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Effect of Low Salt Concentrations on Microbial Changes During Kimchi Fermentation Monitored by PCR-DGGE and Their Sensory Acceptance

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Various salt concentrations (1.0%, 1.3%, 1.6%, 1.9%, and 2.1% labeled as sample A, B, C, D, and E, respectively) were investigated for microbial diversity, identification of Lactic Acid Bacteria (LAB) in salted *kimchi* cabbage, prepared under laboratory conditions. These samples were stored at 4°C for 5 weeks in proper aluminum-metalized pouch packaging with calcium hydroxide gas absorber. A culture-independent method known as polymerase chain reaction – denaturing gradient gel electrophoresis was carried out to identify LAB distributions among various salt concentration samples that had identified 2 *Weissella* (*W. confusa* and *W. soli*), 1 *Lactobacillus* (*Lb. sakei*), and 3 *Leuconostoc* (*Lc. mesenteroides, Lc. lactis,* and *Lc. gelidum)* in the overall *kimchi* samples. The pH, titratable acidity, viable cell counts, and coliform counts were not affected by salt variations. In order to assess sensory acceptance, the conducted sensory evaluation using a 9-point hedonic scale had revealed that samples with 1.3% salt concentration (lower than the manufacturer's regular salt concentration) was more preferred, indicating that the use of 1.3% salt concentration was acceptable in normal *kimchi* fermentation for its quality and safety. Despite similarities in pH, titratable acidity, viable cell counts, coliform counts, and LAB distributions among the various salt concentrations of *kimchi* samples, the sample with 1.3% salt concentration was shown to be the most preferred, indicating that this salt concentration was suitable in *kimchi* production in order to reduce salt intake through *kimchi* consumptions.

Keywords: Low salt, *kimchi*, lactic acid bacteria, PCR-DGGE, sensory evaluation

Introduction

Kimchi, a fermented cabbage, is a traditional Korean food that has become popular worldwide due to its functional properties such as anticarcinogenic and antioxidative activities [5, 7, 14, 44]. *Kimchi* is prepared through a series of processes, including pretreatment of *kimchi* cabbage (or radish), brining, blending with various ingredients, and fermentation. Among these, brining, a salting method, is the most important step in *kimchi* fermentation as it contributes to the maintenance of *kimchi* quality [32]. Mheen [30] reported that the optimum salt concentration of *kimchi* was about 2.0–3.0% and mentioned that *kimchi* fermentation proceeded too fast if the salt concentration was below the optimum concentration, causing quick acidification and softening.

Despite increasing awareness of the health benefits of *kimchi*, consumption of *kimchi* prepared with high salt concentrations is a concern since most Koreans consume *kimchi* as part of the diet that contributes to sodium intake around 16–28% of the total daily sodium dosage [34, 43]. Hence, reducing the salt content in *kimchi* is generally considered to be more health-beneficial, but this has been a challenging situation for food manufacturers due to the negative association between consumer acceptance and lower salt content in the *kimchi* [3].

Various microorganisms originally present in the raw materials initiate *kimchi* fermentation that gradually becomes dominated by lactic acid bacteria (LAB), while suppressing the putrefactive bacteria during salting and fermentation through acid production, ethanol, mannitol, and CO_2 [19]. In the early stage of fermentation, heterofermentative LAB are the major species, whereas homofermentative LAB are the major species in the late stage of fermentation [30]. Changes in microbial communities during fermentation are greatly influenced by salt concentration and temperature [32, 35, 45]. As excessive acids are formed during ripening, the quality of *kimchi* deteriorates.

Cheigh and Park [4], Jo [11], and Mheen [31] concluded that the major genus and species of LAB that are likely to be key players in *kimchi* fermentation and responsible for off-flavor are *Leuconostoc mesenteroides* [2], *Leuconostoc dextranicum*, *Leuconostoc citreum* [2, 24], *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Pediococcus pentosaceus* [41], and *Streptococcus faecalis*. Other species of LAB identified in *kimchi* were *Leuconostoc gasicomitatum* [17], *Leuconostoc kimchii* [15], *Leuconostoc gelidum* [17], *Lactobacillus curvatus* [24], *Lactobacillus sakei* [2, 17, 24], *Weissella confusa* [2, 24], *Weissella kimchii* [6], and *Weissella koreensis* [17]. Other heterofermentative LAB belonging to the genera *Leuconostoc*, *Lactobacillus*, *Pediococcus*, and *Weissella* were also reported by Kim *et al.* [17] and Park *et al.* [36].

