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Impact of sea salt concentration and salting time on *Bacillus cereus* contamination and on the physicochemical qualities of napa cabbage (*Brassica rapa* subsp. Pekinensis)

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ORIGINAL ARTICLE

Abstract

This study examined the effects of varying sea salt concentrations (60, 80, 100, 120, and 140 g/L) and immersion period (6, 8, and 10 h) in brine on the viability of natural microbiota (total viable counts, coliforms, and *Bacillus cereus*) in napa cabbage. Additionally, it assessed how these brine conditions impacted *B. cereus* reduction, along with salinity, Brix, hardness, and moisture content. The results showed that total viable counts were reduced by 0.70–3.81, 1.09–4.65, and 1.85–4.89 log CFU/g after 6, 8, and 10 h of salting at sea salt concentrations of 60–140 g/L, respectively. Coliforms exposed to brine for 6–8 h at 60 g/L salt were reduced by 1.26–1.58 log CFU/g. The inoculated *B. cereus* was reduced by more than 3 log after 6–10 h in 120-g/L salt, which is significant as a 3-log reduction meets the microbial safety threshold set by the Korea Ministry of Food and Drug Safety (MFDS) for vegetables. The moisture content ranged from 89.04 to 84.99% depending on sea salt concentration. Overall, the study suggests that salting napa cabbage with 120–140 g/L salt for 6–10 h effectively reduced *B. cereus* contamination by over 99.9% without compromising its quality.

*Keywords***:** kimchi; napa cabbage; sea salt; physicochemical quality; natural background bacteria

Introduction

Kimchi is a traditional Korean fermented food made from salted vegetables (such as napa cabbage, radish, and cucumber) mixed with various ingredients (such as garlic, ginger, red pepper powder, and fish sauce). Among them, napa cabbage kimchi is the most prevalent variety of kimchi. The mean composition of the ingredients utilized in the preparation of this product is 74–90% napa cabbage, 2.8–13.5% radish, 1.4–2.0% garlic, 0.5–1.0% ginger, 1.8–3.0% chili, and 2.0% anchovy liquor (Patra *et al.*, 2016). The essential ingredient of kimchi is salted napa cabbage, which is produced through four processes of trimming and cutting, salting, washing, and dehydrating (Shim *et al*., 2003). Various methods are used for salting of napa cabbage, namely the mixed, brine salting, and direct salting methods (Sung *et al*., 2011), all of which require considerable time and labor. Thus, ordinary people spend a considerable amount of time preparing salted napa cabbage at home. Because it is difficult to maintain the same consistent salt concentration while preparing napa cabbage, the salted product is commercially available as ready-to-use product to reduce the burden of making kimchi at home or other places. Furthermore, the salting method, duration, temperature, type, and quantity of salt can impact the process of salting of napa cabbage. Salinity and texture are widely recognized as the most significant factors affecting the quality of kimchi (Park, 2022). The sugar content of napa cabbage is known to vary depending on the season (Park, 2022). Regarding sugar content, autumn cabbage had more than twice the amount found in spring cabbage. In terms of hardness, spring cabbage had the highest level, while winter cabbage had the lowest (Ku *et al*., 2014). To achieve the highest quality of salted napa cabbage, it is essential to identify the optimal quality characteristics of napa cabbage as a function of salt concentration during the salting process.

Usually, napa cabbage is cultivated in soil with a rich source of microorganisms, yet the surface of the leaves provides a very favorable environment for bacterial adhesion (Berger *et al*., 2010). A study done by Yang and Scharff (2024) reported that the 2018–2019 Food Net Population Survey estimated that 80.5% of surveyed households had consumed leafy greens within the past 7 days, representing a notable increase from 50.6% observed in the 2006–2007 survey. In addition, the most salted napa cabbage is produced in small processing plants and there is the potential for microbial contamination during processing and at all other stages of the food supply chain (e.g., transport, storage, and display in supermarkets) (Kim *et al*., 2018). In manufacturing plants, microbial contamination may increase because of the repeated use of a salt solution, and the microbes located deep in napa cabbage cannot be destroyed easily. Microbial contamination is a potential issue during the manufacturing process of salted napa cabbage because of the non-thermal process used. Only washing step has the potential to eliminate some contaminating microorganisms from the product. Indeed, Choi *et al*. (2015) demonstrated that pathogenic *E. coli* and *Salmonella* could survive in kimchi for 12 days at 4°C. Similarly, *B. cereus* was detected in 41 out of 100 commercial kimchi product samples (Lee *et al*., 2018).

