Effects of different types of salts on the growth of lactic acid bacteria and yeasts during kimchi fermentation

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Abstract Kimchi was prepared with different types of salts: purified salt (PS), solar salt aged for 1 year (SS1), aged for 3 years (SS3), and bamboo salt (BS). Kimchi inoculated with *Leuconostoc mesenteroides* P30 (starter kimchi), and control kimchi (non-starter kimchi) were prepared, and stored at -1°C for 20 weeks. Titratable acidity values increased slowly and reached 0.96–1.01% (pH 3.73–3.83) at 20 weeks. Proportions of coccus-type lactic acid bacteria (LAB) among total LAB were higher in SS kimchi than PS kimchi. Among non-starter kimchi, the proportions were 44.7, 41.6, 29.7, and 32.1% for SS3, SS1, BS, and PS kimchi, respectively, at 2 weeks, and 11.5, 12.8, 6.7, and 5.8%, respectively, at 20 weeks. SS kimchi had much less yeast counts than PS kimchi. Among starter kimchi, yeasts were detected from PS kimchi at 10 weeks but not detected until 18 weeks from SS1 and BS kimchi and 20 weeks from SS3 kimchi.

Keywords Kimchi · Solar salt · Bamboo salt · Purified salt · *Leuconostoc mesenteroides* · Yeasts

Introduction

Kimchi is a Korean fermented vegetable, containing high levels of vitamins (vitamin B and C), minerals (Na, Fe, K), and many other functional components such as chlorophyll,

allyl compounds, and phytochemicals [1, 2]. Previous studies have shown that kimchi is a functional food with anticancer, antioxidative, antidiabetic, and antiobesity effects [2–4]. Kimchi fermentation is affected by several factors including temperature, salt (types and concentrations), and the ingredients added, which affect microflora of kimchi and eventually the quality of kimchi.

Salt is an important seasoning for most fermented foods including kimchi and it improves the taste of foods and extends the shelf life of foods [5]. Solar salt is obtained from sea water after sea water on the ground is evaporated by sun light and wind. Unlike purified salt, which consists of almost entirely NaCl, solar salt contains significant amounts of minerals such as K, Mg, and Ca [6]. A study indicates that mineral-rich solar salt is better than purified salt for reducing blood pressure [7]. Bamboo salt is a special salt obtained through melting of solar salt in a bamboo container. Solar salt is placed into a bamboo container and the top and bottom of bamboo trunk is sealed with red clay and then heated. Solar salt is melted as the temperature increases above 1000–1500 $^{\circ}\text{C}$ and then recrystallized as the temperature decreases [8]. The melting and recrystallization processes are repeated up to maximum 9 times, and the bamboo salt obtained after 9th cycles is believed to possess significant medicinal effects.

Solar salt and bamboo salt contain more than 70 essential minerals and micronutrients whereas purified salt contains no other minerals besides sodium chloride [6, 8]. For this reason, solar salt has been widely used for fermented foods such as kimchi and doenjang (fermented soybean paste) in Korea. Bamboo salt is becoming popular because of its presumed medicinal effects regardless of its high price. However, few studies have been done for the effects of solar salt or bamboo salt on the quality of

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fermented foods, and especially on the growth of microorganisms during fermentation.

In this study, kimchi samples were prepared by using two solar salts (aged for 1 year and 3 years), a bamboo salt (recrystallized 3 times), and a purified salt. *Leuconostoc mesenteroides* subsp. *mesenteroides* P30 was inoculated into kimchi as a starter for one set of kimchi samples. Total 8 different kimchi samples were prepared. The properties of kimchi samples were examined during the initial fermentation and following 20 weeks of storage at -1°C . The purpose of this study was to examine the effects of different types of salts on the growth of lactic acid bacteria (LAB) and yeasts, two most important groups of microorganisms for the quality of kimchi.

Materials and methods

Preparation of kimchi

Kimchi was prepared with 4 different types of salts: purified salt (PS) (Hanju, Ulsan, Korea, NaCl $99.18 \pm 0.58\%$, moisture content $1.24 \pm 0.21\%$), solar salt aged for 3 years (SS3) (Tae-Pyung salt farm, Sinan, Korea, NaCl $82.55 \pm 0.33\%$, moisture content $5.17 \pm 0.40\%$), solar salt aged for 1 year (SS1) (Tae-Pyung salt farm, NaCl $79.85 \pm 0.33\%$), and bamboo salt recrystallized 3 times (BS) (Insanga, Hamyang, Korea, NaCl $94.54 \pm 0.58\%$, moisture content $1.49 \pm 0.01\%$). Kimchi cabbage was purchased at a local market in Jinju, Korea in the fall of 2014, soaked in brine (10% in terms of NaCl content, w/v) prepared with different types of salts for 10 h, and then washed 3 times with tap water. Washed cabbages were stored in plastic baskets for 3 h to drain excess water. Salted kimchi cabbages were cut into 4×4 cm squares and mixed with ingredients according to a following formula: salted cabbage: red pepper powder: crushed garlic: crushed ginger: jeotgal (salted and fermented sea food): radish: sugar: scallion = 100: 3.5: 1.4: 0.6: 2.2: 13: 1: 2 (w/w). The salt concentration was adjusted to 2.05% in terms of NaCl content (w/w) by using a salimeter (PAL-SALT, Atago, Tokyo, Japan).