It was reported that brining had caused the total microorganisms, such as aerobic counts, in salted cabbage to be reduced to 11−87% and the lactic acid bacteria to be increased 3−4 times [8, 38]. At optimum ripening time, the total microorganisms reach their maximum level ($1 \times 10^{8\text{-}9}$ cells/ml) but decrease slowly for a while and increase again at maximum level ($1 \times 10^{6\text{-}7}$ cells/ml) [30]. At 5°C, this number can be reached at 12 days of fermentation [40]. Meanwhile, the total acidity was more in lower salt concentration (2.25%) than in high concentration at any temperature tested, in which at the lower salt content, maximum acidity was reached in a shorter period of time as discovered by Mheen [30]. The pH and acidity (as lactic acid) of the optimum ripening period of *kimchi* were 4.2 and 0.6−0.8% respectively, as reported by Mheen and Kwon [32].

A culture-independent identification method, denaturing gradient gel electrophoresis (DGGE) of 16S rDNA amplicons, has been shown to be a suitable tool in analyzing microbial communities that enables rapid detection of species and changes in the microflora compositions [33, 45] without discriminating living from dead cells or unculturable cells. Therefore, the current study used a molecular approach, combining PCR amplification of the V3 region of the 16S rDNA gene and DGGE, to monitor the dynamics of the microbial communities involved in the fermentation of *kimchi* and associate the sensory quality for commercial purpose.

The purpose of this study was to evaluate the effect of low salt concentrations (1.0%, 1.3%, 1.6%, 1.9%, and 2.1%) incorporated in *kimchi* preparation on microbial communities and sensory acceptance in order to determine an alternative suitable salt concentration for commercial *kimchi* as well as to reduce intake of salt contributed through *kimchi* consumption. A single batch of *kimchi* that was prepared with the same ingredients under controlled conditions without starter culture in the manufacturer's laboratory was analyzed using PCR-DGGE for identification of LAB communities. Sensory acceptance test was also performed in this study with a 9-point hedonic scale [21].

Materials and Methods

Sample and Storage

A single batch of *kimchi* prepared with the same ingredients under controlled conditions in the manufacturer's laboratory was delivered. The samples were prepared with five different salt concentrations of 1.0%, 1.3%, 1.6%, 1.9%, and 2.1% without addition of starter culture and labeled as sample A, B, C, D, and E, respectively. Among these samples, sample C represented the regular salt concentration of the manufacturer's *kimchi*. Each sample was kept in sealed aluminum-metalized pouch packages with calcium hydroxide gas absorber and stored at 4°C. At 0, 1, 2, 3, 4, and 5 weeks (typical commercial *kimchi*'s shelf-life), the samples were analyzed.

Sample Preparation

Each week, one package that represented each salt concentration was withdrawn from storage. The entire package contents (approximately 300 g) were homogenized using a hand-held blender (Tokebi Origin, Buwon Electronics, South Korea) for 2 min.

Enumeration of Viable Cells from *Kimchi* Sample

Ten grams of each homogenized sample was aseptically weighed and transferred to a sterile Stomacher filter bag (BA6141/STR, Seward, UK) followed by the addition of 90 ml of sterile water before being mixed in a Stomacher (Circular stomacher 400; Seward, UK) for 60 sec. Appropriate decimal dilutions (10^{-1}–10^{-7}) were plated in duplicate in Man, Rogosa, and Sharpe Agar (MRS, Difco, USA) at 30°C for 48 h under anaerobic conditions using Anaeropack (Mitsubishi Gas Chemical, Japan).

pH and Titratable Acidity Determination

Approximately 5 ml of each homogenized sample was diluted with 45 ml of distilled water in duplicate. The pH was measured with a pH meter (Mettler Toledo G20 Compact Titrator, Greifensee, Switzerland). In order to measure titratable acidity as lactic acid, 1 g of homogenized sample was diluted with 49 ml of distilled water in duplicate. The volume of 0.1 N NaOH required to achieve pH 8.3 (V) was recorded. The measured volume was then substituted in the following equation for the titratable acidity value:

$$\text{Titrable acidity (\%)} = \frac{\text{V (ml)} \times \text{Normality of NaOH} \times \text{Lactic acid factor (0.009)}}{\text{Weight of sample (g)}} \times 100$$

DNA Extraction

Each homogenized sample was filtered through two layers of cheesecloth. The samples were centrifuged at 14,000 ×*g* for 15 min at 4°C in order to pellet the cells, which were then washed with sterile water. The DNA extraction was performed using a commercial genomic DNA prep kit (Bacterial Genomic DNA Extraction Kit MB113, BioSolution, Suwon, Korea) according to the protocol described by the manufacturer. The yield and quality of the DNA were visualized electrophoretically on 1% agarose gel.

