

## Changes of Index Microorganisms and Lactic Acid Bacteria of Korean Fermented Vegetables (Kimchi) During the Ripening and Fermentation-Part 2

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### ABSTRACT

The Chinese cabbage kimchi, baechoo-kimchi, is the most popular type of kimchi in Korea. This study was performed to investigate the changes of index microorganisms (aerobic bacteria, psychrotrophic bacteria, coliforms, and *Escherichia coli*), lactic acid bacteria, pH, and acidity of kimchi during the long-term fermentation and ripening. A homemade-style traditional Korean baechoo-kimchi, was prepared from Chinese cabbage, red pepper, green onion, garlic, ginger, and salt-fermented anchovy sauce, and then incubated at 10°C for 28 days. In the baechoo-kimchi, the number of aerobic bacteria increased with time. The number of psychrotrophic bacteria maintained their numbers ( $10^4$  CFU/g) in the kimchi during the fermentation. Coliforms and *E. coli* were not detected in the kimchi. The pH of kimchi decreased and the acidity of kimchi increased over time. Lactic acid bacteria, which are representative of fermentative microorganisms in the kimchi process showed rapid growth in the earlier stage of fermentation and increased steadily after 7 days. The counts of lactic acid bacteria were at a level of  $10^4$  CFU/g early in the fermentation stage, reaching a level of  $10^8$  CFU/g after 14 days, and at this point pH was 4.18 and acidity reached 0.63, indicating that the optimal state of kimchi fermentation. This study suggests that the lactic acid bacteria which were proliferated in kimchi during the ripening and fermentation could contribute to improving the taste and flavor of kimchi and inhibit the growth of pathogenic microorganisms that might exist in kimchi.

**Keywords:** Kimchi, index microorganisms, lactic acid bacteria, pH, acidity

### I. Introduction

Kimchi is a traditional Korean fermented side dish made from several selected vegetables with varied seasonings. It is one of the most important foods for Koreans in their daily life. Recently, kimchi has become popular even in the Western world because of the unique taste and beneficial effects, such as antiaflatoxigenic activity, antioxidant activity, antimutagenic effect, and fibrinolytic activity (Kim and Lee, 2007; Lee and Kunz, 2005; Lee, 1997, Steinkraus, 1997; Cheigh and Park, 1994), which originated from various raw materials and secondary metabolites of fermentative microorganisms (Caplice and Fitzgerald, 1999; Svanberg

and Lorri, 1997).

Among many different types of kimchi, the most frequently consumed and preferred kimchi in Korea is baechoo-kimchi made with Chinese cabbage. Other various vegetables (radish, onion, green onion, carrot, cucumber, leaf mustard, and etc.), spices (red pepper, garlic, ginger, sesame, and etc.) and non-vegetable ingredients (salt-fermented anchovy, salt-fermented shrimp, boiled starch, sugar, oyster, and etc.) are used as minor ingredients (<http://www.kimchi.or.kr>). The procedure of kimchi preparation includes brining of cabbage and mixing the brined cabbage with spices and ingredients. The fermentation and ripening of kimchi are initiated by various microbes originating from the raw materials and environment. The microflora is gradually simplified and finally lactic acid bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, and etc.) and yeast (*Hansenula*, *Pichia*, *Debaryomyces*, and etc.)

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become dominant (Cheigh and Park, 1994).

Several reports isolating the pathogenic microorganisms from raw vegetables indicated that they may harbor potential pathogens (Kim, 2004; Meng and Dolye, 2002; Beuchat 2002; Robertson *et al.*, 2002; McMahon and Wilson, 2001; Beuchat, 1996; Albrecht *et al.*, 1995; Nguyen-the and Carlin, 1994; Doyle, 1990). From their results and the fact that the fermentation of kimchi is a natural process, we can suspect that kimchi could be contaminated from the ingredients, preparer, and environment.