Leuconostoc mesenteroides subsp. *mesenteroides* P30 (*L. mesenteroides* P30) was inoculated into kimchi samples (starter kimchi). The strain was isolated from dongchimi, a type of watery kimchi where the main ingredients are radish and brine. *L. mesenteroides* P30 was grown in lactobacilli MRS broth (Becton, Dickinson and Company, Sparks, MD, USA) overnight and cells were recovered by centrifugation ($7000 \times g$, 20 min, 4°C). Cell pellet was washed with cold sterile water, resuspended in 10 ml of sterile water, and cells were added into kimchi samples (10^7 CFU/g of kimchi) with other ingredients. Another set

of kimchi samples were prepared exactly the same way except no starter was added (control kimchi). Fresh kimchi samples (each 15 kg) were stored in plastic containers ($18 \text{ l}, 19 \times 38 \times 26 \text{ cm}$) specially designed for Dimchae (Winia, Seoul, Korea), a kimchi refrigerator designed for a long-term storage of kimchi. Kimchi samples were first placed into a 15°C incubator until TA values increased to 0.51–0.54% (initial fermentation, 40–64 h). Then, kimchi samples were stored in a kimchi refrigerator (Dimchae) for 20 weeks at -1°C .

Changes in pH, titratable acidity (TA) and salinity of kimchi samples

During storage at -1°C , kimchi samples (100 g) were taken out every 2 weeks, macerated using a laboratory blender (Waring Lab., Sci., Torrington, CT, USA), and then centrifuged for 15 min at $14,000 \times g$. Supernatant was filtered through sterile gauze, and the filtrate was used as a sample for pH, TA, and salinity measurements. The pH was measured with a pH meter. TA was calculated using the following equation after the filtrate was titrated with 0.1 N NaOH to pH 8.3. $\text{TA (\%)} = \text{amount of 0.1 N NaOH (ml)} \times 0.009 \times 10$. The salinity was measured with a salimeter (PAL-SALT).

Changes in total bacteria, LAB, and yeast counts of kimchi samples

Twenty grams of kimchi sample was homogenized with 80 ml of 0.1% peptone water using a stomacher 80 (Seward, Worthing, England) for 2 min. Homogenate was filtered through a Whatman no.2 filter paper (Waters, Milford, MA, USA). The filtrate was serially diluted with 0.1% peptone water and 0.1 ml of each dilution was spread onto agar plates: plate count agar (PCA) plates for total bacteria counts, yeast extract-peptone- dextrose agar (YPD agar, Becton, Dickinson and Company) plates for yeast counts, and lactobacilli MRS agar (Becton, Dickinson and Company) plates for total LAB counts. Phenylethyl alcohol sucrose agar (PES agar) plates were used for the counting of *Leuconostoc* spp. (coccus-type LAB) and *Lactobacillus* selection agar (LBS agar, Becton, Dickinson and Company) plates for the counting of *Lactobacillus* spp. (rod-type LAB) [9–11]. The plates were incubated for 48 h at 30°C or 37°C .

Colonies were randomly picked up and their morphologies were examined using a microscope after Gram staining. For identification, 16S rRNA genes were sequenced for selected colonies. 16S rRNA genes were amplified by PCR using the universal primer set: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGA CTT-3') [12]. PCR was

done using a MJ mini personal thermal cycler (BioRad, Hercules, CA, USA) as follows: denaturation at 94 °C for 5 min, 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C.

Yeast species were examined from PS and SS1 kimchi samples (non-starter) at 10 weeks of storage and from all 4 kimchi samples (non-starter) at 20 weeks. Thirty yeast colonies on YPD plates were randomly selected from each sample (total 180 colonies). ITS (internal transcribed spacer) regions of selected colonies were amplified by PCR using the primer set: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [13]. Sequencing was done at Cosmogenetech (Seoul, Korea). BLAST program provided by National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) was used to find homologous sequences in the data library (<http://www.ncbi.nlm.nih.gov/BLAST>).

Sensory evaluation of kimchi samples

Sensory characteristics of kimchi samples were evaluated by 22 panels (man: woman = 12: 10), average age of 27.5 years old, using a 7-point scale for color, flavor, taste, texture and overall acceptability. The properties were evaluated as follows: very poor (1 point), medium (4 point), and very good (7 point). Panels were provided with drinking water and crackers to rinse their mouth and remove remaining taste before testing other samples.

The statistical analyses of sensory data were performed by least significant difference (LSD) test ($P < 0.05$).

Statistical analyses of microbial counts

All tests were repeated 3 times and the results were shown as mean $\pm$ standard deviation. Data were analyzed by one-way ANOVA and Duncan's multiple range test was done using SPSS ver. 18 (SPSS Inc., Chicago, IL, USA) package ($P < 0.05$).

Results and discussion

Changes in pH, TA, and salinity of kimchi samples

Immediately after preparation (IP), pH and TA values of kimchi samples were 5.34–5.64 and 0.2%, respectively (Fig. 1A, B). Initial fermentation was done at 15 °C and it took 40 h for starter kimchi samples and 64 h for non-starter kimchi samples to reach TA values of 0.51–0.54%. During these periods, the pH decreased to 4.11–4.24. During the initial fermentation and early stage of storage at -1 °C, pH decrease and TA increase occurred faster in