PCR-DGGE Analysis

Based on the protocols described by Kim *et al.* [18], the bacterial community DNA from DNA extraction was amplified for 16S rRNA gene using 27F and 1492R 16S universal primers (Bionics, Korea) under the following conditions; at 95°C for 5 min; 30 cycles of pre-denaturing at 95°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 2 min; and finally 10 min at 72°C followed by cooling to 4°C. The PCR products were re-amplified for the V3 region of the 16S rRNA gene using the DGGE primers GC-338f and 518r primers (Bionics, Seoul, Korea) under the following conditions; 95°C for 5 min; 30 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min; and finally 5 min at 72°C followed by cooling to 4°C. The sequence of the DGGE forward (GC-338f) and reverse (518r) primers were 5′-CGCCCGCCGCGCGGCGGG CGGGGCGGGGGACGGGGGACTCCTACGGGAGGCAGCAG-3′ and 5′-ATTACCGCGGCTGCTGG-3′ respectively.

The PCR products were analyzed by 2% agarose gel electrophoresis before DGGE analysis. PCR amplification was carried out in a final volume of 25 µl that consisted of 5 µl of template, 2.5 µl of 10× PCR buffer, 2 µl of dNTP mixture (2.5 mM each), 0.1 µl of Taq polymerase (5 U/µl; Takara Biotechnology, Japan), and 0.4 pM of each primer. Reactions were performed in a Mastercycler (Eppendorf, Germany).

The subsequent amplicons with added 5 µl of 6× dye were directly loaded onto 8% (w/v) polyacrylamide gels (with a denaturing gradient of 20% to 50% urea-formamide) in a running buffer containing 1× TAE (20 mM Tris, 10 mM acetate, 0.5 mM EDTA (pH 8.0)). Gels were run on a Dcode Universal Mutation Detection System (Bio-Rad, USA) for 30 min at 40 V and 15.5 h at 60 V. Gels were stained with ethidium bromide for 30 min and gel images were captured using a digital camera (COOLPIX 4300, Nikon, Japan) attached to a transilluminator (SL-20 DNA Image Visualizer, Seoulin, Korea).

Sterile blades were used to excise bands of interest from the gels that were then incubated overnight at 4°C in ultra-filtered water to allow passive diffusion of the DNA from the polyacrylamide matrix for re-amplification using the DGGE primers GC-338f and 518r. The PCR products were run once again on polyacrylamide gels of similar constituents in order to improve the band purity for identification resulted from sequencing. The resulted bands were incubated again overnight at 4°C.

Sequencing of the V3 Region of the 16S rRNA Gene

The eluted DNA was amplified using 338F and 518R primers (Bionics, Korea). The PCR products were purified using a QIAquick PCR purification kit (Qiagen, USA). The samples were analyzed with an automated DNA sequencer (Jenotech, Korea). The partial ribosomal DNA sequences from the samples were searched in the GenBank database by BLAST [1] to identify the closest known relatives.

Coliform Bacteria Counts

Coliform counts were performed (on samples B, C, and D only) each week from week 1 to week 4 prior to sensory evaluation to ensure the safety of *kimchi* samples for consumption, using Petrifilm (3M, Microbiology Products, USA) cultures. *Kimchi* samples were first diluted with phosphate buffered saline (PBS) (pH 7.0) at the ratio of 1:10 (*kimchi*:PBS). The dilution carried out was to produce better readability of the Petrifilms as suggested by McCarron *et al*. [29]. A 1 ml aliquot of each *kimchi* mixture was placed on the Petrifilm Coliform Count (CC) plates (Petrifilm 3M) in duplicates before being incubated aerobically at 37°C for 24 h.