However, only a few reports on the hygienic aspects of this fermented vegetable food are found (Kim and Yoon, 2005; Kim *et al.*, 2004; Chung, 1994), although there have been many reports on the food and nutritional characteristics (Kim and Chun, 2005; Mheen and Kwon, 1984). In the previous report, we investigated the microbiological contamination of raw materials of kimchi and microbiological quality changes of kimchi during the 8 days' fermentation (Kim and Yoon, 2005). However, the quality changes of kimchi should be observed more, because kimchi is consumed not only before but also after this short-term fermentation period and sometimes even for a one month.

The purposes of this study were to evaluate the microbiological status of kimchi again and to determine quality changes occurring during the long-term fermentation and ripening. This was done by observing the index microorganisms, lactic acid bacteria, pH, acidity, and their changes during the ripening and fermentation for 28 days.

## II. Materials and Methods

### 1. Chemicals and Media

All reagents used were analytical grade purity or better. A nutrient agar (Difco Lab., MI, U.S.A.) medium was used for the determination of aerobic plate count. Lactose broth, EC broth, and eosin methylene blue (EMB) agar (Difco Lab.) were used for the detection of coliforms and *E. coli*. MRS agar (Difco Lab.) was used for the determination and counting of lactic acid bacteria.

### 2. Preparation of Kimchi

A homemade-style traditional baechoo-kimchi was used for this study. The kimchi was prepared from baechoo (Chinese cabbage). Minor ingredients

of the kimchi were red pepper, green onion, garlic, ginger, and salt-fermented anchovy sauce. The raw materials of kimchi produced in Korea were purchased from a local market.

The preparation methods of kimchi used were those recommended by Kim and Lee (2007), Kim and Yoon (2005), and Han (1999). Chinese cabbages were trimmed, cut into 4 pieces, washed with water, soaked in a 10% (w/v) salt solution for 3 h at room temperature, and then rinsed with water flow to reduce the salt concentration to 2%. After draining the excess water from the salted Chinese cabbages, and cutting the salted Chinese cabbages into 3 cm lengths, kimchi was prepared mixing with the minor ingredients. The recipe of kimchi was as follows: Chinese cabbage 100 g, red pepper powder 2.5 g, green onion 1 g, garlic 1 g, ginger 0.5 g, and salt-fermented anchovy sauce 0.5 g. A total of sixty 200-ml plastic boxes, each containing 100 g of the prepared kimchi were anaerobically packed, and used as a sample. The ripening and fermentation of kimchi was carried out for 28 days using an incubator at 10°C. Analyses of samples were done at initial stage and on every 7 days.

### 3. Sample Preparation

A separate box of kimchi was used at each sampling time. Each sample was diluted and mixed with 9-fold sterile phosphate buffered saline by homogenizing. The juice from each sample was filtered through sterilized gauze. Microbial numbers of the samples were determined by decimal dilution in sterile phosphate buffered saline

### 4. Determination of Aerobic Plate Count (APC) and Psychrotrophic Plate Count (PPC)

The pour plate method was employed to determine the presence of aerobic bacteria using nutrient agar. Serial dilutions of the kimchi samples were prepared in sterile phosphate buffered saline (0.85% sodium chloride). The plates of the nutrient agar were then inoculated with 1.0 ml of the diluted samples, and incubated at 35°C for 48 h for APC and 25°C for 72 h for PPC. Enumeration was done using a Quebec colony counter (Korea Manhattan Co., KMC-1301) and was expressed as CFU/g of sample.

### 5. Determination of Total Coliforms

To determine the presence of total coliforms in

samples, the multi-tube fermentation technique using lactose broth for coliform analysis was used, followed by plating on EMB agar and incubated at 35°C for 48 h (KFDA, 2005). Counts at the highest dilutions using sterile saline were then expressed as MPN/100 g of sample.

#### 6. Detection and Enumeration of *E. coli*

To detect *E. coli*, the multi-tube fermentation technique using EC broth was used at 44.5°C for 24 h. EMB agar plates were inoculated with a loop of positive samples and then incubated at 35°C overnight (KFDA, 2005). Gram stain and IMVic test were necessary in some cases for final identification.

#### 7. Determination of Lactic Acid Bacteria

To determine the growth of lactic acid bacteria, the pour plate method was also employed using MRS agar. Serial dilutions of the juice of kimchi were inoculated on the agar plate, and then incubated at 35°C for 72 h.