Fig. 1 Changes in pH, TA, and salinity of kimchi samples during initial fermentation and storage. (A) pH; (B) TA; (C) salinity. IP stands for immediately after preparation of kimchi and EIF stands for end of initial fermentation at 15 °C. ●, PS kimchi (non-starter); ○, PS kimchi (starter); ▼, SS3 kimchi (non-starter); △, SS3 kimchi (starter); ■, SS1 kimchi (non-starter); □, SS1 kimchi (starter); ◆, BS kimchi (non-starter); ◇, BS kimchi (starter)

starter kimchi samples than non-starter kimchi samples. But differences were not seen as storage period extended. The pH and TA values of well fermented kimchi, best for consumption, are around 4.2 and 0.6–0.8%, respectively [2]. Therefore, kimchi samples were ready-to-eat states after initial fermentation. This was because most LAB

grew rapidly at 15 °C and produced enough acids in short period. During the storage period, TA of kimchi samples increased gradually until 14 weeks, reaching to 0.96–1.01% (pH 3.73–3.83), and these values were maintained stably until 20 weeks (Fig. 1B). There were no significant differences among kimchi samples with different types of salts. The addition of starter did not affect the final TA values of kimchi samples, either. pH changes of kimchi samples followed TA changes in a reverse way. The values decreased rapidly during initial fermentation and then decreased gradually until 4 weeks of storage. After 4 weeks, pH values were maintained stably until the end. Unlike other kimchi samples, SS1 kimchi (non-starter) showed slight increase in pH between EIF (end of initial fermentation) and 2 weeks (4.16–4.27). But the pH decreased again after 2 weeks, and all kimchi samples showed stable pH values after 4 weeks.

Salinities of kimchi samples were measured (Fig. 1C). The initial salt concentration of kimchi samples was adjusted to 2.05% in terms of NaCl content. After initial fermentation, the salinities of SS and PS kimchi samples were 1.92–1.94% and 1.97–1.98%, respectively (Fig. 1C). The salinities decreased further to 1.73–1.76 and 1.69–1.70% at 20 weeks of storage, respectively. The salinities of BS kimchi were 1.93–1.95% at the end of initial fermentation and decreased further to 1.69–1.70% at 20 weeks of storage. Decrease in salinity of kimchi samples was due to the osmotic phenomenon of kimchi cabbages. Kimchi cabbage was cut into small pieces (4 × 4 cm) for the complete mixing of seasonings and starter organisms. Due to small size, water release from cabbage was accelerated by osmosis, causing decrease in salinity of kimchi samples during storage.

Changes in total bacteria, LAB, and yeast counts of kimchi samples

Total bacteria, LAB, and yeasts were counted from kimchi samples (Tables 1 and 2). Immediately after kimchi preparation (IP), total bacteria counts were 6.54–6.70 log CFU/g for non-starter kimchi, and 7.31–7.59 log CFU/g for starter kimchi samples. Total bacteria counts increased to 8.97–9.11 log CFU/g for non-starter kimchi samples and 9.02–9.10 log CFU/g for starter kimchi samples after initial fermentation (EIF), and increased continuously until 2 weeks of storage. After 2 weeks, the counts decreased gradually, reaching about 5 log CFU/g at 20 weeks. LAB counts of non-starter kimchi samples, determined by using MRS agar plates, increased from 6.52 to 6.64 log CFU/g immediately after kimchi preparation to 8.84–9.05 log CFU/g after initial fermentation (Table 1). LAB counts increased until 2 weeks of storage, reaching to 9.10–9.90 log CFU/g, and then decreased continuously, reaching to

5.04–5.21 log CFU/g at 20 weeks. LAB counts of starter kimchi samples increased from 7.25 to 7.45 log CFU/g immediately after kimchi preparation to 9.01–9.06 log CFU/g after initial fermentation (Table 2). LAB counts increased to 9.48–9.81 log CFU/g at 2 weeks of storage, and then decreased gradually, reaching to 5.17–5.29 log CFU/g at 20 weeks.

Proportions of coccus-type and rod-type LAB to total LAB were estimated by dividing counts of coccus-type LAB (colonies on PES agar plates) and rod-type LAB (colonies on LBS agar plates) by total LAB counts (colonies on MRS agar plates). The dominant LAB were rod-types for non-starter kimchi samples. The proportions of rod-type LAB were 67.9, 55.3, 58.4, and 70.3% for PS, SS3, SS1, and BS kimchi, respectively, at 2 weeks of storage (Table 1). The proportions of rod-types increased continuously and were 94.2, 88.5, 87.2, and 92.3% for PS, SS3, SS1, and BS kimchi, respectively, at 20 weeks. Among non-starter kimchi samples, BS kimchi had the highest proportion of rod-type LAB (lactobacilli), and PS kimchi the second highest until 8th weeks of storage (Table 1). After 8 weeks, PS kimchi had the highest proportion followed by BS kimchi until 20 weeks. SS kimchi samples showed significantly lower lactobacilli proportions than PS kimchi throughout the storage period.

For starter kimchi samples, coccus-type LAB were the major group in the early stage of storage, and the proportions of coccus-type LAB were 62.5, 79.3, 77.9, and 68.1% for PS, SS3, SS1, and BS kimchi, respectively, at 2 weeks. The proportions of coccus-type LAB decreased continuously as storage period was extended.