Bacterial pathogens were the most prevalent causative agents in South Korea and were related to 63.7% of foodborne diseases in 2022 (Ministry of Food and Drug Safety [MFDS], 2023). Bacterial pathogens, such as *Salmonella*, *Campylobacter*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Bacillus cereus* are reported to be major sources of these outbreaks (MFDS, 2023). *B. cereus* is a ubiquitous soil bacterium known for contaminating various plant-derived foods, including vegetables and cereals. Recent studies have highlighted its prevalence in fresh produce (Lin *et al*., 2023). *B. cereus* is a bacterium that can cause food poisoning and, on occasion, acts as an opportunistic human pathogen (Jovanovic *et al*., 2021). Napa cabbage crop is typically contaminated by this bacterium during cultivation and storage procedures. The maximum salt concentration tolerated by *B. cereus* without impeding its growth is reported to be 7.5% (Food and Drug Administration [FDA], 2012). In contrast, the European Food Safety Authority (EFSA, 2005) recommends a maximum acceptable level of 10^2 CFU/g for *B. cereus* in ready-to-eat foods, including fermented vegetables. Notably, *B. cereus* poses particular concerns in fermented foods such as kimchi because of its ability to form endospores that are highly resistant to environmental stresses, such as heat, desiccation, and pH variation. Few studies have examined the microbiological quality of salted napa cabbage distributed in the market. Despite the increasing consumption of salted napa cabbage, bacterial contamination remains a potential risk. In particular, 85% of consumers in 2016 and 91% of consumers in 2021 stored the product at room temperature, and 60% and 58%, respectively, and used it without washing, which could affect quality of the food, as microorganisms could multiply during storage (Kim *et al*., 2022). Additionally, Tambekar and Mundhada (2006) reported that *B. cereus* was commonly found in leafy vegetables, such as cabbage, because of poor sanitation and handling practices during post-harvest stages. These findings highlight that napa cabbage can serve as a significant carrier of *B. cereus*, particularly when hygienic handling and processing conditions are inadequate. Microbiological data collection is crucial to ensure the hygienic status of commercial salted napa cabbage, as most consumers do not wash it at home. Such microbial contamination not only threatens public health by causing foodborne illnesses but also leads to economic losses because of product spoilage, recalls, and damage to consumer trust.

Salted napa cabbage, a ready-to-use food, is processed to increase consumer convenience before making kimchi (Ragaert *et al*., 2007). However, kimchi factories need to optimize and standardize the brining conditions for different cabbage varieties depending on the season (Ku *et al*., 2014). Sea salt is mostly composed of sodium chloride (NaCl), but it also contains some calcium chloride ($CaCl_2$), potassium chloride (KCl), magnesium chloride ($MgCl_2$), and phosphorus (Nowzari *et al*., 2022). These ingredients contribute to microbial safety by reducing water activity and chlorine removes pathogens by breaking the chemical bonds in their molecules (Jeong *et al*., 2019).

Generally, sea salt is used as a food seasoning, preservative, fermentation regulator, and toothpaste additive, thus it can be used as a natural antimicrobial agent (Jeong *et al.*, 2019). Shim *et al.* (2003) demonstrated that brining for 6 h at a high concentration of 15% salt is economically advantageous. Additionally, Choi *et al.* (2014) found that brining for 12–15 h at a 5% concentration provided sufficient salinity for napa cabbage. Therefore, it is necessary to conduct research to ensure uniform quality of salted cabbage supplied to consumers throughout the year.

Therefore, in the first part of the present study, the effects of sea salt concentration (60, 80, 100, 120, and 140 g/L) and exposure time to brine (6, 8, and 10 h) on the viability of natural microbiota, including total viable counts, total coliforms, *E. coli*, and *B. cereus* in the napa cabbage, was investigated. The second part of this study specifically examined the anti-*B. cereus* effects of these brine conditions on the survival of *B. cereus* inoculated in the salted napa cabbage and their effects on physicochemical quality (salinity, Brix, hardness, and moisture content) of napa cabbage.

Materials and Methods

Preparation of raw materials

Fresh napa cabbage (*Brassica rapa*, subsp. Pekinensis) was purchased from online marketplace, with one cabbage head selected per region (Jeolla-do, Chungcheong-do, Gyeongsang-do, and Gangwon-do) based on the regional origin indicated on product label. Sinan sea salt (NaCl 80%) was purchased from Chungjungone Co. Ltd. (Seoul, South Korea). After acquiring, the cabbage was transferred to a refrigerator at 4°C and used within 24 h.

The salting method for napa cabbage is a variation of the methodology proposed by Rhee and Park (2015). The inedible parts of napa cabbage weighing 2–3 kg were trimmed off and the cabbage was divided into two parts (approximately 500–600 g). Then, salted napa cabbage was prepared by soaking cabbage in salt water. The salt water was prepared by adding sea salt to tap water, of which the temperature was 15±1°C (room temperature), to prepare sea salt water with concentrations of 60, 80, 100, 120, and 140 g/L. The sea salt water was added to cabbage halves at a ratio of 1:1.5 (w/v). The temperature of the brining place was 18±1°C. Prior to use, the sea salt water was autoclaved for 15 min at 121°C to remove preexisting microorganisms. Napa cabbages were salted for 6, 8, and 10 h, after which they were washed thrice to remove salt and then drained for 1 h at room temperature. No additional salt was added to the samples (0% salt) in the control group.

Microbiological analyses

All microbiological analyses were performed according to the Korea Food Code (MFDS, 2022). A sterile stomacher filter was used to transfer 25 g of homogenized salted napa cabbage sample, which was then mixed in the stomacher (BagMixer 400; Interscience, Saint-Nom la Breteche Arpents, France) with 225 mL of 8.5-g/L saline solution for 2 min. The solution containing homogenized specimen was diluted stepwise with 9 mL of 8.5 g/L of saline solution.