Sensory Acceptance Test

Overall preference, appearance, flavor, color, color intensity, spiciness, saltiness, sourness and crunchiness of the *kimchi* samples (samples B, C, and D only) were evaluated by 49 woman as the panel, age between 30 and 40 years old, using a 9-point hedonic scale, with 1 representing the least score and 9 the highest score (1, Dislike extremely; 2, Dislike very much; 3, Dislike moderately; 4, Dislike slightly; 5, Neither like or dislike; 6, Like slightly; 7, Like moderately; 8, Like very much; 9, Like extremely) [35]. The sensory data were further analyzed by one-way ANOVA using Minitab ver. 14. Sensory evaluation data were expressed as means ± standard deviations (SD). The confidence limits were set as $p < 0.05$.

Results and Discussion

Changes in pH, Titratable Acidity, and Viable Cell Count

Changes in pH and titratable acidity are shown in Fig. 1. At week 1, the difference in salt concentrations created lower pH in low salt samples A and B. Since, sample C

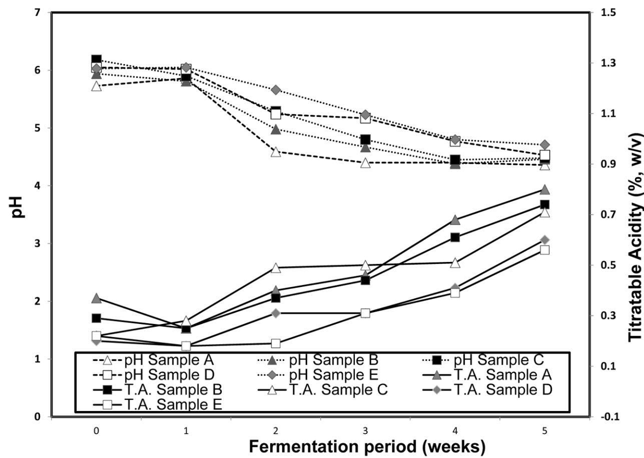

Fig. 1. Changes in pH and titratable acidity (TA) in *kimchi* samples (A, 1.0%; B, 1.3%; C, 1.6%; D, 1.9%; and E, 2.1%) during fermentation.

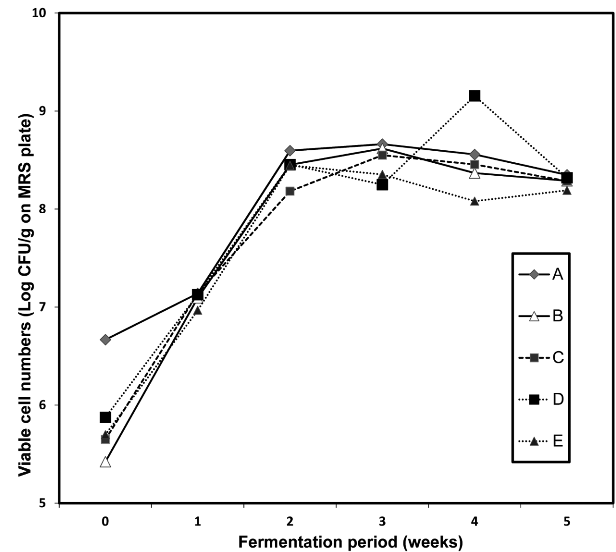

Fig. 2. Changes in viable cell numbers (Log CFU/ml) in *kimchi* samples (A, 1.0%; B, 1.3%; C, 1.6%; D, 1.9%; and E, 2.1%) during fermentation.

represented the regular salt concentration of the manufacturer's *kimchi*, the results for pH and titratable acidity will be described with reference to sample C. The pH of sample B was close to sample C at weeks 1, 3, 4, and 5 while sample D had closer pH to sample C at weeks 1 and 2. This suggests that the salt concentrations used in preparing samples B and D could generate a similar pH as sample C, which could lead to similar titratable acidity production and the taste of *kimchi*. However, all of the samples had not reached pH 4.2, which was reported as the optimum pH of *kimchi* by Mheen and Kwon [32] at week 5. This also means that the quality of *kimchi* samples was still acceptable since the pH values were not lower than 4.0 where *kimchi* will no longer be acceptable [25].

