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Microbial Evaluation of Commercially Packed *Kimchi* Products

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Abstract Commercially packed *kimchi* products from 6 different manufacturers, which are exported overseas as well as sold domestically, were analyzed to determine their microorganism distributions and presence of pathogenic bacteria. All samples showed decreasing pH levels (from 5.7-6.2 to 3.9-4.3) and increasing titratable acidities (from 0.3-0.4 to 0.8-1.2%) during 15 days of storage at 4°C. Total bacterial counts ranged from 2.1×10^5-1.9×10^6 CFU/mL in the initial *kimchi* samples, and then increased to 1.1×10^8-1.8×10^9 CFU/mL. The coliform numbers decreased from approximately 2.5×10^2-1.7×10^4 CFU/mL to zero. Major foodborne pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus*, *Yersinia enterocolitica*, and *Shigella* spp. were not detected in any of the samples. However, 2 out of the 6 samples carried *E. coli*, emphasizing the need for improved hygiene practice. Interestingly, *Hafnia alvei*, belonging to the *Enterobacteriaceae* family, was isolated in all of the samples. Further study is needed on this newly reported bacterium in *kimchi*.

Keywords: *kimchi*, hygiene, safety, microorganism, *Hafnia alvei*

Introduction

Kimchi is a traditional Korean fermented dish containing salted vegetables, and was originally developed to give foods an extended storage period (1). Approximately 190 different types of *kimchi* are made with various vegetables, including Asian cabbage (*baechu*) and radish (2). The main ingredients of *kimchi* include *baechu*, garlic, red pepper, green onion, ginger, and salt (3). Other optional ingredients are fruits, glutinous rice paste, nuts, fermented seafood (*jeotgal*), sesame seeds, sugar, and wheat flour paste (3).

There are various reports about the functional properties of *kimchi*, including anticancer, antioxidant, antimutagenic, and antibacterial effects (4-7). Park *et al.* (6) reported that the methanol extract of *kimchi* presented antimutagenic activities. Kim (7) reported that lactic acid bacteria isolated from *kimchi*, such as *Lactobacillus plantarum* and *Leuconostoc mesenteroides*, showed antibacterial effects. However, until recently, only limited reports pertaining to the microbial safety of *kimchi* were available.

Since the registration of *kimchi* on Codex Alimentarius in 2001 (3), the status of *kimchi* has been promoted to a global food, and the *kimchi* market continues to expand worldwide. *Kimchi* has traditionally been made at home. Today, however, due to changes in the family structure and an apartment-centered housing culture, many food manufacturing plants commercially produce *kimchi* in massive amounts (8).

Recently, issues on the microbial safety of *kimchi* have held consumers' attention, as a result of outbreaks in 2005 (9). These outbreaks revealed that *kimchi* can be exposed to diverse sources of contaminants. The tainting of raw ingredients as well as contamination during the manufacturing process may occur through human and animal excreta, polluted water, natural pathogens, flies and pests, cross-contamination, etc. (10).

The objective of this study was to evaluate microbial distributions in commercially packed *kimchi*, especially for products that are both exported overseas and consumed domestically. The numbers of total bacteria and coliforms were counted, and the presence of pathogenic bacteria such as *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Shigella* spp., *Bacillus cereus*, and *Yersinia enterocolitica* was monitored. Identification of the isolated microorganisms was also conducted.

Materials and Methods

Preparation of sample *Baechukimchi*, which is exported worldwide and sold domestically, was purchased at local supermarkets in the Daegu and Gyeongbuk areas, as well as through the internet. The *kimchi* samples were designated as A, B, C, D, E, and F. When purchased, samples A, B, and E were 3 days aged, and samples C, D, and F were 2 days aged from their manufacturing dates. In the laboratory, the *kimchi* was stored at 4°C according to the manufacturer's recommendation, and examined every 3 days over 15 days total. To prepare the samples, *kimchi* was aseptically taken and homogenized with a blender for 1 min. The homogenized *kimchi* was filtered through sterile gauze. For the microbial count analysis, 1 mL of the filtrate was diluted with sterile saline solution as required.