The plate count method was used to measure total aerobic bacteria. Then, the culture medium of plate count agar (PCA, BD Difco, Sparks, MD, USA) was mixed with the diluted samples at each stage. The samples were cultured at 37°C for 48 h before the number of colonies was counted.

The coliform/*E. coli* was measured by means of 3M Petri film. The diluted solution (1 mL) was plated on 3M Petri film (Coliform/*E. coli* Count Plate, 3M, Seoul, South Korea) by the plate count method and was incubated for 24–48 h at 35±1°C. The total coliform count was indicated by red colonies with gas production.

To enumerate *B. cereus*, a 25-g sample of treated salted napa cabbage was taken and transferred to a stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada). Subsequently, 225 mL of 8.5-g/L saline solution was added to the bag, homogenizing the contents in a stomacher for 2 min. The diluted solutions were treated with the same method used for total aerobic bacterial analysis, and 1 mL of each diluted solution was plated on selective agar of *B. cereus* (Mannitol Egg Yolk Polymyxin Agar, MYP, BD Difco, Sparks, MD, USA) by a spreading culture method and incubated for 24 h at 37°C. After processing, all samples were stored at 4°C until analysis to ensure microbiological stability and reproducibility of results. The data presented here represent the mean ± standard deviation (SD) of the results of three assays.

Bacterial strain and inoculation

Three strains of *B. cereus* (NCCP 10623, NCCP 14579, and ATCC 11778) were tested. The bacterial stock was stored at -80°C in Tryptic Soy Broth (TSB) with 30% glycerol. *B. cereus* (10 μL) was activated in 5-mL TSB by incubation at 37°C for 24 h. The culture was centrifuged at 32,254 ×g for 15 min at 4°C (SUPRA22K, Hanil Science Industrial Co., Daejeon, South Korea). This process was repeated twice to ensure the bacteria were optimally activated. The final pellets were resuspended in 10 mL of 8.5 g/L sterile saline solution, which matched to approximate 10^7–10^8 CFU/mL.

The salted napa cabbage was cut into pieces of 250 g each using sterilized scissors, whereupon the surface was disinfected with 70% ethanol to remove any residual microorganisms. The sample of salted napa cabbage was widely spot-inoculated to contaminate it with 1 mL of *B. cereus*. The samples were placed on a clean bench at room temperature of 25±1°C for 1 h to allow the bacteria adhere easily to the surface. The inoculation procedure was adapted from previous studies involving *B. cereus* contamination in food matrices (Kim *et al.*, 2017). The concentration of final samples was 7.02±0.22 CFU/g. The data presented here represent the mean ± SD of three assays per sea salt concentration/time.

For *B. cereus* enumeration, samples were plated on MYP agar (BD Difco, Sparks, MD, USA) and incubated at 37°C for 24 h. Presumptive *B. cereus* colonies were identified based on typical morphological characteristics: pink-red colonies with lecithinase activity (opaque zone).

Physicochemical analysis

The physicochemical properties were analyzed to characterize any potential changes in the quality of salted napa cabbage prepared using different sea salt concentrations (60, 80, 100, 120, and 140 g/L) and brine periods (6, 8, and 10 h) by determining salinity, Brix, hardness, and moisture content. Subsamples of salted napa cabbage were selected from three random locations. The samples were measured using three assays, and the results were averaged.

Salinity and Brix measurement

The salinity and Brix value were measured by adding 10 g of salted napa cabbage to a mixer, after which the salted napa cabbage was filtered using qualitative filter paper. The salinity of the filtered liquid was measured using a salinity meter (PAL-03S, Atago Shrine, Tokyo, Japan) and Brix meter (PAL-1, ATAGO Co., Tokyo, Japan). Both instruments were calibrated according to the manufacturer's instructions prior to each set of measurements. Calibration of salinity meter was performed using standard saline solutions, and the Brix meter was calibrated using a set of standard sucrose solutions with known refractive indices.

Hardness measurement

The CT3 texture analyzer (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) was used to perform hardness measurements. Napa cabbage was cut to a specific size (W×L: 40×30 mm) after the salting process. The "hardness" was measured in texture profile analysis (TPA) mode. A stainless-steel probe (type TA18, 12.7 mm) was used together with the following measurement conditions: test speed, 1.0 mm/s; force threshold, 20 g; distance threshold, 0.50 mm; and compression limit, 50% deformation. To minimize the influence of weight or size of samples, the results were recorded after measurements were repeated twice within an error range of ±0.5 g using a sample of the same size and from the same part of napa cabbage.

Moisture content

Salted napa cabbage was finely crunched, weighed in 1-g portions, and measured using a moisture meter (Moisture Analyzer, MB-95, OHAUS, Parsippany, USA).

Statistical analysis

Each experiment was conducted in triplicate. The data are presented as mean ± SD. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to compare mean values across different treatments. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were conducted using the SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA). Although confidence intervals (CI) were not reported, triplicate independent experiments and presentation of SD ensured sufficient representation of variability.