The optimum value of titratable acidity (0.6%) according to Mheen and Kwon [32] was reached by samples A and B at week 4. Sample C reached the optimum titratable acidity between weeks 4 and 5, and sample D at week 5, while sample E did not reach the optimum titratable acidity at week 5. Samples with lower salt concentrations reached optimum acidity faster than those with higher salt concentrations which was consistent with the findings documented by Mheen and Kwon [31]. Between 20 to 30 days, all of the samples reached optimum titratable conditions similar to those reported by Lee *et al.* [22]. Since, *kimchi* quality is no longer acceptable when the titratable acidity is 1.5–2.0% [25], all the *kimchi* samples in this study were still acceptable at week 5. However, most of the *kimchi* samples (samples A, B, and C) were beyond the optimum titratable acidity (0.8%, 0.74%, and 0.71% respectively) at week 5. This also indicates that the *kimchi* samples used in this study had a shelf-life of up to 4 weeks.

The viable cell counts on MRS media are shown in Fig. 2. There were three outliers considered in the data, which were cell counts on MRS for sample A at week 0 (because initially, the number of viable cells should be the same for all samples), whereas the other two were samples D and E at week 4 (due to their outstanding values). Generally, the microbial population increased from week 0 to week 2 (5.42–8.60 log CFU/ml; data not shown), which was quite consistent with a study performed by Shin *et. al.* [41] where they found that the maximum total microbial population had reached >8.0 log CFU/ml after 12 days of fermentation at 5°C and became stable until week 5 (8.34–8.62 log CFU/ml). This was easily correlated to the titratable acidity that was gradually increasing throughout the fermentation process. These findings did not demonstrate the effect of low salt concentrations on the number of viable cells on MRS.

Overall, the pH and titratable acidity of samples at weeks 2 and 3 showed variations at which the pH value increased with increasing salt concentrations whereas the titratable acidity deceased with decreasing salt concentration, except for samples E and D at week 2. Although the viable cell counts remained stationary at weeks 2 and 3, the difference in salt concentrations provided variations in the production of lactic acid that eventually accumulated in the sample and hence, lowered the pH accordingly.

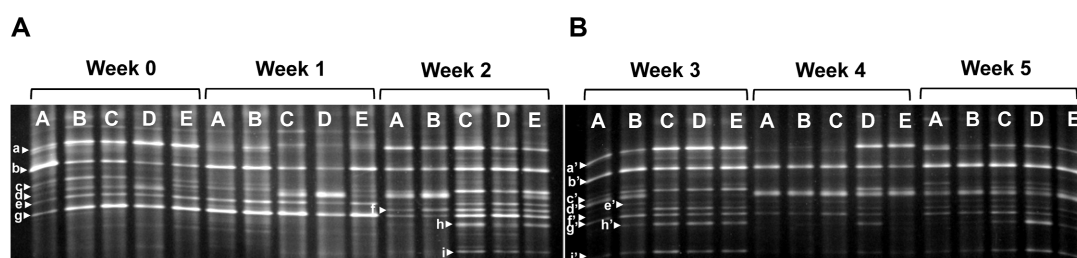

Fig. 3. PCR-DGGE patterns of 16S V3 rRNA gene sequences in *kimchi* samples (A, 1.0%; B, 1.3%; C, 1.6%; D, 1.9%; and E, 2.1%) from weeks 0 to 5 ((**A**), weeks 0–2; (**B**), weeks 3–5).

PCR-DGGE Analysis

The PCR-DGGE patterns of 16S rRNA are shown in Fig. 3 and the sequencing results of 16S rRNA fragments are tabulated in Table 1. LAB identified were *Weissella, Leuconostoc,* and *Lactobacillus,* which were previously reported by Bae *et al.* [2], Cho *et al.* [5], Kim *et al.* [16], and Lee *et al.* [23] to play key roles in *kimchi* fermentation. The overall microflora identified in this study were 2 *Weissella* (*W. confusa* and *W. soli*), 1 *Lactobacillus* (*Lb. sakei*), and 3 *Leuconostoc* (*Lc. mesenteroides, Lc. lactis,* and *Lc. gelidum)* species. *Kimchi* fermentation is dominated by *Leuconostoc* species at 5°C [27], which was close to the storage condition in this study.

W. confusa, *W. soli, Lc. mesenteroides,* and *Lc. lactis* (bands a, a′, b, b′, c, c′, d, d′, g, and g′ in Fig. 3 and Table 2) remained present throughout the fermentation process in samples A, B, C, D, and E. In general, microflora patterns were similar for weeks 0 and 1. Weeks 2 and 3 showed more diverse bands for samples C, D, and E before optimum acidity was reached, which was between weeks 4 and 5 for those samples. Patterns became fewer at weeks 4 and 5 when optimum acidity was reached. This indicates that the diversity of LAB in samples C, D, and E were influenced by acidity that was generated by variations in the salt concentrations.