Chemical analysis The sample pH levels were measured with a pH meter (Thermo Electron Co., Beverly, MA, USA). Twenty mL of the *kimchi* filtrate (*kimchi* juice) was titrated with 0.1 N NaOH to pH 8.1±0.2 for titratable acidity (11). The titratable acidity was calculated on the basis of lactic acid.

$$\text{Acidity(\%, as lactic acid)} = \frac{0.009\times\text{mL of 0.1 N NaCl}\times F\times 100}{\text{Sample (mL)}}$$

F: factor of 0.1 N NaOH

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Total bacteria and total coliforms Each sample of *kimchi* juice was diluted with 0.85% sterilized saline by the serial dilution method. Each 0.1 mL of the diluted solution was plated onto a plate count agar plate (Becton, Dickinson Co., Sparks, MD, USA) for the total bacterial counts, as well as onto a desoxycholate lactose agar plate (Becton, Dickinson Co.) for the total coliform counts, according to the pour-plate method (12). The plates were then incubated at 35°C for 48 hr. The colonies formed on the plates were counted and expressed as colony-forming units per mL (CFU/mL).

Isolation of *Salmonella* spp. The isolation of *Salmonella* spp. was carried out using 2 enrichment steps (12). First, 10 mL of *kimchi* juice was aseptically taken, mixed with 90 mL of peptone water (peptone 10 g, NaCl 5 g, distilled water 1 L), and incubated at 35°C for 18 hr. Following incubation, 0.1 mL of the culture was transferred into 10 mL of Rappaport Vassiliadis broth (Merck, Darmstadt, Germany) for the second enrichment, and then incubated at 42°C for 24 hr. One loop of the second enrichment broth was spread onto a MacConkey agar plate (Becton, Dickinson Co.) and incubated at 35°C for 24 hr. The colonies on the MacConkey agar plate were transferred to a triple sugar iron agar slant (TSI, Becton, Dickinson Co.) and incubated at 35°C for 24 hr. The colonies on the TSI agar slant were chosen for identification using an API 20E kit (Bio-Merieux, Marcy I'Etoile, France)

Isolation of *E. coli* O157:H7 The isolation of *E. coli* O157:H7 was conducted using the method of the Korean Food Standards Codex (12). Here, 10 mL of *kimchi* juice was aseptically taken, mixed in 90 mL of modified *E. coli* broth with novobiocin (Merck), and incubated at 35°C for 24 hr. One loop of the enrichment broth was streaked onto a sorbitol MacConkey agar plate (SMAC, Becton, Dickinson Co.) and incubated at 35°C for 24 hr. For the second isolation, the colonies on the SMAC agar plate were transferred to an eosin methylene blue agar plate (EMB, Becton, Dickinson Co.) and incubated at 35°C for 24 hr. The colonies on the EMB agar plate were selected for *E. coli* O157:H7 confirmation. To confirm *E. coli* O157:H7, O157 and H7 antisera (Becton, Dickinson Co.) and an API 20E kit (Bio-Merieux) were used.

Isolation of *S. aureus* Tryptic soy broth (TSB, Becton, Dickinson Co.) with 10% NaCl was used for the enrichment of *S. aureus* (12). Ten mL of *kimchi* juice was aseptically taken and mixed with 90 mL of TSB with 10% NaCl. After 16-18 hr of incubation at 35-37°C, one loop of the culture was transferred onto a mannitol-salt-egg yolk agar plate (MSEY, Becton, Dickinson Co.) and incubated for another 24 hr at 37°C. The colonies on the MSEY agar plate were identified using an API Staph kit (Bio-Merieux).

Isolation of *L. monocytogenes* Ten mL of *kimchi* juice was aseptically taken, mixed in 90 mL of *Listeria* enrichment broth (Becton, Dickinson Co.), and incubated at 30°C for 24 hr (12). One loop of the enrichment broth was streaked on an Oxford agar plate (Becton, Dickinson Co.). After 48 hr of incubation at 30°C, the colonies on the Oxford agar plate were identified using an API Listeria kit (Bio-Merieux).

Isolation of *Y. enterocolitica* Ten mL of *kimchi* juice was aseptically taken, mixed in 90 mL of peptone sorbitol bile broth (disodium phosphate 8.23 g, monosodium phosphate 1.2 g, bile salts No. 3 1.5 g, sodium chloride 5 g, *d*-sorbitol 10 g, peptone 5 g, distilled water 1 L), and incubated for 10 days at 10°C (12). Following incubation, 0.1 mL of the culture was mixed into 1 mL of 0.5% NaCl solution containing 0.5% KOH. One loop of the mixture was streaked onto MacConkey agar and cefsulodin irgasan novobiocin agar (CIN, Oxoid, Basingstoke, Hampshire, UK) plates. After 24 hr of incubation at 30°C, the colonies on the MacConkey agar and CIN agar plates were transferred to a TSI agar slant and further identified using an API 20E kit (Bio-Merieux).