Results and Discussion

Effects of sea salt concentration and immersion period in brine on natural microbiota in napa cabbage

Table 1 shows the counts of natural indigenous bacteria, such as total viable counts, coliforms, and *B. cereus* in salted napa cabbage after 6, 8, and 10 h of brining at sea salt concentrations of 60, 80, 100, 120, and 140 g/L. Experiments were conducted to determine sea salt concentration and brine time required to reduce microorganisms on salted napa cabbage. In this study, the initial total viable counts of microorganisms on raw napa cabbage were 7.90 log CFU/g. The total viable counts of microorganisms on salted napa cabbage, brined for 6 and 8 h at sea salt concentrations of 60, 80, 100, 120, and 140 g/L, were reduced significantly. At 6 h, at sea salt concentrations of 60, 80, 100, 120, and 140 g/L, the reduction was 0.70, 1.77, 2.64, 3.44, and 3.81 log CFU/g, respectively. At 8 h, the respective reduction was 1.09, 2.40, 3.22, 4.45, and 4.65 log CFU/g. The total viable counts of microorganisms on salted napa cabbage immersed in sea salt at concentrations of 60, 80, 100, and 120 g/L for 10 h were reduced by 1.85, 3.38, 3.79, and 4.89 log CFU/g, respectively.

After exposure to brine for 6 h and 8 h at a sea salt concentration of 140 g/L, the total viable counts were

Table 1. Effects of sea salt concentration and immersion time in brine on natural microbiota (total viable counts, total coliforms, and *Bacillus cereus*) in napa cabbage.

Bacterium	Brine time (hours)	Mean (± SD) reduction value (Log CFU/g)					
		Sea salt concentration (g/L)					
		0	60	80	100	120	140
Total viable counts	6	5.65 ± 0.22^{a,A}	4.95 ± 0.18^{b,A}	3.88 ± 0.20^{c,A}	3.01 ± 0.15^{d,A}	2.21 ± 0.28^{e,A}	1.84 ± 0.11^{f,A}
	8	5.64 ± 0.12^{a,A}	4.55 ± 0.18^{b,B}	3.24 ± 0.26^{c,B}	2.42 ± 0.13^{d,B}	1.19 ± 0.20^{e,B}	0.99 ± 0.18^{f,B}
	10	5.64 ± 0.22^{a,A}	3.79 ± 0.24^{b,C}	2.26 ± 0.11^{c,C}	1.85 ± 0.24^{d,C}	0.75 ± 0.21^{e,C}	ND
Total coliforms	6	2.11 ± 0.30^{a,A}	0.85 ± 0.22^{b,A}	ND	ND	ND	ND
	8	2.08 ± 0.15^{a,A}	0.50 ± 0.28^{b,B}	ND	ND	ND	ND
	10	2.12 ± 0.10^{a,A}	ND	ND	ND	ND	ND
Bacillus cereus	6	1.45 ± 0.18^{a,A}	0.95 ± 0.11^{b,A}	ND	ND	ND	ND
	8	1.49 ± 0.20^{a,A}	0.55 ± 0.20^{b,B}	ND	ND	ND	ND
	10	1.55 ± 0.18^{a,A}	0.18 ± 0.15^{b,C}	ND	ND	ND	ND

The data indicate mean values and standard deviations (three samples/treatment).
Within the same row, mean values with different superscript lower case alphabets (a–f for sea salt concentration) differ significantly ($P < 0.05$) according to Duncan's multiple range test.
Within the same column, mean values with different superscript upper case alphabets (A-C for brine time) differ significantly ($P < 0.05$) according to Duncan's multiple range test.
ND: not detected; represents values <10 CFU/g for total coliforms or *B. cereus*.

significantly ($P < 0.05$) reduced by 3.81 log CFU/g and 4.65 log CFU/g, respectively. Finally, total viable counts were not detected (ND: <10 CFU/g) after exposure to brine for 10 h at a sea salt concentration of 140 g/L. In South Korea, the mean total aerobic bacteria count on napa cabbage from whole markets were in the range 6–9 log CFU/g (Kim *et al.*, 2014). Kim *et al.* (2010) further observed that an analysis of salted napa cabbage (min 0.5 to max 7.0 salinity) sold on the market revealed total viable counts ranging from a minimum of 3.37 log CFU/g to a maximum of 6.05 log CFU/g. This finding aligned with prior research indicating that increased levels of sea salt may impede bacterial growth (Wang *et al.*, 2020). The use of sea salt reduces the moisture value and indirectly inhibits bacterial growth; hence, salted napa cabbage is recognized as safe at these levels of salinity (Gan *et al.*, 2021). Sea salt is able to eliminate bacteria because of a process known as osmosis, in which water passes out of a bacterium to balance sea salt concentrations on each side of its cell membrane (Dayma *et al.*, 2016).