#### 8. Determination of pH and Acidity

The pH of juice of kimchi was measured with a pH meter (Istek 750, Korea) after calibration with standard pH buffers. Acidity measurement was by titration (AOAC, 2005).

#### 9. Statistical Analysis

The microbial prevalence in the kimchi samples at every 7 days was compared. Also pH and acidity of juice of kimchi were compared. The data from samples were compared by analysis of variance. Significant differences among means were determined by Duncan's multiple range tests.

### III. Results and Discussion

In this study, a type of kimchi made from Chinese cabbage, namely baechoo-kimchi was used to evaluate the microbiological status and quality changes because it is the most popular type and the most frequently consumed in Korea. The changes of index microorganisms (aerobic mesophiles, total coliforms, and *E. coli*) of the kimchi examined are summarized in Table 1. During the 28 days' ripening and fermentation at 10°C, there appeared to be overall increased microbial numbers in aerobic plate count. The APCs were 10<sup>6.15</sup> CFU/g at the initial stage of fermentation, and increased for 28 days' fermentation (p<0.05). The increase in the counts of aerobic bacteria during the fermentation of kimchi has also been described by other authors (Yi *et al.*, 1998; Chung, 1994). However, microbiological analysis results showed that the counts of psychrotrophic bacteria in the kimchi (10<sup>3.97</sup>~10<sup>4.96</sup> CFU/g) did not show remarkable changes during the fermentation period. These results are partially in agreement with those obtained by previous reports (Kim and Yoon, 2005).

The changes of lactic acid bacteria are shown in Fig. 1. The counts of lactic acid bacteria were increased during the fermentation. Initial numbers of lactic acid bacteria were 10<sup>4.32</sup> CFU/g, and reached 10<sup>7.95</sup> CFU/g after 28 days' fermentation (p<0.05). These results are in agreement with previous studies by other investigators (Yi *et al.*, 1998; Chung, 1994; Mheen and Kwon, 1984).

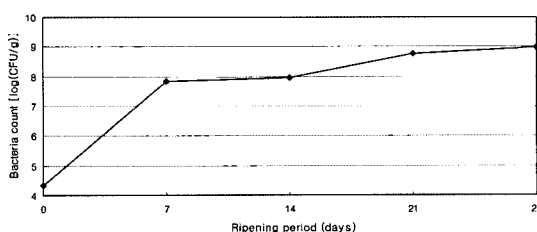
The pH of kimchi was decreased while acidity of kimchi was increased during fermentation as the time goes (Table 2). The pH of kimchi started at 5.35, then decreased to 4.74, 4.18, 4.06, and 3.89

**Table 1.** Changes of microbiological quality of kimchi during the ripening and fermentation for a 28-day period

Fermentation period (days)	Aerobic plate count [log(CFU/g)]	Psychrotrophic plate count [log(CFU/g)]	Total coilforms [log(MPN/100 g)]	Fecal coilforms [log(MPN/100 g)]
0	6.15±0.09 <sup>b</sup>	4.96±0.05 <sup>a</sup>	— <sup>*</sup>	—
7	8.06±0.05 <sup>a</sup>	4.54±0.05 <sup>b</sup>	—	—
14	8.95±0.04 <sup>a</sup>	3.97±0.16 <sup>c</sup>	—	—
21	8.83±0.04 <sup>a</sup>	4.39±0.04 <sup>b</sup>	—	—
28	8.27±0.04 <sup>a</sup>	4.30±0.06 <sup>bc</sup>	—	—

\*—: Not detected.

Values with the different superscript letters in a column are significantly different (p<0.05).



**Fig. 1.** Changes of lactic acid bacteria of kimchi during the ripening and fermentation for a 28-day period.

**Table 2.** Changes of pH and acidity of kimchi during the ripening and fermentation for a 28-day period

Fermentation period (days)	pH	Acidity
0	5.35±0.02 <sup>a</sup>	0.21±0.10 <sup>b</sup>
7	4.74±0.03 <sup>b</sup>	0.45±0.09 <sup>a</sup>
14	4.18±0.04 <sup>b</sup>	0.63±0.15 <sup>a</sup>
21	4.06±0.03 <sup>b</sup>	0.70±0.19 <sup>a</sup>
28	3.89±0.06 <sup>b</sup>	0.81±0.20 <sup>a</sup>

Values with the different superscript letters in a column are significantly different ( $p < 0.05$ ).

after 7, 14, 21, and 28 days of fermentation at 10°C, respectively. The more decrease of pH was, the more increase of acidity in the kimchi.