It has been well known that *Leuconostoc* species are dominant during early and middle stages of kimchi fermentation but lactobacilli showing more acid tolerance become dominant during later stages of kimchi fermentation [14–16]. Significant decrease in the proportions of coccus-type was observed between 4 and 6 and 10 and 12 weeks (Table 2). The proportions of coccus-type LAB were 8.1, 13.5, 12.1, and 9.4% for PS, SS3, SS1, and BS kimchi, respectively, at 20 weeks. One interesting observation was that the proportions of coccus-type LAB were significantly higher in SS kimchi samples than PS kimchi samples for both starter and non-starter kimchi samples. These results indicated that SS stimulated the growth of coccus-type LAB such as *Leuconostoc* spp. In other words, *Leuconostoc* species are more sensitive than *Lactobacillus* species to minerals such as Mg which are present in high concentrations in SS but absent in PS. The same results were reported by our group. When two kimchi samples, MRS-kimchi (same as SS3-kimchi) and PS-kimchi, were fermented for 6 weeks, the proportions of lactobacilli were 74 and 90%, respectively [17]. The differences in the portions of lactobacilli were interpreted to be caused by

Table 1 Changes in microbial populations of kimchi (non-starter) prepared with different types of salts

Counts (log CFU/g)		Kimchi	IP	EIF	Storage (weeks)							
		2	4	6	8	10	12	14	16	18	20	
Total viable cells	PS	6.61 ± 0.02 ^b	9.11 ± 0.02 ^b	9.04 ± 0.03 ^c	8.54 ± 0.06 ^b	8.31 ± 0.04 ^b	7.23 ± 0.05 ^b	6.64 ± 0.03 ^b	5.63 ± 0.05 ^b	5.21 ± 0.06 ^b	5.01 ± 0.04 ^a	
	SS3	6.54 ± 0.04 ^a	8.97 ± 0.03 ^a	9.01 ± 0.02 ^a	8.68 ± 0.03 ^c	8.39 ± 0.02 ^c	7.31 ± 0.02 ^b	6.86 ± 0.03 ^d	5.71 ± 0.08 ^c	5.32 ± 0.05 ^c	5.25 ± 0.03 ^b	
	SS1	6.68 ± 0.03 ^c	9.08 ± 0.02 ^b	9.93 ± 0.04 ^c	8.78 ± 0.05 ^d	8.42 ± 0.01 ^c	7.32 ± 0.03 ^b	6.77 ± 0.04 ^c	5.58 ± 0.03 ^b	5.36 ± 0.05 ^c	5.31 ± 0.02 ^b	
	BS	6.70 ± 0.03 ^c	9.09 ± 0.01 ^b	9.65 ± 0.03 ^b	9.13 ± 0.02 ^b	9.03 ± 0.01 ^a	8.01 ± 0.02 ^a	7.94 ± 0.03 ^a	6.38 ± 0.02 ^a	5.21 ± 0.04 ^a	5.05 ± 0.03 ^a	
LAB	PS	6.55 ± 0.03 ^a	8.84 ± 0.01 ^a	9.57 ± 0.06 ^b	9.12 ± 0.07 ^a	9.02 ± 0.01 ^b	8.46 ± 0.03 ^b	8.28 ± 0.02 ^b	7.21 ± 0.02 ^b	6.54 ± 0.04 ^d	5.51 ± 0.03 ^c	
	SS3	6.52 ± 0.02 ^a	8.95 ± 0.02 ^b	9.10 ± 0.02 ^a	9.02 ± 0.02 ^a	8.89 ± 0.03 ^a	8.62 ± 0.02 ^c	8.34 ± 0.01 ^b	7.22 ± 0.04 ^b	6.41 ± 0.02 ^b	5.40 ± 0.03 ^b	
	SS1	6.64 ± 0.02 ^b	9.05 ± 0.02 ^c	9.90 ± 0.04 ^d	9.33 ± 0.04 ^b	9.12 ± 0.01 ^c	8.71 ± 0.01 ^d	8.35 ± 0.02 ^c	7.27 ± 0.01 ^b	6.47 ± 0.03 ^c	5.42 ± 0.02 ^b	
	BS	6.63 ± 0.02 ^b	9.04 ± 0.01 ^c	9.66 ± 0.02 ^c	9.12 ± 0.07 ^a	9.04 ± 0.03 ^b	7.94 ± 0.03 ^a	7.61 ± 0.06 ^a	6.82 ± 0.03 ^a	6.33 ± 0.01 ^a	5.25 ± 0.04 ^a	
Yeast	PS	-	-	-	-	-	-	1.20 ± 0.03 ^b	3.60 ± 0.04 ^b	4.10 ± 0.02 ^b	5.40 ± 0.03 ^b	
	SS3	-	-	-	-	-	-	-	-	-	1.60 ± 0.06 ^b	
	SS1	-	-	-	-	-	-	1.00 ± 0.01 ^a	1.70 ± 0.02 ^a	1.90 ± 0.04 ^a	2.20 ± 0.02 ^a	
	BS	-	-	-	-	-	-	-	-	-	1.10 ± 0.04 ^a	
Ratio* (<i>Leuconostoc</i> / <i>Lactobacillus</i> , %)	PS	-	-	32.1/67.9	33.1/66.9	27.3/72.7	24.2/75.8	20.3/79.7	14.4/85.6	6.7/93.3	6.1/93.9	
	SS3	-	-	44.7/55.3	42.4/57.6	39.1/60.9	34.4/65.6	32.1/67.9	22.4/77.6	14.6/85.4	13.1/86.9	
	SS1	-	-	41.6/58.4	40.6/59.4	40.7/59.3	35.1/64.9	33.4/66.6	24.1/75.9	16.9/83.1	14.8/85.2	
	BS	-	-	29.7/70.3	29.6/70.4	27.1/72.9	22.5/77.5	21.1/78.9	15.2/84.8	7.5/92.5	6.6/93.4	