Coliforms are commonly used as an indicator of fecal contamination, resulting from cross-contamination or inadequate food processing (Lee *et al.*, 2009). The initial coliform on raw napa cabbage was 3.54 log CFU/g. The coliform populations were 0.85 log CFU/g and 0.50 log CFU/g after immersion in brine for 6 and 8 h in sea salt with a concentration of 60 g/L, and the counts were significantly reduced by 2.69 log CFU/g and 3.04 log CFU/g, respectively. Coliforms were neither detected after the cabbage was soaked in brine for 10 h, nor were they detected in any cabbage sample exposed to sea salt concentrations of 80–140 g/L. *E. coli* was not detected in any of the samples (data not shown). Tambekar and Mundhada (2006) reported that the main indigenous bacteria, including foodborne pathogens commonly detected in fresh vegetables, are coliform, *E. coli*, and *B. cereus*. In general, sea salt can inhibit the growth of many spoilage and pathogenic bacteria, yeasts, and molds, *albeit* to a different extent depending on the microbial group (Burgess *et al.*, 2016). According to Atter *et al.* (2014), the coliform population reduced from 5.79 log CFU/g to 3.68 log CFU/g after treating cabbage with a sea salt concentration of 5%. In addition, Breidt and Caldwell (2011), in the United States, observed that *E. coli* was reduced by 5 log after cucumber was pickled for 9 h with 6% sea salt concentration. Atter *et al.* (2014) also found that cabbage treated with 5% sea salt showed a 2-log reduction in coliforms. Based on these studies, coliforms could be reduced largely by treating with a sea salt concentration exceeding 6%. However, excessive brining affects appearance, texture, flavor, sensory properties, and fermentation quality of the final product (Kim, 1997). Furthermore, the antimicrobial effect of salt on coliform group cells appeared to depend on the sea salt concentration and storage temperature (Lee and Kang, 2016).

B. cereus is found in various plants, including rice (*Oryza sativa*), potato (*Solanum tuberosum*), napa cabbage (*Brassica rapa* sp. Pekinensis), soybean (*Glycine max*), and wheat (*Triticum aestivum*). This is due to its widespread distribution in the environment, such as in soil, plant roots, food products, and water (Browne and Dowds, 2001). Owing to the adhesive nature of its endospores, *B. cereus* is also often found in food production environments (Arnesen *et al*., 2008). This characteristic allows the bacterium to spread to all types of foods. This result indicates that if *B. cereus* is not eliminated from raw ingredients used for kimchi, it can survive during the distribution thereof. We evaluated the effects of various concentrations of salt on the growth of *B. cereus* in napa cabbage salted with a sea salt solution. We also determined the extent to which *B. cereus* could be eliminated by immersing napa cabbage in brine for three different periods of 6, 8, and 10 h. The population of *B. cereus* decreased significantly ($P < 0.05$) with immersion in brine and increase in sea salt concentration (60, 80, 100, 120, and 140 g/L), similar to the trends observed for total viable counts and total coliforms. The overall average populations of *B. cereus* in napa cabbage exposed to brine containing 60 g/L sea salt for 6, 8, and 10 h were 0.95 log CFU/g (reduction of 1.16 log), 0.55 log CFU/g (reduction of 1.56 log), and 0.18 (reduction of 1.93 log) log CFU/g, respectively, compared to the initial *B. cereus* population of 2.11 log CFU/g. Complete inactivation by sea salt was achieved for concentrations >80 g/L. Kim *et al.* (2017) observed that *B. cereus* populations in shrimp inoculated with this bacterium decreased by 1.89, 2.78, and 4.44 log CFU/g at sea salt concentrations of 50, 100, and 150 g/L, respectively. In addition, Shahbazi and Shavisi (2018) reported a significant decrease in the survival of *Listeria monocytogenes* with a stepwise increase in sea salt concentration. Cheese treated with sea salt concentrations of 80, 120, and 150 g/L showed levels below the detection limit after 10 days (initial 5 log CFU/g). These results aligned with the findings of Khemmapas *et al.* (2023), who demonstrated that 300-g/L salt reduced *B. cereus* on coconut by 1.49 log CFU/g within 10 min. Compared to our results in salted napa cabbage, this suggests that antimicrobial efficacy of brining depends not only on salt concentration and time but also on food matrix characteristics, such as surface structure and moisture content. Different degrees of reduction revealed in our research could be credited to differences in some characteristics of the target food, such as topology and nutritional constituents, between studies. However, the mechanism underlying the antimicrobial effects of sea salt and dependence on the concentration of sea salt of salted napa cabbage is not explained completely. The inhibition of *B. cereus* in salted napa cabbage could be related to changes in water activity (aw), rather than the presence of chloride anions. Water activity necessary to inhibit the growth of most bacteria is approximately 0.91, but the water activity of salted napa cabbage is 0.97–0.99, which could be sufficient to allow the bacteria to survive (Song *et al*., 2019).

Based on the above results, the coliform was more sensitive to saline brine conditions (sea salt concentration and duration of immersion in brine) than *B. cereus* because the coliform treated with a 60-g/L solution of sea salt for 6 h was reduced by more than 1 log CFU/g, whereas *B. cereus* was reduced by 0.5 log CFU/g.