W. confusa (bands a and a′ in Fig. 3 and Table 2) was predominant at the beginning of fermentation but disappeared at week 1 and re-appeared again at weeks 2 and 3 for all samples. However, these bands started to disappear from week 4, especially at low salt concentration (samples A, B and C), due to high acidity after optimum acidity has been reached. *W. confusa* bands appeared at week 0 since it originated from raw ingredients such as garlic and green onion used in the making of *kimchi* [12].

The presence of *W. soli* (bands b and b′ in Fig. 3 and Table 2) was consistent throughout the fermentation process regardless of salt concentration and fermentation period but began to grow faint during week 5. It was reported that *W. soli* grows very well between 4°C and 40°C [28], which was consistent with the storage temperature of *kimchi* samples during the fermentation process in this study. *W. soli* has not been detected from Korean *kimchi* but has been detected only from Chinese *kimchi* in previous studies [24], which indicates that there is a possibility that the *kimchi* in this study was prepared with raw ingredients obtained from China.

Table 1. Identification of the bacteria in *kimchi* samples by sequencing the 16S V3 rDNA fragments excised from PCR-DGGE.

Bands[a]	Species identification	Homology (%)	Accession No.
a, a′	*W. confusa*	100	HQ711354.1
b,b′	*W. soli*	99	GU470977.1
c, c′	*Lc. mesenteroides*	100	KF746910.1
d, d′	Uncultured eukaryote	99	JQ243530.1
e, e′	*Lc. lactis*	98	AB904777.1
f, f′	*Lc. mesenteroides*	97	KJ187158.1
g, g′	*Lc. lactis*	99	KC753458.1
h, h′	*Lb. sakei*	99	JN851763.1
i, i′	*Lc. gelidum*	98	KF577569.1

[a]Bands refer to Fig. 3.

Table 2. Changes in bacteria profile in *kimchi* samples (A, 1.0%; B, 1.3%; C, 1.6%; D, 1.9%; and E, 2.1%) during fermentation[a].

LAB strains identified	Bands	Sample week																													
		0					1					2					3					4					5				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
W. confusa	a, a′	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+
W. soli	b, b′	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lc. mesenteroides	c, c′	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uncultured eukaryote	d, d′	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lc. lactis	e, e′	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
Lc. mesenteroides	f, f′	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Lc. lactis	g, g′	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lb. sakei	h, h′	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	-	-	+	-	-	-	+	+	+
Lc. gelidum	i, i′	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] the entire data

+, detected; -, not detected

Lc. mesenteroides (bands c and c′ in Fig 3. and Table 2), described as psychrotrophic, microaerophilic, and resistant to salt by Franz and Von Holy [9], was present throughout the fermentation process although there was only one band that was undetected by DGGE that belongs to sample D at week 1. Although *Lc. mesenteroides* was expected to be present at the beginning of fermentation, as it was previously shown to be present in both garlic and green onion (raw ingredients in *kimchi*) by Jung *et al.* [12] and in both garlic and *kimchi* cabbage by Kim *et al.* [13], the other *Lc. mesenteroides* (bands f and f′ in Fig 3. and Table 2) was not detected at weeks 0 and 1, probably due to its low number. Although, according to study conducted by Mheen and Kwon [32], the number of *Lc. mesenteroides* reached its maximum at the optimum ripening periods of *kimchi*, which were between weeks 3 and 4 for lower salt concentration (samples A and B) and between weeks 4 and 5 for higher salt concentration (samples C, D, and E) in this study, and decreased when *kimchi* becomes acidic, the bands did not disappear after these ripening periods. Our

Table 3. Results of sensory acceptance test of low salt *kimchi* samples (B, 1.3%; C, 1.6%; and D, 1.9%) from weeks 1 to 4.

