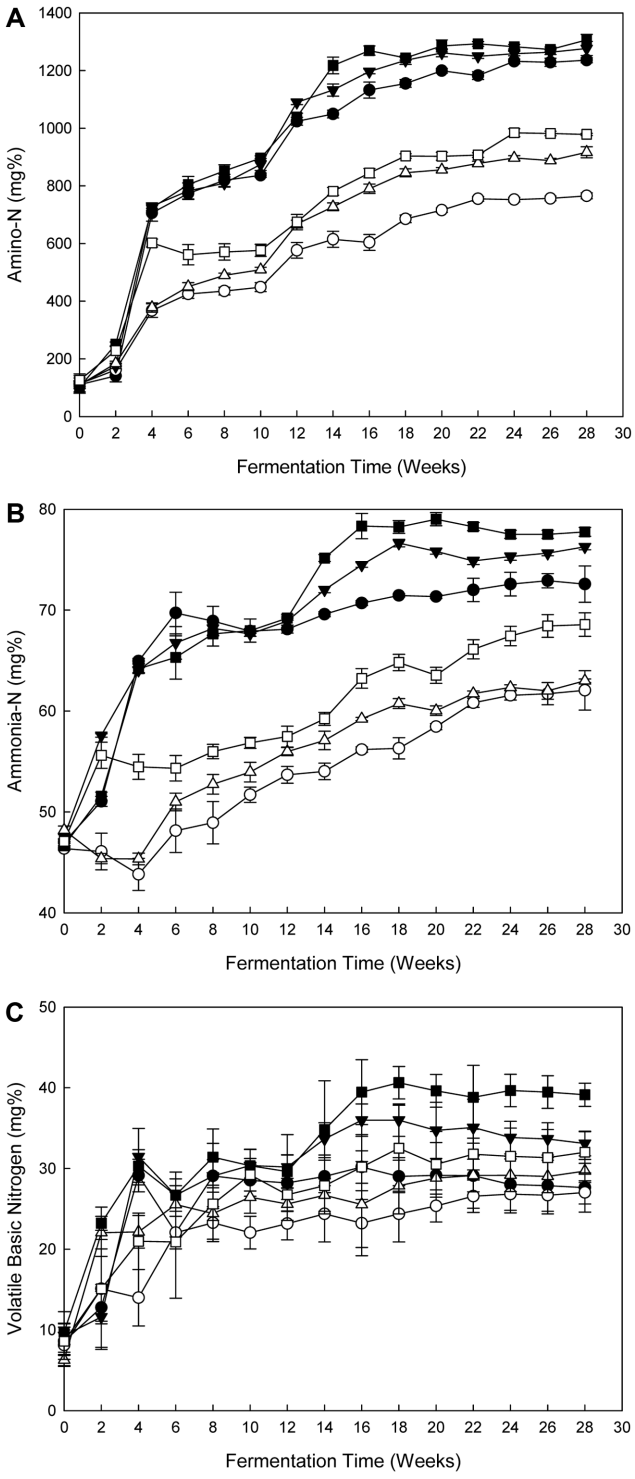

Fig. 2. Changes in amino-type nitrogen (**A**), ammonia-type nitrogen (**B**), and volatile basic nitrogen (**C**) of myeolchi jeotgals prepared with different types of salts during fermentation.
●, Purified salt (10%); ○, purified salt (20%); ▼, solar salt (10%); △, solar salt (20%); ■, bamboo salt (10%); □, bamboo salt (20%).

jeotgal with high salinity [20]. Similar results were also reported for jeotgal prepared from *Haliotis discus hannai* Ino (abalone) viscera where the NaCl concentration was 13% to 25% [21]. BS and SS MJs showed higher AAN contents than PS MJs, and this was due to the higher mineral contents of SS and BS. Minerals are required for the growth of microorganisms and protease activities. Differences in the AAN contents were more significant among MJs with 20% salt.

The AMN content is another index for the degree of fermentation of foods. Excessive degradation of proteins leads to production of ammonia, a cause of bad flavor of fermented foods. The AMN contents of MJs increased in a similar way as the AAN contents during fermentation (Fig. 2B). They increased rapidly until 4 weeks and then increased gradually until the end of fermentation in MJs with 10% salt. The AMN contents of MJs with 10% salt were higher than those of MJs with 20% salt. The AMN contents were 46.65–47.55 and 46.36–48.20 mg/g for MJs with 10% and 20% salt, respectively, just after preparation. After 28 weeks, the AMN contents of BS and SS MJs with 10% salt were 77.76 ± 0.44 and 76.28 ± 0.30 mg/g, respectively, which were higher than that of PS MJ (72.58 ± 1.8 mg/g).