Isolation of *Shigella* spp. One loop of *kimchi* juice was streaked onto a MacConkey agar plate and incubated at 35°C for 24 hr (13). The colonies on the MacConkey agar plate were transferred to a TSI agar slant and incubated at 35°C for 24 hr. The colonies on the TSI agar slant were identified using an API 20E kit (Bio-Merieux).

Isolation of *B. cereus* Ten mL of *kimchi* juice was aseptically taken and mixed in 90 mL of sterile phosphate buffered diluent (12). One loop of the mixture was streaked onto a mannitol egg yolk polymyxin agar plate (MYP, Oxoid) and incubated at 30°C for 24 hr. The colonies on the MYP agar plate were identified using an API 50CH kit (Bio-Merieux).

Results and Discussion

pH and acidity Figure 1 and 2 show the pH and acidity changes, respectively, of the *kimchi* during 15 days of storage at 4°C. While ripening, pH tended to decrease, and titratable acidity tended to increase. At the beginning of storage, the pH and acidity were distributed as 5.7-6.2 and 0.3-0.4%, respectively. On the 3rd day of storage, the pH

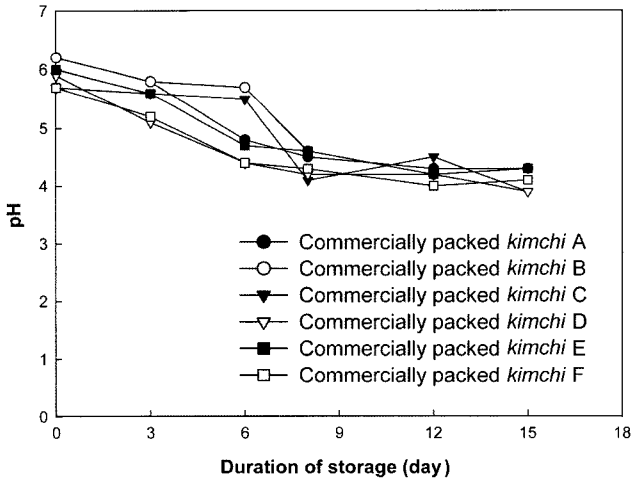

Fig. 1. Changes in pH of commercially packed *kimchi* during storage at 4°C.

Table 1. Changes in total bacteria and total coliforms of commercially packed *kimchi* during storage at 4°C

Storage days	Sample A			Sample B			Sample C			Sample D			Sample E			Sample F		
	pH	Total bacteria (CFU/mL)	Total coliforms (CFU/mL)	pH	Total bacteria (CFU/mL)	Total coliforms (CFU/mL)	pH	Total bacteria (CFU/mL)	Total coliforms (CFU/mL)	pH	Total bacteria (CFU/mL)	Total coliforms (CFU/mL)	pH	Total bacteria (CFU/mL)	Total coliforms (CFU/mL)	pH	Total bacteria (CFU/mL)	Total coliforms (CFU/mL)
0	6.2	3.2×10^5	1.7×10^4	6.0	1.2×10^6	3.5×10^3	5.7	2.1×10^5	2.3×10^3	5.9	1.9×10^6	7.3×10^2	6.0	1.6×10^6	2.5×10^2	5.7	1.7×10^6	1.2×10^4
3	5.8	1.1×10^7	1.1×10^4	5.8	3.2×10^8	1.5×10^3	5.6	1.7×10^7	1.3×10^3	5.1	6.8×10^7	3.9×10^2	5.6	3.5×10^6	2.3×10^3	5.2	1.0×10^6	1.2×10^5
6	4.8	6.6×10^7	6.3×10^3	5.7	6.9×10^8	1.2×10^2	5.5	2.0×10^7	7.8×10^2	4.4	1.1×10^8	1.0×10	4.7	2.8×10^7	8.6×10	4.4	1.5×10^7	5.0×10
9	4.5	6.3×10^8	nd[1)]	4.6	9.9×10^8	nd	4.1	8.0×10^7	6.9×10	4.2	7.1×10^8	nd	4.6	1.3×10^8	nd	4.3	2.5×10^7	nd
12	4.3	3.3×10^8	nd	4.2	1.7×10^8	nd	4.5	1.6×10^8	nd	4.2	1.4×10^9	nd	4.2	1.1×10^8	nd	4.0	1.9×10^8	nd
15	4.3	5.5×10^8	nd	4.3	1.8×10^9	nd	3.9	1.4×10^8	nd	3.9	1.1×10^8	nd	4.3	6.0×10^8	nd	4.1	1.6×10^8	nd

[1)]Not detected; Each value of the total bacteria counts is the mean of 3 replicates.