Reduction of inoculated *B. cereus* in salted napa cabbage: concentration and duration of exposure to sea salt

B. cereus, a major cause of outbreak of foodborne illnesses globally, can tolerate severe stress conditions, such as strong acids, extreme heat, and salty conditions, and can survive in the environment despite most cleaning and decontamination processes of food processing phase (Glasset *et al*., 2021). Kristine *et al*. (2006) reported the contamination of vegetables (cabbage, tomatoes, cucumbers, peppers, and root vegetables) by *B. cereus*, and Flores-Urbán *et al.* (2014) observed that *B. cereus* was isolated from 32%, 44%, 84%, and 68% of broccoli, carrots, lettuce, and coriander samples, respectively.

We evaluated the effect of sea salt and period of immersion in brine on the reduction of *B. cereus* present in napa cabbage (Figure 1). The level of *B. cereus* decreased with increasing sea salt concentration (60, 80, 100, 120, and 140 g/L) and time of exposure to brine (6, 8, and 10 h). *B. cereus* levels declined with improved concentration of sea salt and immersion period, with a >3-log decease achieved at ≥120 g/L for 6–10 h. The maximum decrease of 4.80 log CFU/g was observed at 140 g/L for 10 h, satisfying the microbiological safety threshold (MFDS, 2023). These observations were consistent with previous findings of Lee *et al.* (2013), who showed that the *B. cereus* biofilm formed in glass wool decreased from approximately 7 log CFU/g to 4 log CFU/g after treatment with 100 g/L sea salt. In the present study, a decrease of 2.90 log CFU/g was observed at this level of sea salt under similar conditions. Moreover, Feng *et al.* (2022) observed that the growth activity of *S. aureus* cells decreased gradually with increase in salinity. Sutherland *et al.* (1996) reported the growth of *B. cereus* in the presence of 7% sea salt *in vitro*, whereas 10% sea salt was shown to be inhibitory.

The antimicrobial effect observed was probably due to a combination of factors. As salt concentration increased, osmotic pressure on bacterial cells mounted, causing water to exit the cells, leading to cellular dehydration and reduced metabolic activity. Furthermore, increased salinity reduced the water activity of the environment, limiting microbial growth by creating osmotic stress (Ahillah *et al*., 2017; Gurtler *et al*., 2010). On the other hand, Sanjaya *et al*. (2023) indicated that the microorganisms in fish sauce inoculated with *B. subtilis* decreased from 8.74 log CFU/g to 7.07 log CFU/g after treatment with 120-g/L sea salt (1.67 log CFU/g decrease).

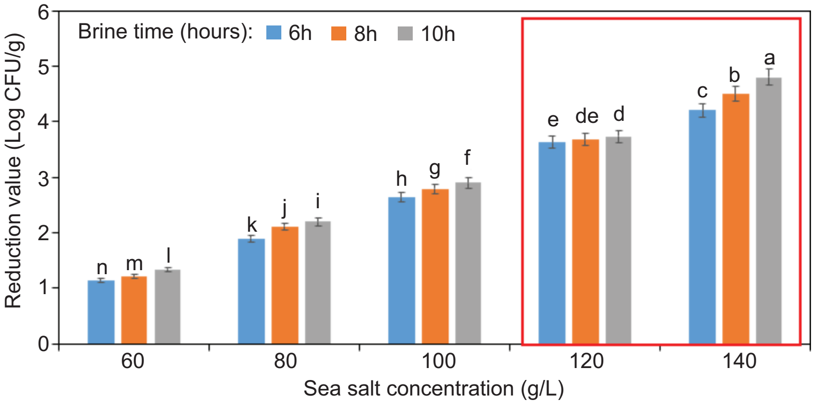

Figure 1. Reduction in *B. cereus* (log CFU/g) population on napa cabbage after treatment with sea salt. For the sea salt treatments resulting in >3-log reductions, the red box highlights groups with significant differences (indicated by different letters [a–e], $P < 0.05$) as determined by Duncan's test.

Although this decrease was low, compared with the present results, their results were expected considering that *B. subtilis* is a halotolerant bacterium because of its strong viability in the environments containing sea salt concentrations of 0–300 g/L. Therefore, it is speculated that the survival of *B. cereus* cells as well as their growth inhibition could additionally be dependent on the concentration of hyperosmotic sea salt. However, the effect of concentration of hyperosmotic sea salt on the viability of *B. cereus* is not fully assessed yet. While the effect of concentration of hyperosmotic sea salt on *B. cereus* viability remains unclear, the recommended brine concentrations could be approximated using simple ratios (e.g., 120–140 g/L), making the method more practical for small-scale producers. These findings are especially relevant for food safety and public health. Since kimchi is typically consumed without a heat treatment step, effective microbial reduction during the salting of raw napa cabbage is critical to minimizing consumer exposure to foodborne pathogens, such as *B. cereus*. Achieving more than a 3-log reduction through optimized brining conditions (e.g., 140 g/L for 10 h) ensures that microbial levels remain well below the threshold set by regulatory authorities. Although this study did not include a complete quantitative microbial risk assessment, the data presented here could contribute to the future exposure modeling and provide scientific support for establishing microbiological control strategies in kimchi production and other fermented vegetable processes.