VBN compounds are low molecular weight, basic nitrogen compounds with volatility, such as ammonia, dimethylamine, and trimethylamine [9]. The VBN content is often used to determine the degree of fermentation of jeotgal and the freshness of fishes [9]. The amount of VBN increases either by spoilage of foods or enzymatic degradation of proteins. The initial VBN contents of MJs were 8.13–9.75% mg/g and the contents increased rapidly until 4 weeks, and then increased gradually until the end of fermentation (Fig. 2C). BS MJ with 10% salt showed the highest VBN content of 39.14 ± 2.01 mg% at 28 weeks. PS MJ with 10% salt showed the highest value, 30.18 ± 2.01 mg%, at 16 weeks, and then decreased gradually until 28 weeks, reaching to 27.63 ± 3.48 mg% at 28 weeks.

Changes in Salinities and Neutral Proteases of MJs

Immediately after preparation, the salinity of BS MJ (20% salt) was quite low, 9.32 ± 0.18%, and then increased to 17.88 ± 0.10% after 2 weeks (Fig. 3). This was due to the low solubility of BS. It was reported that 2.65 wt% of BS is insoluble in water due to formation of less water-soluble $Mg(OH)_2$ during preparation of BS [22]. As fermentation proceeded, the solubility of BS increased, causing an increase of salinity. The final salinities were 11.50–11.65% and 16.50–17.10% for MJs with 10% and 20% salt, respectively.

Table 4. Changes in neutral protease activities of myeolchi jeotgals.

		Fermentation period (weeks, U/g)				
		0	4	12	20	26
10%	PS	0.91 ± 0.24	4.02 ± 0.22	4.73 ± 0.22	5.01 ± 0.30	5.39 ± 0.33
	SS	0.75 ± 0.19	5.11 ± 0.19	5.33 ± 0.26	6.62 ± 0.22	6.62 ± 0.15
	BS	0.35 ± 0.30	4.68 ± 0.15	6.43 ± 0.28	6.09 ± 0.23	6.65 ± 0.46
20%	PS	0.38 ± 0.21	2.64 ± 0.21	3.12 ± 0.26	2.94 ± 0.36	3.70 ± 0.25
	SS	0.82 ± 0.19	2.88 ± 0.11	3.70 ± 0.22	3.79 ± 0.30	4.46 ± 0.32
	BS	0.95 ± 0.14	3.46 ± 0.20	4.11 ± 0.11	4.29 ± 0.31	5.01 ± 0.40

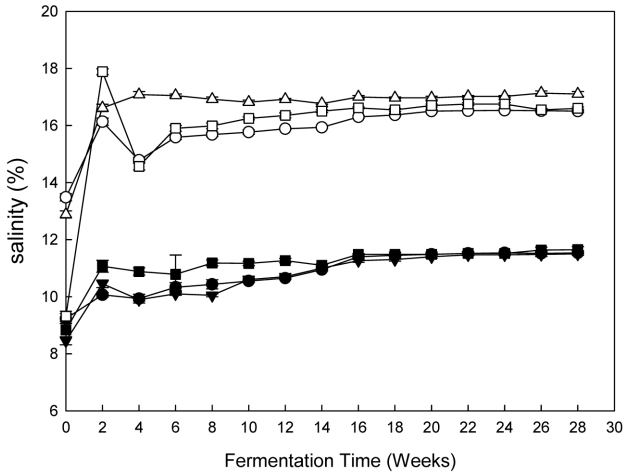

Fig. 3. Changes in the salinity of myeolchi jeotgals prepared with different types of salts during fermentation.
●, Purified salt (10%); ○, purified salt (20%); ▼, solar salt (10%); △, solar salt (20%); ■, bamboo salt (10%); □, bamboo salt (20%).

There were no significant differences between MJs with different types of salts.

The neutral protease activities of MJs increased continuously during fermentation (Table 4). The activities of SS and BS MJs were higher than those of PS MJs. The mineral contents of SS and BS were higher than that of PS and this caused growth stimulation of microorganisms in the BS and SS MJs. MJs with 10% salt showed higher protease activities than MJs with 20% salt, and this was due to growth inhibition of microorganisms at 20% salt.

Growth of microorganisms was affected by salt types during MJ fermentation. Yeast counts were higher in PS MJs than SS and BS MJs. SS and BS MJs showed higher AAN, AMN, and VBN contents than PS MJs. Neutral protease activities were also higher in SS and BS MJs. SS and BS seem more appropriate than PS for the preparation of high-quality MJs. *T. halophilus* and *S. epidermidis* were the most dominant bacterial species of MJs with 10% salt, as shown by a culture-independent method. Further studies are necessary for determining the effect of different salt types on the growth of *T. halophilus* and *S. epidermidis*, and the roles of these bacteria in the quality of MJ.

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