Values are mean ± SD from triplicate determinations. Significant differences (a-d) in the same tested counts are tested ($P < 0.05$, Duncan's multiple range test) IP, immediately after preparation of kimchi; EIF, end of initial fermentation

*Ratio is the proportion of coccus-type (*Leuconostoc* spp.) or rod-type (*Lactobacillus* spp.) counts to the total LAB counts

Table 2 Changes in microbial populations of kimchi (starter) prepared with different types of salt

Counts (log CFU/g)		Kimchi	IP	EIF	Storage (weeks)							
		2	4	6	8	10	12	14	16	18	20	
Total viable cells	PS	7.51 ± 0.03 ^b	9.02 ± 0.02 ^a	9.83 ± 0.06 ^c	9.54 ± 0.08 ^c	9.35 ± 0.03 ^d	8.54 ± 0.06 ^b	8.34 ± 0.02 ^b	7.27 ± 0.02 ^b	6.46 ± 0.03 ^b	5.43 ± 0.02 ^a	5.25 ± 0.02 ^a
	SS3	7.59 ± 0.01 ^c	9.06 ± 0.03 ^a	9.47 ± 0.04 ^a	9.23 ± 0.04 ^a	9.13 ± 0.02 ^b	8.68 ± 0.03 ^c	8.34 ± 0.04 ^b	7.30 ± 0.03 ^b	6.54 ± 0.04 ^b	5.46 ± 0.03 ^a	5.28 ± 0.03 ^a
	SS1	7.58 ± 0.01 ^c	9.10 ± 0.01 ^b	9.55 ± 0.02 ^b	9.31 ± 0.03 ^b	9.21 ± 0.02 ^c	8.78 ± 0.05 ^c	8.35 ± 0.02 ^b	7.33 ± 0.02 ^b	6.61 ± 0.02 ^c	5.51 ± 0.05 ^b	5.43 ± 0.02 ^b
	BS	7.31 ± 0.02 ^a	9.09 ± 0.02 ^b	9.61 ± 0.06 ^b	9.33 ± 0.05 ^b	9.03 ± 0.03 ^a	8.05 ± 0.02 ^a	7.93 ± 0.02 ^a	6.88 ± 0.03 ^a	6.37 ± 0.02 ^a	5.40 ± 0.02 ^a	5.38 ± 0.04 ^a
LAB	PS	7.25 ± 0.02 ^a	9.01 ± 0.01	9.81 ± 0.05 ^c	9.48 ± 0.02 ^c	9.21 ± 0.02 ^c	8.66 ± 0.04 ^b	8.37 ± 0.01 ^b	7.26 ± 0.01 ^b	6.41 ± 0.03 ^b	5.40 ± 0.03 ^b	5.31 ± 0.04 ^b
	SS3	7.45 ± 0.01 ^b	9.05 ± 0.01	9.48 ± 0.08 ^a	9.19 ± 0.06 ^a	9.08 ± 0.03 ^a	8.71 ± 0.02 ^b	8.41 ± 0.03 ^b	7.28 ± 0.03 ^b	6.51 ± 0.02 ^c	5.44 ± 0.03 ^b	5.25 ± 0.03 ^b
	SS1	7.44 ± 0.02 ^b	9.05 ± 0.03	9.58 ± 0.09 ^b	9.18 ± 0.03 ^a	9.14 ± 0.02 ^b	8.73 ± 0.04 ^b	8.38 ± 0.02 ^b	7.24 ± 0.03 ^b	6.42 ± 0.02 ^b	5.39 ± 0.05 ^b	5.29 ± 0.04 ^b
	BS	7.27 ± 0.04 ^a	9.06 ± 0.01	9.59 ± 0.08 ^b	9.27 ± 0.04 ^b	9.05 ± 0.01 ^a	8.27 ± 0.05 ^a	8.18 ± 0.03 ^a	6.71 ± 0.02 ^a	6.31 ± 0.04 ^a	5.27 ± 0.03 ^a	5.24 ± 0.04 ^b
Yeast	PS	–	–	–	–	–	–	1.1 ± 0.02	1.9 ± 0.01	3.1 ± 0.03	4.2 ± 0.04	5.1 ± 0.02 ^d
	SS3	–	–	–	–	–	–	–	–	–	–	1.5 ± 0.05 ^a
	SS1	–	–	–	–	–	–	–	–	–	1.8 ± 0.03 ^b	2.1 ± 0.04 ^c
	BS	–	–	–	–	–	–	–	–	–	1.1 ± 0.02 ^a	1.7 ± 0.03 ^b
Ratio* (<i>Leuconostoc</i> <i>Lactobacillus</i> , %)	PS	–	–	62.5/37.5	62.1/37.9	48.4/51.6	49.4/50.6	46.6/53.4	30.2/69.8	20.4/79.6	10.5/89.5	9.2/90.8
	SS3	–	–	79.3/20.7	77.4/22.6	54.4/45.6	54.2/45.8	52.7/47.3	38.3/61.7	27.4/72.6	16.8/83.2	15.4/84.6
	SS1	–	–	77.9/22.1	76.7/23.3	53.2/46.8	51.4/48.6	49.8/50.2	36.2/63.8	26.1/73.9	13.4/86.6	13.6/86.4
	BS	–	–	68.1/31.9	68.3/31.7	49.0/51.0	49.1/50.9	46.5/53.5	33.1/66.9	23.4/76.6	12.1/87.9	10.9/89.1

