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Improvement in the Quality of Kimchi by Fermentation with *Leuconostoc mesenteroides* ATCC 8293 as Starter Culture

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To investigate the effect of the predominant microorganisms in kimchi on quality, *Leuconostoc mesenteroides* ATCC 8293 was used as starter culture during kimchi fermentation. A higher number of lactic acid bacteria and lower initial pH were observed in starter kimchi than in non-starter kimchi in the early stage of fermentation. The concentrations of the main metabolite, lactic acid, were 69.88 mM and 83.85 mM for the non-starter and starter fermented kimchi, respectively. The free sugar concentrations of starter kimchi decreased earlier than those of non-starter kimchi, and the levels of free sugars in both kimchi samples decreased during fermentation. At the end of fermentation, non-starter kimchi had a softer texture than starter kimchi, suggesting that *L. mesenteroide*s is useful in extending shelf life. Sensory evaluation showed that starter kimchi had higher sourness and lower bitterness and astringency values, resulting in high sensory quality. These results suggest that the *L. mesenteroides* ATCC 8293 strain could be a potential starter culture in kimchi.

Keywords: **Kimchi, *Leuconostoc mesenteroides*, starter, quality**

Introduction

Kimchi is a traditional fermented food in Korea, it is popular because of its nutritional and health effects including antioxidative, anticancer, immune-stimulatory, and cholesterol-lowering activities, therefore its market has increased worldwide [1, 2]. Kimchi is a lactic acid-fermented vegetable product, it is fermented by lactic acid bacteria (LAB) which plays an important role in maintaining the quality of kimchi product.

Among the LAB, *Leuconostoc* spp., *Lactobacillus* spp., and *Weissella* spp. play a key role in kimchi fermentation [3]. In kimchi fermentation, heterofermentative bacteria *Leuconostoc* spp. predominate in the early and middle stages of fermentation, while homofermentative *Lactobacillus* spp. are dominant in later stages [2]. In previous studies showed that heterofermentative LAB are considered to be better kimchi starter compared to the homofermentative LAB [4]. *Leuconostoc* spp. convert sugar into lactic acid, acetic acid, CO_2, and several aromatic compounds [5]. In this way, these compounds were contributed in taste, flavor, sensory, and texture of fermented foods. *Leuconostoc* spp. mostly affect the kimchi quality during the first 1–2 weeks of fermentation [6]. Many studies have been conducted to use *Leu. mesenteroides* or *Leu. citreum* as a starter during the early stage of fermentation to improve sensory properties in kimchi [4, 7]. As shown in previous study, *Leu. mesenteroides* is known to produce mannitol and keep cabbage in firm texture [8]. It also used as starter culture for kimchi fermentation to improve the quality [7]. Therefore, changes in the communities of these bacteria

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during fermentation are critical for kimchi quality and sensory properties [9, 10]. Several kinds of LAB have been developed for starter cultures for the dairy and meat industries [11]. However, few LAB cultures like *Leuconostoc* spp. and *Lactobacillus* spp. were used in industrial kimchi production. It is required to develop the effective LAB starter for kimchi. Starter culture in kimchi fermentation improve the sensory characteristics, prolong the shelf life, and improve some health and nutritional properties. In addition, microorganisms belonging to the genera of Leuconostoc are considered for probiotics [12]. Therefore, it is necessary to determine whether *Leuconostoc* spp. uses as starter have effect on kimchi quality, especially on texture and taste.

This study was aimed to determine the effects of heterofermentative LAB for controlling kimchi fermentation to improve quality. For this, we used *Leu. mesenteroides* ATCC 8293 as a starter during the early fermentation stage of kimchi fermentation to evaluate the effects on microbial content, pH, metabolites, texture, and sensory properties.

Materials and Methods

Bacterial strain and culture condition

Leu. mesenteroides ATCC 8293 was used as starter was cultured in MRS broth (Difco) at 30℃. After 24 h cultivation, the cells were harvested by centrifugation. The harvested cells were washed and resuspended with 0.85% saline to a concentration of 10^8 CFU/ml used as starter. NaCl, lactic acid, acetic acid, malic acid, citric acid, glucose, and fructose were purchased from Sigma Chemical Co. (USA).