Week	Samples	Sensory Attributes								
		Overall preference	Appearance	Flavor	Color	Color intensity	Spiciness	Saltiness	Sourness	Crunchiness
1	B	6.3 ± 1.1	6.6 ± 0.9^{a}	6.2 ± 1.1	6.5 ± 1.0^{a}	6.2 ± 1.0^{a}	5.0 ± 1.3	4.2 ± 1.1^{b}	3.4 ± 1.6	6.2 ± 1.2^{a}
	C	6.0 ± 1.0	6.2 ± 1.0^{ab}	6.0 ± 1.0	6.1 ± 1.1^{ab}	5.8 ± 1.1^{ab}	4.9 ± 1.3	4.6 ± 1.2^{ab}	3.5 ± 1.6	5.8 ± 1.0^{ab}
	D	5.9 ± 1.4	6.1 ± 1.2^{b}	5.9 ± 1.4	5.7 ± 1.3^{b}	5.5 ± 1.1^{b}	5.0 ± 1.4	5.1 ± 1.4^{a}	3.3 ± 1.6	5.4 ± 1.3^{b}
2	B	6.4 ± 1.1	6.8 ± 0.8^{a}	6.6 ± 1.0	6.3 ± 1.2	6.3 ± 1.0^{a}	4.8 ± 1.0	4.5 ± 1.2	5.2 ± 1.7	6.2 ± 1.1
	C	6.4 ± 1.1	6.3 ± 1.1^{ab}	6.6 ± 1.1	6.2 ± 1.0	5.9 ± 1.0^{ab}	5.0 ± 1.2	4.9 ± 1.2	5.7 ± 1.6	6.0 ± 1.0
	D	6.2 ± 1.2	6.2 ± 1.2^{b}	6.3 ± 1.2	6.1 ± 1.1	5.7 ± 1.1^{b}	4.9 ± 1.2	4.9 ± 1.2	5.2 ± 1.2	5.8 ± 1.3
3	B	5.7 ± 1.6	6.2 ± 1.4	5.5 ± 1.5	5.5 ± 1.4	5.9 ± 1.4	4.3 ± 1.3	4.3 ± 1.3^{b}	5.6 ± 1.7	6.1 ± 1.5
	C	6.1 ± 1.5	6.4 ± 1.3	6.1 ± 1.5	5.6 ± 1.4	5.3 ± 1.3	4.6 ± 1.4	4.8 ± 1.2^{ab}	6.0 ± 1.4	6.1 ± 1.3
	D	5.6 ± 1.6	6.3 ± 1.6	5.7 ± 1.7	6.0 ± 1.4	5.7 ± 1.3	4.9 ± 1.2	5.0 ± 1.2^{a}	6.1 ± 1.6	6.0 ± 1.4
4	B	5.4 ± 1.7	5.9 ± 1.3	5.3 ± 1.7	5.4 ± 1.2^{b}	5.7 ± 1.2	4.6 ± 1.3^{b}	4.7 ± 1.1^{b}	6.0 ± 1.5	5.7 ± 1.5
	C	5.7 ± 1.5	6.0 ± 1.2	5.6 ± 1.4	5.6 ± 1.1^{b}	5.7 ± 1.1	4.9 ± 1.2^{ab}	4.7 ± 1.2^{b}	5.8 ± 1.4	5.4 ± 1.4
	D	5.7 ± 1.5	5.9 ± 1.3	5.5 ± 1.8	6.3 ± 1.2^{a}	5.6 ± 1.4	5.3 ± 1.5^{a}	5.0 ± 1.4^{a}	5.9 ± 1.5	5.8 ± 1.4

Sensory preference tests were carried out with the *kimchi* samples collected at weeks 1, 2, 3, and 4 using a 9-point scale (from 1 to 9) that corresponds to Dislike extremely, Dislike very much, Dislike moderately, Dislike slightly, Neither like or dislike, Like slightly, Like moderately, Like very much, and Like extremely, respectively. Values are the mean ± SD (n = 49). Values with different superscripts (a, b, c) for each attribute differ significantly ($p < 0.05$).

findings showed that *Lc. mesenteroides* was not affected by salt concentrations, although it was reported that there was more *Lc. mesenteroides* found at lower salt content than at higher salt content [32].

Lc. lactis (bands e, e′, g, and g′ in Fig. 3 and Table 2) was present throughout the fermentation process for all samples, indicating that *Lc. lactis* was initially present at the beginning of the fermentation process since they originated from garlic and green onions [12]. *Lc. lactis* has been isolated by Hong *et al.* [10] in their attempt to compare bacterial community changes in fermenting *kimchi* at two different temperatures using DGGE analysis, where their findings suggested that *Lc. lactis* was one of the major strains detected in their *kimchi* sample fermented at 4°C. However, in this study, one of the *Lc. lactis* (band e′ in Fig. 3 and Table 2) disappeared at high salt concentration (sample E) at weeks 4 and 5, which might be caused by their low concentration to form visible DGGE bands.