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

IP, immediately after preparation of kimchi; EIF, end of initial fermentation

*Ratio is the proportion of coccus-type (*Leuconostoc* spp.) or rod-type (*Lactobacillus* spp.) counts to the total LAB counts

different mineral contents of two salt types. Among the minerals, two showed significant differences in the amounts. Mg content of MRS-kimchi was 17.34 ± 0.59 mg/100 g kimchi at 6 weeks but it was 9.26 ± 0.36 mg/100 g kimchi for PS-kimchi. Mn content of MRS-kimchi was 0.27 ± 0.02 mg/100 g kimchi at 6 weeks whereas that of PS-kimchi was 0.51 ± 0.12 mg/100 g kimchi [17]. It was suspected that different Mg and Mn contents somehow affected the growth of *Leuconostoc* spp. The amount of major minerals were shown in our previous report [17]. Higher proportions of *Leuconostoc* spp. in SS kimchi was previously reported by other group. When Chang et al. examined the proportion of *Leuconostoc citreum* GJ7, a starter used, among total LAB from kimchi samples prepared with different types of salts, 80.2, 75.8, and 61.3% were observed for SS4 (SS aged for 4 years), SS1 (SS aged for 1 year), and PS kimchi, respectively, after 33 days at 7 °C [18]. The proportions of rod type LAB among total LAB were 19.8, 21.6, and 38.7% for SS4, SS1, and PS kimchi, respectively after 33 days [18]. They concluded that growth of LAB and especially *Leuconostoc* was accelerated in SS kimchi samples than PS kimchi, and this was caused by minerals present in SS [18].

To verify the selecting power of PES and LBS media for coccus-type and rod-type LAB, respectively, 10 colonies were randomly selected from both agar plates inoculated with kimchi samples at 2, 8, 14, and 20 weeks of storage. Total 320 colonies were picked up from PES agar plates (8 samples and 4 time points) and another 320 colonies from LBS agar plates. They were identified by 16S rRNA gene sequencing as described in the methods section. Sequences of 600–650 nucleotides in length were determined and analyzed by BLAST. Colonies from PES agar plates were identified to be *Leu. mesenteroides* (284/320, 88.7%), *Leu. citreum* (13/320, 4.1%), *Lactococcus lactis* (21/320, 6.6%), and *Streptococcus thermophilus* (2/320, 0.6%) (Table 3). Another 320 colonies from LBS agar plates were all identified as lactobacilli. They were identified to be *Lb. plantarum* (261/320, 81.6%), and *Lb. sakei* (59/320, 18.4%) (Table 3). The results clearly showed that PES and

LBS agar plates were very effective for selecting coccus-type and rod-type LAB, respectively, from kimchi samples. In addition to *Leu. mesenteroides*, which was added as the starter, *Leu. citreum*, *Lac. lactis*, *Lb. plantarum*, and *Lb. sakei* were detected. They are well known as the major LAB species during kimchi fermentation [19].

SS contains significant amounts of minerals such as Mg, K, and Ca as reported by Chang et al. [18]. These minerals stimulate growth of LAB during kimchi fermentation. The Mg content of SS1 kimchi immediately after preparation was 19.03 ± 1.58 mg/100 g kimchi, higher than that of PS kimchi (10.52 ± 0.80 mg/100 g kimchi). Although SS contained minerals such as Mg and Ca at significant amounts compared to PS, most minerals were removed from kimchi during washing step after cabbage was brined. *Leuconostoc* spp. are obviously more dependent on the minerals for growth than *Lactobacillus* spp. In fact, *L. mesenteroides* P30 showed much higher degree of growth stimulation than *Lb. sakei* BN17 when SS was added into mineral-deficient medium where both strains had been inoculated (results not shown).

Heterolactic fermenters such as *Leuconostoc* spp. are major LAB during early and middle stages of kimchi fermentation and play important roles for developing kimchi taste and flavor by producing lactic acid, acetic acid, carbon dioxide, and ethanol [18]. These metabolites are responsible for the increased acidity and the refreshing taste of well fermented kimchi. As fermentation continues further, acid resistant lactobacilli become dominant and they produce more acids, causing overacidification of kimchi, a state where excessive acids severely deteriorate the eating quality of kimchi [9, 14, 16]. If a method is developed by which heterofermenting LAB remain as the dominant flora for an extended period, the shelf-life of kimchi could be extended. Efforts have been made to isolate acid-resistant *Leuconostoc* spp. to be used as starters [16]. An *Enterococcus* strain which produced a bacteriocin inhibiting *Lb. plantarum* was isolated and tested as a starter for extending shelf-life of kimchi [20]. In this respect, SS and BS are more appropriate than PS for kimchi

Table 3 Identification of LAB selected on PES and LBS agar plates

Types of medium	Most matched ^a	Matched colony counts/total colony counts (%)
PES agar	<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	284/320 (88.7%)
	<i>Leuconostoc citreum</i>	13/320 (4.1%)
	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	21/320 (6.6%)
	<i>Streptococcus thermophilus</i>	2/320 (0.6%)
LBS agar	<i>Lactobacillus plantarum</i>	261/320 (81.6%)
	<i>Lactobacillus sakei</i>	59/320 (18.4%)

^a16S rRNA genes (700 bp) were amplified and sequenced. Blast was used to identify the species

preparation since they encourage growth of *Leuconostoc* spp, which is expected to expand shelf-life of kimchi.