Preparation and fermentation of kimchi

The Chinese cabbages (1200 g) were soaked in a salt solution for 12 h, until it became soft. Then, it mixed with various seasoning ingredients including radish (350 g), ginger (5 g), red pepper powder (80 g), garlic (25 g), and leek (70 g). Kimchi without starter addition was used as a non-starter sample, and starter kimchi was inoculated with 1% *Leu. mesenteroides* ATCC 8293 containing 10^8 CFU/ml of cells. Kimchi samples were fermented at 20℃ for first 24 h, and then fermented at 10℃ for almost 4 weeks. Samples were taken at intervals of 0, 3, 7, 14, 21, and 28 days.

Microbial and pH analysis

For viable cell counting of LAB during kimchi fermentation, each sample was diluted with 0.85% physiological saline and 20 µl samples were spread on MRS agar plates that incubated at 30℃ for 48 h. The growth of LAB in kimchi was expressed as log CFU/ml. The pH of kimchi fermentation was measured with a pH meter (IQ 240, I.Q. Scientific Inc., USA).

Organic acids and free sugars analysis

In order to investigate the effect of the starter on kimchi fermentation, the kimchi metabolites were analyzed using an Agilent 1260 Infinity high-performance liquid chromatography (HPLC) (Agilent Technology, USA) system. The samples were centrifuged at 10,000 ×*g* and analyzed immediately after filtration using a 0.45 µm pore size filter. For organic acid analysis, HPLC system was equipped with TC-C18 column (250 × 4.6 mm). 0.1% phosphoric acid and methanol (97.5:2.5 v/v) were used as the mobile phase. The flow rate of the mobile phase was 1 ml/min and it was monitored at an absorbance of 215 nm using a UV spectrophotometric detector. In case of sugar analysis, ZORBAX carbohydrate column (4.6 mm × 250 mm) and refractive index (RI) detector was used in the same HPLC analysis system. Acetonitrile and water (70:30 v/v) were used as the mobile phase and the flow rate was fixed as 1 ml/min.

Textural hardness analysis

Hardness is an important factor of kimchi that affects processing, handling, shelf-life, and consumer acceptance. The hardness index of fermented kimchi (0, 14, 28 days samples) was determined using a TA-XT Plus Texture analyzer (Stable Microsystems, UK). Cut kimchi into 3 × 3 cm pieces with around 4 mm thickness. Each piece of kimchi was subjected to one bite using a P2-5mm cylinder probe. The measurement conditions were at distance 10 mm, the test speed was 1 mm/s.

Sensory analysis

To evaluate kimchi quality based on flavor assessment and recognition by using electronic tongue system (TS-5000Z) which employs the same mechanism as that of the human tongue. TS-5000Z was equipped with 6 lipid membrane sensors indicating different taste qualities and 3 corresponding reference electrodes (TA.XTplus,

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Stable Micro System Ltd., UK). There are bitterness sensor (SB2C00), gustatory stimuli umami sensor (SB2AAE), saltiness sensor (SB2CT0), sourness sensor (SB2CA0), astringency sensor (SB2AE1), and sweetness sensor (SB2GL1). The test sensors can evaluate two types of taste, namely initial taste, which is the taste perceived when food first enters the mouth (sourness, saltiness, acidic bitterness, umami, astringency, and sweetness) and aftertaste, which is the persistent taste that remains in the mouth after the food has been swallowed. Two washing solutions for negatively and positively charged sensors were 100 mM HCl in 30% distilled ethanol solution, 100 mM KCl and 10 mM KOH in 30% distilled ethanol solution, respectively. The measurement procedure was repeated 4 times for each sample, and the mean values of the last 3 cycles were used for statistical analysis.

Statistical analysis

Each experiment results were expressed as mean ± standard deviation (SD) after triplicate analysis measurements for each starter and non-starter samples.