Lb. sakei (bands h and h′ in Fig. 3 and Table 2) appeared from week 2 for samples C, D, and E and disappeared at week 3 for sample A (lowest salt concentration), and week 4 for samples B, C and E, and re-appeared at week 5 for samples C and E. These findings disagreed with study conducted by Leroy and De Vuyst *et al.* [26], where they reported that an increase in salt concentration decreases the growth rate of *Lb. sakei* CTC 494. The presence of *Lb. sakei* was also reported to be associated with the ripening process in fermentation and thus appeared at a later stage of fermentation when fermented food turned sour [23], which agreed with our findings in which *Lb. sakei* appeared during the ripening periods from weeks 3 to 4 for samples C, D, and E.

Although *Lc. gelidum* (bands i and i′ in Fig. 3 and Table 2) has been found previously as a dominant LAB in *kimchi* samples at lower temperatures [4, 13, 23, 35, 39, 42], it started to appear from week 2 for samples C, D, and E, and appeared from week 3 for samples A and B, which were probably undetected owing to its low number from the beginning of the fermentation until reaching pH 5.66 to 4.80. According to Kim *et al.* [13], *Lc. gelidum* was only found in garlic, and their study indicated that garlic and *kimchi* cabbage may be the original sources for *Lc. gelidum*, which also means that *Lc. gelidum* bands should have appeared from the beginning of the fermentation period.

Table 2 shows the changes in LAB distribution among various salt concentrations from week 0 to week 5 fermentation using PCR-DGGE. Based on the distribution profiles, it can be generalized that these salt concentrations did not influence the appearance and disappearance of LAB owing to similarities in band patterns as a whole.

Coliform Counts

Regulation stated in food standards of traditional Korean fermented foods for coliform groups only applies to sterile packaged commercial *kimchi*, in which there must be no coliform detected in those products [20]. Since our samples were not sterile, the coliform counts ranging from 2.22 to 2.83 (log CFU/ml) was considered within acceptable value to be present in *kimchi* samples, as typical *kimchi* manufacturers have to maintain coliform counts below 4.00 (log CFU/ml). This indicates that these salt concentrations did not support desirable environment for coliform to grow but controlled their growth, and low salt *kimchi* is safe to be consumed both for commercial and sensory evaluation purposes.

Sensory Acceptance Test

Performing sensory acceptance test on the five samples of *kimchi* was too much for a panelist to evaluate at one time; hence, only samples very close to regular commercial *kimchi* were selected for this purpose. In addition, owing to the fact that samples B and D had similar pH and titratable acidity throughout the fermentation periods, the sensory acceptance test was narrowed down to three samples (B, C, and D). Since the best taste of *kimchi* was acquired after 2 to 3 days of fermentation at 20°C with 2–3% of salt [33], our samples that were stored at 4°C might have taken longer to reach their best taste, and therefore, the sensory acceptance test was performed only from week 1 instead of week 0. Mheen and Kwon [32] also mentioned that fermentation in samples prepared with lower salt concentration than the optimum concentration (2.0–3.0%) might proceed too fast, causing quick acidification and softening; the latter being the reason to perform sensory evaluation up to week 4 rather than week 5. Samples A and E were not sensory evaluated because their pH values and titratable acidity were not similar to values of commercial *kimchi* (sample C) and sample A had reached beyond the optimum titratable acidity as early as week 4. Therefore, samples A and E were not suitable for sensory evaluation for this study.

Table 3 shows the result of sensory acceptance test of *kimchi* samples in this study that was performed from week 1 to week 4 of *kimchi* fermentation. Nine attributes, including overall preference, were scored under a 9-point hedonic scale. Among these attributes, appearance, color, color intensity, spiciness, saltiness, and crunchiness differed significantly between samples and determined which sample was preferred over the other. Overall, samples B

and D were equally more accepted over sample C, indicating the use of lower salt concentration in preparing *kimchi* can be applied without affecting major changes in *kimchi* sensory quality. Based on the sensory evaluation data, the sample B salt concentration can replace the manufacturer's regular salt concentrations in association with the objective to reduce salt intake through *kimchi* consumption.

This study suggests that various salt concentrations did not affect the general pH, titratable acidity, viable cell counts, coliform counts, and PCR-DGGE band patterns among the samples. Based on the sensory acceptance test results, sample B prepared with 1.3% salt concentration has shown its potential to replace the manufacturer's regular salt concentration in *kimchi* production.

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