Yeasts were detected from PS kimchi samples at 10 weeks of storage, and the numbers were 1.20 log CFU/g for non-starter kimchi and 1.10 log CFU/g for starter kimchi. Thereafter yeast counts increased continuously and reached to 5.91 log CFU/g for non-starter kimchi and 5.10 log CFU/g for starter kimchi at 20 weeks. Yeast counts of SS1 kimchi (non-starter) were 1.00 log CFU/g at 10 weeks and 2.90 log CFU/g at 20 weeks. Yeasts were not detected from SS3 and BS kimchi samples (non-starter) until 18 weeks, and the numbers were 1.90 log CFU/g for SS3 kimchi and 1.40 log CFU/g for BS kimchi at 20 weeks. Among the starter kimchi samples, yeasts were detected only from PS kimchi (1.10 log CFU/g) at 10 weeks. Yeasts were not detected until 18 weeks from SS1 and BS kimchi and 20 weeks from SS3 kimchi. The yeast counts were 5.10, 2.10, 1.70, and 1.50 log CFU/g for PS, SS1, BS, and SS3 kimchi, respectively at 20 weeks (Table 2). The yeast counts were much higher in PS kimchi samples than SS and BS kimchi samples for both starter and non-starter kimchi samples. The same phenomenon was observed from doenjang samples prepared with the same batch of salts (PS, SS1, SS3, and BS) used for kimchi preparation in this work. Doenjang samples (12% NaCl, w/w) were prepared and fermented for 13 weeks at 25 °C [21]. During the fermentation period, SS and BS doenjang samplers showed much lower yeast counts than PS doenjang. After fermentation (13 weeks), the yeast counts of doenjang samples (no starters) were 7.17, 5.05, 5.92, and 4.54 log CFU/g for PS, SS3, SS1, and BS doenjang, respectively [21]. Doenjang samples with starters showed the same results for yeast counts. A similar result was reported for kimchi fermentation by other group. Chang et al. prepared kimchi with SS4 (4 year aged SS), SS1, and PS. After 5 month storage at -1 °C, yeast counts were 1.3, 4.7, and 5.1 log CFU/ml for SS4, SS1, and PS kimchi, respectively [9]. The same authors reported that yeasts were not detected from SS4 kimchi after 33 days of fermentation at 7 °C but detected from PS kimchi (1.94 log CFU/ml) at 33 days [18]. The salts used were different from those used by our group, but still very similar results were obtained, indicating different salt types affected significantly the growth of yeasts.

Non-starter PS and SS3 kimchi samples at 10 weeks and all four non-starter kimchi samples at 20 weeks were spread onto YPD agar plates. Thirty colonies were randomly selected from each sample and ITS sequencing was done to identify the colonies. Results showed that most were *Candida albicans* and some were *Saccharomyces cerevisiae* (Table 4). Among the 30 colonies from PS kimchi at 10 weeks, 22 were *Candida albicans* (22/30, 73.3%), 6 were *Saccharomyces cerevisiae* (6/30, 20.0%),

and 2 were *Pichia onychis* (2/30, 6.7%). From SS3 kimchi at 10 weeks, 26 were *Candida albicans* (26/30, 86.7%) and 4 were *Saccharomyces cerevisiae* (4/30, 13.3%). In PS kimchi at 20 weeks, the proportion of *C. albicans* increased to 90.0% (27/30) and that of *S. cerevisiae* decreased to 10.0% (3/30) (Table 4). In SS3 kimchi at 20 weeks, the proportion of *C. albicans* was the same, 86.7% (26/30), with kimchi at 10 weeks but that of *S. cerevisiae* decreased to 10% (3/30), and a *P. onychis* (1/30, 3.3%) was detected. Among the 120 yeast colonies from non-starter kimchi samples at 20 weeks, *C. albicans* was the most dominant (106/120, 88.3%), followed by *S. cerevisiae* (13/120, 10.9%). A *Pichia onychis* (1/120, 0.8%) was detected from SS3 kimchi.

The increase in yeast counts, especially during later stages of kimchi fermentation, affects negatively the quality of kimchi because they are responsible for the softening of kimchi by producing pectinases and deteriorating taste by producing off-flavors [9, 14, 16]. Jeong et al. prepared kimchi after Chinese cabbage was soaked in 10% solar salt solution and stored for 120 days at 4 °C [22]. *Saccharomyces cerevisiae* appeared at the beginning of storage and reached the maximum number around 40 days of storage and then decreased continuously until the end of storage. *Candida* spp. were detected at 100 days and the numbers increased thereafter [22]. Appearance of *Candida* spp. was closely related to deterioration of kimchi quality during the later stages of kimchi fermentation [18].

Although the conditions under which kimchi was prepared and stored were different from those of this work, appearance of *S. cerevisiae* and *Candida* spp. during the middle and late stages of kimchi fermentation was the same. In this work, yeasts appeared first from PS kimchi at 10 weeks of storage, quite earlier than the detection times of SS and BS kimchi samples. The yeast counts of PS kimchi were significantly higher than those of SS and BS kimchi samples. The differences were suspected to be caused by different types of salts since all other conditions were the same. It is not known why different types of salts caused different yeast counts among kimchi samples. It can be guessed that SS and BS somehow discouraged the growth of yeasts whereas PS didn't. Detailed studies are necessary on the responsible compounds in SS and BS by which the growth of yeasts was discouraged. From above results, it can be concluded that SS, especially aged for 3 years, and BS possess significant advantages over PS for kimchi fermentation. SS and BS seem to improve quality and extend shelf-life of kimchi by encouraging growth of *Leuconostoc* spp. and suppressing growth of yeasts during kimchi fermentation.