Results and Discussion

Total viable LAB and pH changes during fermentation

The total viable LAB cells were monitored during the kimchi fermentation for 28 days as shown in Fig. 1. The initial counts were 6.7 log CFU/ml for non-starter kimchi and 7.4 log CFU/ml for starter kimchi. The higher initial number of LAB in starter kimchi was due to the inoculation of *Leu. mesenteroides* starter culture. In non-starter kimchi, the total viable LAB cells increased from 6.7 log CFU/ml to 10.0 log CFU/ml. In case of kimchi with starter, the total viable LAB increased markedly within 3 days, reaching a maximum of 10.5 log CFU/ml. Then, it decreased slowly to 8.4 log CFU/ml until 28 days of fermentation. Total LAB cell number of starter kimchi were slightly higher than those of non-starter kimchi after 14 days of fermentation.

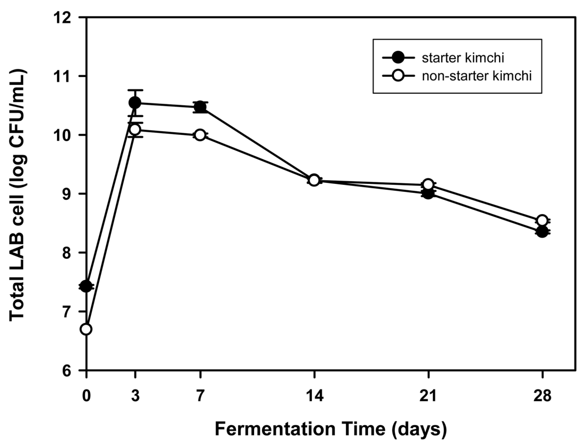

Fig. 1. Changes in viable LAB cell number of kimchi between non-starter and starter kimchi.

Table 1. Changes in pH values of non-starter and starter kimchi during fermentation.

Fermentation time (days)	Non-starter	Starter
0	6.03 ± 0.01	5.75 ± 0.05
3	4.83 ± 0.04	4.43 ± 0.02
7	4.38 ± 0.02	4.41 ± 0.01
14	3.83 ± 0.01	3.84 ± 0.01
21	3.63 ± 0.01	3.63 ± 0.05
28	3.60 ± 0.00	3.59 ± 0.03

Values are means ± SD from triplicate determination

During the early stage of fermentation (3 days), pH values of non-starter kimchi and starter kimchi decreased to 4.83 and 4.43, respectively. After 7 days of incubation period, the pH values became similar between non-starter and starter kimchi. After 28 days fermentation, pH values in both kimchi reached approximately 3.6 (Table 1). As results, the pH of starter kimchi decreased faster than non-starter kimchi within 3 days. These results showed that *Leu. mesenteroides* had effect on inducing the fermentation rate in the early stage. However, based on the time which reach the lowest optimal pH (4.2) of fermented kimchi, the starter-kimchi have no effect on extend the shelf-life [13].

Organic acids changes during fermentation

Changes in the organic acid profiles of the non-starter and starter kimchi fermentation are shown in Fig. 2. A total of four organic acids including lactic acid, malic acid, acetic acid, and citric acid were identified. Lactic acid was detected as the major organic acid in kimchi and exhibits the highest content during the fermentation. The initial concentrations of lactic acid of non-starter and starter kimchi were 13.63 mM and 11.34 mM, respectively. Lactic acid concentrations for both samples are gradually increased during the fermentation. At the