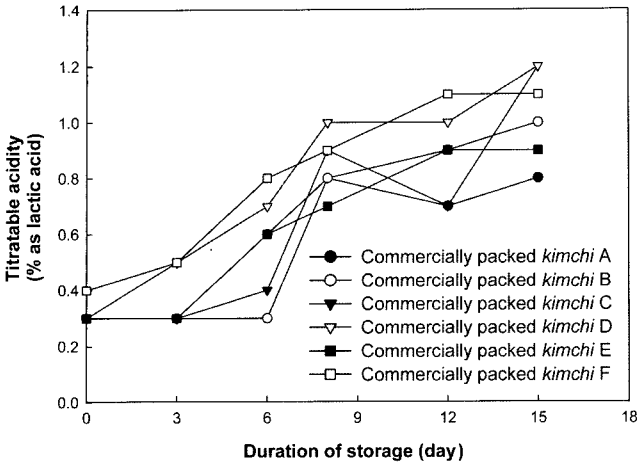

Fig. 2. Acidity changes of commercially packed *kimchi* during storage at 4°C.

and titratable acidity were near 5.1-5.8 and 0.3-0.5%, respectively. As the 15th day of storage approached, pH decreased to 3.9-4.3, and titratable acidity increased to 0.8-1.2%. Yoo (14) reported that changes in pH and acidity may be influenced by the acid produced from lactic acid bacteria. It was also reported that optimum taste is attained when the pH and acidity are in approximate ranges of 4.2-4.4 and 0.6-0.8%, respectively (15). In this study, the commercially packed *kimchi* reached optimal pH and acidity between 6-12 days of storage, with differences occurring among the samples. These differences between samples may be attributed to the optional ingredients, or to the *kimchi* manufacturing process itself.

Total bacteria and total coliforms In this study, we examined total bacteria and coliforms to determine the distribution of microorganisms during the storage period. The results are listed in Table 1. The number of total bacteria on the day of purchase ranged from 2.1×10^5 to 1.9×10^6 CFU/mL in the samples. Late in the storage period (15 days), the number of microorganisms reached 1.1×10^8-1.8×10^9 CFU/mL. The total bacteria tended to increase during storage. According to a report by Park *et al.* (16), total microbial counts were approximately 6.01 log CFU/mL at pH 5.79 in the initial sample, and 9.11 log CFU/mL at pH 4.05 during the late storage period. The findings of Park *et al.* (16) show good agreement with our results. The total bacteria during the initial period of fermentation are reported to be influenced by the *kimchi* raw materials and the cleaning process (17). Kang *et al.* (17) reported that the number of total bacteria increased during ripening, however, the number of total bacteria had a tendency to decrease when the pH was below 3.5. Similarly, Yoo *et al.* (18) reported that total bacteria tended to increase until their numbers reached a peak. After the peak, the number of total bacteria decreased due to the acids produced.

Total coliform counts were shown to be between 2.5×10^2 and 1.7×10^4 CFU/mL on the day of *kimchi* purchase (Table 1). When the pH dropped below 5.0, the numbers of total coliforms started to decline remarkably in most of the samples. Kim *et al.* (19) reported levels of 1.31-2.79 log CFU/mL of coliform bacteria in their laboratory-made *kimchi*. Coliforms are commonly found in the guts of mammalian animals and humans (20). Coliforms are indicative of the quality of food and possible fecal contamination, as well as the potential presence of other enteric pathogens (21). Coliforms, which appeared in the early storage period, gradually disappeared and were not found at the end. This implies that *kimchi* becomes relatively safe as its fermentation proceeds.

Occurrence of foodborne pathogenic bacteria According to the Korean Food Standards Code published by the Korea Food Industry Association (12), *Salmonella* spp., *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *B. cereus*, and *Y. enterocolitica* should not be isolated from foods. In this study, we tested for the presence of the above bacteria along with *Shigella* spp. As the results show, *Salmonella* spp., *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *B. cereus*, *Y. enterocolitica*, and *Shigella* spp. were not detected in any of the *kimchi* samples during 15 days of storage at 4°C (Table 2). This suggests that *kimchi* is microbiologically safe against such critical foodborne pathogenic bacteria.