Lactic acid bacteria are major players in many fermented vegetable foods, such as kimchi, cassava sour starch (Omar *et al.*, 2000). Mexican pozol, an acid beverage produced by the natural fermentation of nixtamal (heat-and alkali-treated maize) dough (Escalante *et al.*, 2001; Ampe *et al.*, 1999). The taste and flavor of kimchi is dependent on the ingredients, fermentation conditions, and lactic acid bacteria involved in the fermentation process (Cheigh and Park, 1994; Lee *et al.*, 1992; Mheen and Kwon, 1984). In this study, the counts of lactic acid bacteria in the kimchi fermentation were a level of  $10^4$  CFU/g early in the fermentation stage, reaching about  $10^8$  CFU/g after 14 days (Fig. 1), and at this point acidity reached up to 0.63 and pH was 4.18. In our previous report on the short-term fermentation of kimchi, the pH and acidity of the kimchi were 4.39 and 0.47 after 8 days' although the counts of lactic acid bacteria reached up to  $10^{7.86}$  CFU/g at that time (Kim and Yoon, 2005). The results of our studies are supported by several

investigators (Marriott and Robertson, 1997; Hayes, 1992; Nout and Motarjemi, 1984; Mheen and Kwon, 1984). They indicated that fermentation with lactic acid bacteria may be the best at pH 4, and this could inhibit harmful pathogens. It is also suggested that the taste and flavor of kimchi may be the best when the pH of the kimchi reached approximately 4.2~4.4, and acidity 0.6~0.8. On the basis of this suggestion, it is generally recommended that kimchi should be consumed in 2 weeks after preparation, if we wish to enjoy the optimal state of kimchi.

The microbial results indicated a generally acceptable level of hygiene in the raw materials of kimchi employed in the previous report (Kim and Yoon, 2005). However, they observed that there was appearance ( $10^{1.78} \sim 10^{2.23}$  CFU/g) and disappearance of coliforms in kimchi during the 8 days' fermentation. Kim *et al.* (2004) also observed viable cells on the SS agar plate were  $10^4$  CFU/g at initial fermentation stage of kimchi, and they also observed the cell counts were gradually reduced, and not detected after 10 days. Therefore, it is suspected that the possibility of contamination of pathogenic microorganisms may be fairly high in the kimchi fermentation process, although coliforms and *E. coli* were not detected in the kimchi of this study during the long-term fermentation. It is inferred from previous studies (Cheigh *et al.*, 2002; Alakomi *et al.*, 2000) that the bacteriocins and/or organic acid, produced from various fermentative microbes, induce inactivation of pathogenic microorganisms during the fermentation of kimchi. However, it should be reminded that we eat kimchi not only after fermentation but also early in the preparation, before fermentation is complete. Therefore, use of fresh and safe raw materials for kimchi, hygienic practice and personal hygiene of during the preparation, and elimination of pathogenic microorganisms in the early stage of fermentation are absolutely required.

#### IV. Summary

In this study the changes of aerobic bacteria, psychrotrophic bacteria, coliforms, *Escherichia coli*, lactic acid bacteria, pH, and acidity of traditional Korean baechoo-kimchi were investigated during the fermentation at 10°C for 28 days. Aerobic bacteria and lactic acid bacteria of kimchi showed increasing tendency during the fermentation period.

The kimchi showed optimal state after 14 days' fermentation, and at this point the growth of lactic acid bacteria was almost highest level. Although coliforms and *E. coli* were not detected in the kimchi, we should not overlook the possibility of contamination from the raw materials of kimchi and environment. The data presented in this study should provide a useful background data for further studies of the microbial community, the selection of safe raw materials, and control of the fermentation process and long-term preservation of kimchi.

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