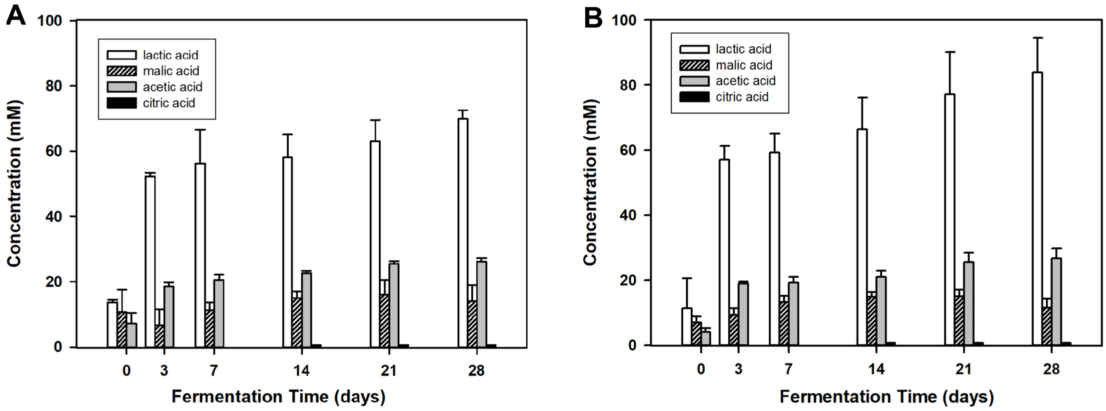

Fig. 2. Organic acid content in non-starter kimchi (A) and starter kimchi (B).

final days of fermentation (28 days), lactic acid concentration of starter kimchi (83.85 mM) was higher than that of non-starter kimchi (69.88 mM). During the fermentation process, more amount of lactic acid was detected in starter kimchi due to it mainly produced by *Leu. mesenteroides*. Except for lactic acid, both non-starter and starter samples showed almost similar results with no significant difference of malic acid, citric acid, and acetic acid. The lactic acid concentration of non-starter culture slowly increased, it is similar to the result found in previous study which was used *Lactobacillus plantarum* as a starter culture [14]. The composition and levels of organic acids produced from kimchi fermentation mainly depend on the microorganism composition and fermentation condition [15]. Particularly, the production of lactic acid depends mainly on the sugar metabolism of LAB [16].

Free sugars changes during fermentation

The concentration changes of two major free sugars (glucose and fructose) were measured (Table 2). Glucose and fructose play an important role as a carbon sources for LAB growth during fermentation, and produce the unique flavor and aroma components. Therefore, it is important to realize the changes of free sugar and estimate the microbial growth, flavor, and taste. At the beginning of fermentation, the concentrations of glucose in non-starter and starter kimchi were 225.31 mM and 215.76 mM, respectively. Then, it decreased to 210.09 mM (non-starter kimchi) and 183.44 mM (starter kimchi) at 3 days. The levels of glucose in both samples decreased during fermentation, which was in accordance with previous studies [7, 14]. The decrease of glucose was caused by the consumption of sugar by the growth of LAB in kimchi. The utilization rate of glucose in starter kimchi is higher than non-starter kimchi. The content of fructose increased at the beginning of fermentation and decreased gradually after 7 days. Generally, free sugar concentrations of starter kimchi decreased earlier than non-starter kimchi, and the levels of free sugars in both

Table 2. The concentration of free sugars during kimchi fermentation.

Fermentation time (days)	Non-starter		Starter	
	Glucose (mM)	Fructose (mM)	Glucose (mM)	Fructose (mM)
0	225.31 ± 13.34	3.63 ± 0.74	215.76 ± 9.25	3.16 ± 0.28
3	210.09 ± 6.52	4.50 ± 1.15	183.44 ± 12.06	3.12 ± 0.44
7	210.54 ± 5.69	8.97 ± 1.02	191.69 ± 11.44	6.98 ± 0.83
14	199.01 ± 6.16	4.84 ± 0.31	181.81 ± 13.22	3.72 ± 0.38
21	165.22 ± 1.99	4.22 ± 0.13	150.60 ± 19.92	2.83 ± 0.35
28	157.93 ± 3.38	3.07 ± 0.30	136.31 ± 6.27	2.08 ± 0.05

Values are means ± SD from triplicate determination.

kimchi samples decreased during fermentation.