Other major microorganisms isolated Table 3 shows the average incidence of major microorganisms isolated from the commercially packed *kimchi* during 15 days of storage at 4°C. *E. coli* was found in some samples depending on the storage day (Table 3). The detection of *E. coli* is in accordance with a previous report, where Shin

Table 2. Occurrence of foodborne pathogens in commercially packed *kimchi* during 15 days of storage at 4°C

Foodborne pathogenic bacteria	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
B. cereus	nd[1)]	nd	nd	nd	nd	nd
E. coli O157:H7	nd	nd	nd	nd	nd	nd
L. monocytogenes	nd	nd	nd	nd	nd	nd
Salmonella spp.	nd	nd	nd	nd	nd	nd
Shigella spp.	nd	nd	nd	nd	nd	nd
S. aureus	nd	nd	nd	nd	nd	nd
Y. enterocolitica	nd	nd	nd	nd	nd	nd

[1)]Not detected.

Table 3. Average incidence of *E. coli* and *H. alvei* in commercially packed *kimchi*

Storage day	Average incidence[1)] (%)	
	E. coli	*H. alvei*
0	0	50
3	33	17
6	0	33
9	17	50
12	0	83
15	0	50

[1)]n=6.

et al. (22) also reported its presence in commercially packed *kimchi* products. *E. coli* is an indicator of fecal contamination; therefore, the presence of *E. coli* indicates that the food was in (direct or indirect) contact with feces at some point. Unfortunately, our data does not offer where *E. coli* contamination occurred during the *kimchi* manufacturing process. In fact, it was reported that *E. coli* can not be traced back to the origin of contamination (23, 24), but it may occur from the production practices used with vegetables, from human or animal feces, or through storage, processing, and handling. The use of hygienic practices with ingredients and tools, and other preventive measures, should be taken by manufacturers to remove *E. coli*.

Another major microorganism isolated from the commercially packed *kimchi* was *Hafnia alvei* (Table 3). *H. alvei* belongs to the *Enterobacteriaceae* family (25). In this study, *H. alvei* was frequently isolated from the samples during the entire testing period (Table 3). We have been unable to find reports on the presence of *H. alvei* in *kimchi*. Therefore, our finding is worth noting. Other reports have stated the occurrence of *H. alvei* in milk products and cheese (26, 27). Meats and fish are also reported to contain *H. alvei* (28, 29). However, vegetables are not known to be frequent reservoirs for *H. alvei* (25). Based on this information, we suspect that the *H. alvei* found in *kimchi* could have originated from *jeotgal*, because all the manufacturers used *jeotgal* as a common ingredient.

H. alvei is known to be the third most common identified enteric species, following *E. coli* and *Enterobacter cloacae* (30). Over the past decade, *H. alvei* has received increased attention from the medical community due to its possible association with gastroenteritis (25). However, very little is known about the *Hafnia* genus in regard to its role(s) as both a human and veterinary pathogen, and there are a limited amounts of data available on disease states associated with *H. alvei* (25). Therefore, further researches on its health impacts and the microorganism's prevalence in *kimchi* are needed.

Implications of the study Once *kimchi* is prepared, it is ready to eat at any stage of fermentation, depending on an individual's taste. Since there is no sterilizing process during *kimchi* processing, microbial hygiene and safety are very important issues. According to the Korean Food Standards Code (12), for sterilized products, test results for coliforms should be negative. For non-thermally processed products like *kimchi*, the Korean Food Standards Code contains no guidelines of microbial standards.

In our microbial evaluation of commercially packed *kimchi* products, coliforms appeared in the early storage period and then rapidly disappeared during fermentation. *E. coli*, which is indicative of food hygiene, was isolated from 2 of the 6 samples. Thus, preventive measures to reduce coliforms and *E. coli* are needed to improve the quality of *kimchi*. Foodborne pathogenic bacteria such as *B. cereus*, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., *S. aureus*, and *Y. enterocolitica* were not detected in the *kimchi* at any period of storage, suggesting that *kimchi* is sufficiently safe from a health point of view, with regard to the foodborne pathogenic bacteria selected. *H. alvei*, belonging to *Enterobacteriaceae*, was newly reported in this study. Currently, it is known that much more work and evidence needs to be accumulated to support *Hafnia* as a cause of gastroenteritis. Lastly, for future studies, monitoring the presence of *H. alvei* in *kimchi* ingredients would be of great interest.

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