Effects of sea salt concentration and salting time in brine on physicochemical characteristics (salinity, Brix, hardness, and moisture content) of salted napa cabbage

The results of the measurements (salinity, Brix, hardness, and moisture content) according to sea salt concentration and salting period are presented in Table 2. The brining process affected the characteristics of napa cabbage, with variations depending on operating conditions, such as temperature, sea salt concentration, and duration of immersion in brine. In this study, differences in the salinity of cabbages stored in sea salt concentrations of 60–140 g/L for 6, 8, and 10 h were analyzed. The salinity of untreated napa cabbage was 0.23%. At sea salt concentrations of 120 g/L and 140 g/L, the salinity of napa cabbage reached 1.87–1.89% and 2.11–2.14%, respectively. Extension of salting period (6, 8, and 10 h) at the same sea salt concentration did not affect the outcome significantly. Brine salting is carried out in a 100–150-g/L salt solution for 5–10 h, with an optimum sea salt concentration of about 2–3% in salted napa cabbage (Ryu *et al.*, 2014). In addition, Lee *et al.* (2011) reported that the salinity of traditional brined napa cabbage ranged from 2.34 to 4.62, a value that was deemed high due to the absence of a washing and dehydration process. The results of this study were similar to the findings of Park (2002), who reported that immersion of Chinese cabbage in 10% and 15% NaCl solution resulted in a salinity of 1.85% and 2.49%, respectively. However, Ryu *et al.* (2014) found that salted napa cabbage brined for 4 h at a high salt concentration of 29.6% had a salinity ranging from 2.46% to 2.88%. Choi *et al.* (2014) noted that exposure to brine for more than 12 h was not recommended. Thus, the quality of napa cabbage changed according to the duration of treatment with brine, type of salt, salting method, and sea salt concentration, and a decreasing trend in springiness was observed with increase in brining period.

A comparison of the changes in Brix and salinity revealed an ideal negative linear correlation with decreasing Brix and salinity. The Brix of all samples decreased slowly with sea salt concentration and brine period. These results were found for sea salt concentrations of 60 g/L (3.90–3.93 Bx), 80 g/L (3.80–3.87 Bx),

Table 2. Physicochemical characteristics of salted napa cabbage.

Sea salt concentration (g/L)	Brine time (hours)	Salted napa cabbage			
		Salinity (%)	Brix	Hardness (kg$_f$)*	Moisture content (%)
0	–	0.23 ± 0.03^{l}	5.82 ± 0.03^{a}	7.7 ± 0.4^{a}	93.7 ± 0.3^{a}
60	6	0.44 ± 0.02^{k}	3.93 ± 0.05^{b}	6.2 ± 0.4^{b}	89.0 ± 0.4^{b}
	8	0.57 ± 0.01^{j}	3.93 ± 0.02^{b}	6.2 ± 0.5^{b}	88.9 ± 0.5^{b}
	10	0.63 ± 0.01^{i}	3.90 ± 0.03^{b}	6.0 ± 0.5^{b}	88.8 ± 0.5^{b}
80	6	0.82 ± 0.02^{h}	3.87 ± 0.03^{c}	6.1 ± 0.4^{b}	87.1 ± 0.4^{c}
	8	0.95 ± 0.02^{g}	3.87 ± 0.05^{c}	6.1 ± 0.2^{b}	86.9 ± 0.5^{c}
	10	1.05 ± 0.05^{f}	3.80 ± 0.04^{c}	6.0 ± 0.5^{b}	86.7 ± 0.5^{c}
100	6	1.20 ± 0.05^{e}	3.81 ± 0.01^{c}	6.0 ± 0.2^{b}	85.2 ± 0.4^{d}
	8	1.44 ± 0.01^{d}	3.80 ± 0.01^{c}	6.0 ± 0.5^{b}	84.4 ± 0.3^{d}
	10	1.53 ± 0.02^{c}	3.80 ± 0.02^{c}	5.9 ± 0.3^{b}	84.7 ± 0.5^{d}
120	6	1.87 ± 0.05^{b}	2.30 ± 0.01^{d}	5.9 ± 0.3^{b}	83.4 ± 0.5^{e}
	8	1.87 ± 0.04^{b}	2.25 ± 0.02^{e}	6.0 ± 0.2^{b}	83.2 ± 0.6^{e}
	10	1.89 ± 0.04^{b}	2.24 ± 0.03^{e}	6.0 ± 0.6^{b}	83.0 ± 0.4^{e}
140	6	2.11 ± 0.05^{a}	2.18 ± 0.02^{f}	5.7 ± 0.3^{b}	83.3 ± 0.7^{e}
	8	2.12 ± 0.02^{a}	2.19 ± 0.03^{f}	5.8 ± 0.4^{b}	83.2 ± 0.4^{e}
	10	2.14 ± 0.01^{a}	2.18 ± 0.04^{f}	5.8 ± 0.4^{b}	83.1 ± 0.5^{e}

The data indicate mean values and standard deviations (three samples/treatment).
Within the same column, mean values with different superscript lowercase letters (a–l) differ significantly ($P < 0.05$) according to Duncan's multiple range test.
*kg$_f$: kilogram-force.