Texture changes during fermentation

Texture changes were measured in the starter and non-starter kimchi stem samples in 0, 14, and 28 days. Hardness values of kimchi decreased during storage. In non-starter kimchi, hardness values decreased from 25.90 N to 15.84 N within 14 days, then it sharply decreased to 3.05 N in two weeks later. In case of starter kimchi, it had sharply decrease in hardness from 25.90 N to 7.72 N within 14 days. Nevertheless, it exclusively decreased to 6.27 N in two weeks later. Although the hardness of starter kimchi decreased faster than non-starter kimchi in first two weeks, it will maintain in the forwarding two weeks. At the end of fermentation, non-starter kimchi had softer texture compared to the starter kimchi. It proposed that *Leu. mesenteroides* ATCC 8293 starter culture could retard the softening of kimchi. At later stage of kimchi fermentation, yeasts and mold were grown on the surface of kimchi. The soft texture of kimchi is mostly associated with the changed solubility of the cell wall pectin [11]. Several researchers have attempted to use LAB as starter to control the growth of yeast and mold to prolong the shelf-life of kimchi. As results, *Leu. mesenteroides* ATCC 8293 starter is useful for extend the shelf life of fermentation foods. It is related to the antimicrobial and antifungal effects of LAB. The result was in agreement with many other reports that other species of LAB used in various fermented foods. In previous study, non-starter kimchi displayed more softer than sample kimchi which was inoculated *Lactobacillus sakei* as a starter [17].

Sensory evaluation

Electronic tongue sensor system (TS-5000Z) as an advance taste sensor equipment, employs the same mechanism as human tongue, converts the taste of various substances into numerical data [18]. As shown in Fig. 3, there were no differences of umami and richness values between non-starter and starter kimchi. The sourness value of starter kimchi was higher than non-starter kimchi. As shown in previous study, some metabolites such as free sugars, glycerol, and lactic acid play an important role in the taste of kimchi [19]. Sour taste was mainly influenced by the content of lactic acid which was produced by LAB. As a starter sample which contains abundant number of *Leu. mesenteroides* microbial strain, it will be converts more free sugar into organic acids. It confirmed that adding *Leu. mesenteroides* as starter culture mostly affect sour taste of kimchi. The result was in agreement with previous study which using *Leu. citreum* as starter in kimchi [20]. In addition, the bitterness and astringency values of non-starter were slightly higher than starter kimchi ($p < 0.001$), it will be more acceptable by consumer. The electronic tongue sensor system converts the taste of various sub-

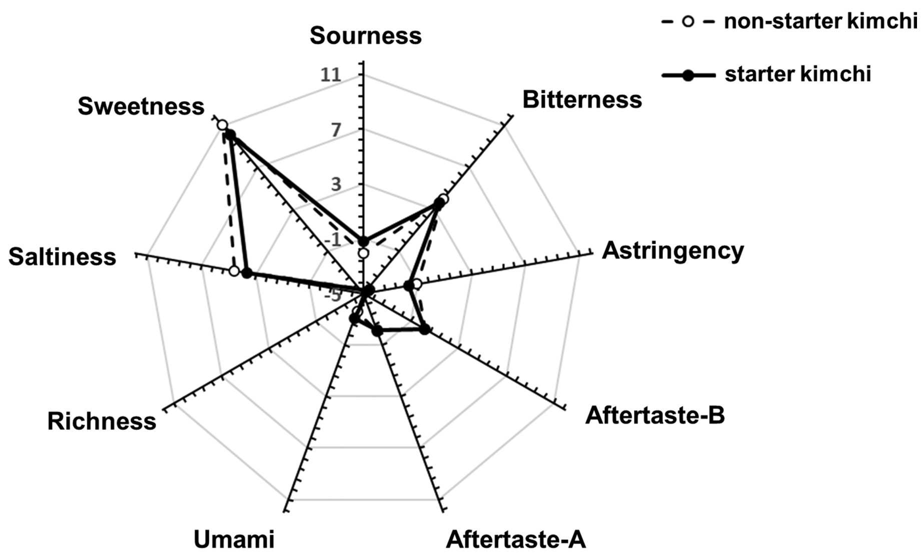

Fig. 3. Radar chart of sensory evaluation of non-starter and starter kimchi at 28 days.

stance into numerical data, it useful to evaluate the quality of kimchi. Further studies are needed to determine the correlation between sensory characteristics and metabolites produced by *Leu. mesenteroides* ATCC 8293.

In conclusion, the application of *Leu. mesenteroides* ATCC 8293 as a starter exerts higher number of LAB, higher lactic acid concentration, higher sourness value, and lower bitterness and astringency values. Furthermore, *Leu. mesenteroides* ATCC 8293 can be positively recommended as a probiotic starter culture for shelf-life extension and sensory quality improvement in kimchi fermentation.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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