Table 4 Identification of yeasts isolated from non-starter kimchi samples at 10 and 20 weeks

Time of storage (weeks)	Kimchi	Most matched yeast (% identity) ^a	Matched colonies/total colonies (%)
10	PS	<i>Candida albicans</i> (99.0%)	22/30 (73.3%)
		<i>Saccharomyces cerevisiae</i> (100%)	6/30 (20.0%)
		<i>Pichia onychis</i> (99.0%)	2/30 (6.7%)
	SS3	<i>Candida albicans</i> (99.0%)	26/30 (86.7%)
		<i>Saccharomyces cerevisiae</i> (100%)	4/30 (13.3%)
20	PS	<i>Candida albicans</i> (99.0%)	27/30 (90.0%)
		<i>Saccharomyces cerevisiae</i> (100%)	3/30 (10.0%)
	SS1	<i>Candida albicans</i> (99.0%)	28/30 (93.3%)
		<i>Saccharomyces cerevisiae</i> (100%)	2/30 (6.7%)
	SS3	<i>Candida albicans</i> (99.0%)	26/30 (86.7%)
		<i>Saccharomyces cerevisiae</i> (100%)	3/30 (10.0%)
		<i>Pichia onychis</i> (99.0%)	1/30 (3.3%)
	BS	<i>Candida albicans</i> (99.0%)	25/30 (83.3%)
		<i>Saccharomyces cerevisiae</i> (100%)	5/30 (16.7%)

^aITSs (550 bp) were amplified and sequenced. Blast was used to identify the species

Sensory evaluation of kimchi samples

Sensory evaluations for kimchi samples were done twice, immediately after initial fermentation and at 3 weeks of storage (Table 5). After initial fermentation, SS1 kimchi (non-starter and starter) received the highest scores for

flavor. The overall acceptability scores of SS3 kimchi (non-starter) and SS1 kimchi (starter) were higher than those of other kimchi samples. Among the kimchi samples stored for 3 weeks, SS1 kimchi (starter) and SS3 kimchi (non-starter) got higher overall acceptability scores than other kimchi samples. SS and BS kimchi samples got higher

Table 5 Sensory properties of kimchi samples prepared with different types of salts

After initial fermentation	PS-A	SS3-A	SS1-A	BS-A	PS-B	SS3-B	SS1-B	BS-B
Color	4.71	4.94	4.84	4.87	4.97	5.10	4.87	4.77
Flavor	3.71	4.23	4.45*	4.35	3.84	4.55	4.67*	4.16
Texture	4.74	5.06	5.42	4.81	4.81	5.10	5.23	5.29
Saltiness	3.32	3.61	3.55	3.32	3.45	3.87	3.77	3.94
Spiciness	3.26	3.19	3.42	2.94	3.06	3.42	3.35	3.29
Sweetness	3.13	3.13	2.97	2.94	3.19	3.26	2.94	2.87
Bitterness	3.13	3.03	3.10	2.84	3.23	3.13	2.77	2.77
Overall acceptability	3.48	4.16*	3.84	3.74	3.45	3.97	4.41*	3.87
After 3 weeks	PS-A	SS3-A	SS1-A	BS-A	PS-B	SS3-B	SS1-B	BS-B
Color	4.05	5.09	4.73	4.68	4.27	4.91	5.14	4.86
Flavor	3.73	4.00	3.91	3.95	3.86	3.64	3.64	3.95
Texture	4.05	5.09*	4.72*	4.68*	4.27	4.91	5.14*	4.86
Saltiness	3.68	4.00	3.91	3.86	3.55	4.18*	4.05	4.14
Spiciness	3.32	3.59	3.32	3.45	3.09	3.27	3.23	3.64
Sweetness	3.09	3.09	2.91	3.14	3.14	2.86	3.09	3.09
Bitterness	3.64	3.41	3.86	3.82	4.05	3.23	3.18*	3.45
Overall acceptability	2.91	4.41*	3.91*	4.05*	3.14	4.23*	4.68*	4.09*

A, non-starter kimchi; B, starter kimchi

*Significant difference between the values in the same tested items ($P < 0.005$, by least significant difference test)

overall acceptability scores than PS kimchi. Similar result was reported previously. Three different kimchi samples were prepared with solar salt aged for 4 years (SS4), 1 year (SS1), and pure salt (PS) [9]. Kimchi samples were ripened for 12–21 days at 7 °C until the acidities reached 0.5–0.6%, and then stored at –1 °C for 5 months. When the sensory properties of kimchi samples were evaluated at 2 and 5 months of storage, SS4 kimchi was the best and SS1 kimchi the second. PS kimchi received the lowest scores [9].

Kimchi prepared with solar salt or bamboo salt had better sensory properties than kimchi with purified salt. This might be due to different proportions of coccus-type LAB and yeasts, two most important groups of microorganisms for kimchi fermentation. In this respect, SS and BS have an advantage over PS for kimchi preparation as they encourage the growth of coccus-type LAB and discourage the growth of yeasts at the same time. These two effects might be the major reasons for the better sensory properties of SS kimchi samples. Further studies are necessary to characterize the responsible compounds present in SS and BS. If the compounds are identified, they could be used to extend the shelf-life and improve sensory properties of kimchi. The results obtained through this work can be applied for other fermented foods where salt is an important component. Use of solar salts or bamboo salts instead of purified salt can result in changes in microbial populations of a specific food, which can positively affect the quality of corresponding food.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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