100 g/L (3.82–3.86 Bx), 120 g/L (2.24–2.30 Bx), and 140 g/L (2.18–2.19 Bx) ($P < 0.05$). The highest Brix was observed for the control, which was not brined with sea salt, with a value of 5.82 Bx. This behavior may be related to the presence of sugar, which could decrease the saltiness threshold and thus mask the salt content (Amerine *et al.*, 1965). Kim *et al.* (2018) reported an average Brix value of 4.9 Bx (from 4.3 to 6.6 Bx), determined by collecting 500 commercial salted napa cabbages in retail market. Additionally, Ku *et al.* (2003) determined that the Brix of spring, summer, autumn, and winter napa cabbage samples were 5.95 Bx, 6.18 Bx, 6.29 Bx, and 7.76 Bx, respectively. Generally, winter season samples had a slightly higher Brix value than that of napa cabbages salted in other seasons (spring, summer, and autumn). These current results indicated relatively low sugar content because the cabbage was grown from spring to summer and was found to be entirely free from sea salt concentration and brine period, with the difference being insignificant ($P > 0.05$).

The hardness of untreated napa cabbage was 7.7 kg$_f$, but the hardness results of salted napa cabbage were found for various sea salt concentrations: 60 g/L (6.0–6.2), 80 g/L (6.0–6.1), 100 g/L (5.9–6.0), 120 g/L (5.9–6.0), and 140 g/L (5.7–5.8). This fact is somewhat related to the report by Park and Park (1998), who noted that an increase in Na$^+$ ions in napa cabbage disrupted hydrogen bonding, thereby weakening the ability of calcium (Ca^{2+}) ions to support cellulose. This, in turn, decreased the hardness and increased the flexibility of salted napa cabbage. The primary reason for decrease in hardness was the disruption of hydrogen bonds between pectin and cellulose during the salting process as well as the formation of ionic bonds between the carboxyl groups of pectin and potassium ions (Park and Park, 1998).

The moisture content of untreated napa cabbage was 93.7%. A significant difference ($P < 0.05$) was observed in the sea salt concentration and brine time of salted napa cabbage samples. These results were found for various sea salt concentrations: The moisture content of the samples was found to be 60 g/L (89.0–88.9%), 80 g/L (87.1–86.9%), and 100 g/L (85.2–84.7%). A reduced water activity increased the lag phase of microorganisms and a concomitant decrease in growth rate. Nevertheless, no significant difference ($P > 0.05$) was observed between the sea salt concentrations of 120 g/L (83.4-83.0%) and 140 g/L (83.3-83.1%). Such conditions may even lead to inactivation of the pathogen, as was observed by Wemmenhove *et al.* (2014). The authors also observed that a low salt content or high water content in cheese could have a detrimental effect on the inhibition of microbial pathogens. Although moisture content decreased with increasing sea salt concentration, the values remained within a range that was typical for commercial salted napa cabbage, and the reduction likely contributed to enhanced microbial safety without substantially compromising juiciness or texture.

Conclusions

The results of the study showed that the populations of natural indigenous bacteria (total viable counts, total coliforms, and *B. cereus*) in napa cabbage decreased gradually ($P < 0.05$) with increase in both salting period (6–10 h) and sea salt concentration (60–140 g/L). The levels of *B. cereus* were lowered by more than 3 log by immersing napa cabbage in sea salt solutions with concentrations of >12 g/L (120–140 g/L) for 6–10 h. This treatment is necessary because *B. cereus* levels of >3-log CFU/g in vegetables are regarded as unacceptable by South Korea MFDS. Additionally, when the cabbage was brined with 140 g/L sea salt for 10 h, the levels of *B. cereus* were reduced by nearly 5 logs (4.80-log reduction). The salinity of cabbage was not significantly influenced ($P > 0.05$) by brining period at sea salt concentrations of 120 g/L and 140 g/L, and at these sea salt concentrations, the Brix of salted napa cabbage was like that of commercial salted napa cabbage available in retail markets. The hardness of salted napa cabbage was unaffected by sea salt concentration and duration of salting ($P > 0.05$). The moisture content of napa cabbage was significantly influenced ($P < 0.05$) by brining time at different sea salt concentrations. Among all tested conditions, the most effective treatment was brining of napa cabbage in 140 g/L sea salt for 10 h, which achieved a 4.80 log CFU/g reduction in *B. cereus* while maintaining acceptable physicochemical quality. Therefore, these parameters are recommended for application by the kimchi manufacturing industry. Moreover, these findings may also be applicable to the processing of other fermented or minimally processed vegetable products that require microbial safety control without compromising texture and flavor. The future research could investigate the effects of alternative salting agents, such as potassium chloride (KCl), to reduce sodium content, or the combined impact of salting with other preservation methods, including fermentation and refrigeration, to enhance microbial safety and product stability.

Data Availability Statement

All data generated or analyzed in this study are included in this published article.

Author Contributions

Eun Bi Jeon and Sung-Hee Park: data curation, investigation, and writing – original draft. Min Jung Lee: methodology. Shin Young Park: writing – review and editing, and funding. All authors had read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declared no conflict of interest.

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