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The Relationship between Physiological Activity and Cell Number in Dolsan Leaf Mustard *Kimchi* (*Brassica juncea*)

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Abstract

Changes in antioxidative activity and Angiotensin converting enzyme (ACE) inhibitory activity in juice prepared from Dolsan leaf mustard *kimchi* (DLMK) at various fermentation temperatures were investigated. Antioxidative activity of juice from optimally ripened DLMK at 20 and 30°C showed 80 and 83%, respectively. Juice from 10-day fermented DLMK at 30°C showed 62% inhibitory activity against the ACE. In the juice fermented DLMK at 20°C ~ 30°C, physiological activity was higher than that of the 4~10°C. In particular, optimally ripened DLMK at 30°C showed the highest physiological activity. The physiological activity in DLMK juice at the fermentation period increased significantly with an increase in the growth of microbes. Consequently, a maximum physiological activity was shown at the maximum cell number. These results suggest that the microorganisms in DLMK juice would play an important role in the physiological activity.

Key words: Dolsan leaf mustard *kimchi* (DLMK), antioxidative activity, angiotensin converting enzyme (ACE) inhibitory activity

INTRODUCTION

Recently, much attention has been focused on health and life extension world-wide. Synthetic antioxidants or anticancer reagents have been widely used as food appendices and medical cures, but they are suspected of being toxic and carcinogenic (1). Hence, the physiological activity of natural plants and the development of functional food have been studied extensively. Especially, studies on physiological activity of fermented food have been the most prolific.

Kimchi is a traditional fermented vegetable food in Korea. *Kimchi* is classified into many different categories depending on the raw ingredients, processing methods, season and locality. Leaf mustard *kimchi* (LMK) is made of leaf mustard (*Brassica juncea*). A major ingredient such as leaf mustard is salted, blended with various spices and other minor ingredients, and then fermented. The leaf mustard consists of high amounts of ascorbic acid, β-carotene, chlorophylls, dietary fiber and flavonoids which are known to be physiologically active (2,3). As fermentation goes, *kimchi* contains high levels of lactic acid bacteria, various organic acids, vitamin C, and so forth. In addition, it could help digestion, prevent constipation and control intestinal microflora, and it has health benefit functions (4) as well. The fermentation of *kimchi* strongly depends on the fermentation temperature (5). According to the fermentation temperature of LMK, different microbial flora and chemical changes were present. Recently, it has been reported that extracts from LMK possess physiological activity (5~7). However, studies on the relationship between the fermentation pattern and physiological activity of Dolsan leaf mustard *kimchi* (DLMK) at various fermentation temperatures were not extensively studied. In this study, DLMK juice at various fermentation temperatures was investigated to determine any changes in physiological activity. The physiological activity of DLMK was examined for both antioxidative and Angiotensin converting enzyme (ACE) inhibitory activities. ACE inhibitor results in the decrease of blood pressure. The antioxidative activity was evaluated by α, α'-diphenyl-β- picrylhydrazyl (DPPH) (8), and the ACE inhibitory activity was measured by using ACE (peptidyldipeptide hydrolase, EC 3.4.15.1).

MATERIALS AND METHODS

Preparation and fermentation of DLMK

The leaf mustard was obtained from Dolsan, Jeollanamdo, Korea. The other ingredients such as garlic, ginger, red pepper powder and green onion were purchased from a local market in Yeosu, Korea. The leaf mustard was cut into 2 to 3 cm sizes, salted with 10% brine for 3 hr, and washed twice with distilled water. After draining, other minor ingredients were added and mixed. The ratio of minor ingredients for DLMK was 1.5 of garlic, 0.5 of ginger, 1.5 of red pepper powder and 1.5 of green onion to 100 salted leaf mustard (9). The final salt concentration in the DLMK was adjusted to 2%. The DLMK was fermented for 50 days at 4, 10, 20 and 30°C.

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Sample preparation

One hundred grams of DLMK was crushed by a crusher (Hanil HMF-340, Korea) and filtered through sterilized gauze. The filtrate was centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through a Whatman No. 4 paper and then stored at -20°C.

Measurement of pH and acidity

The blended DLMK (20 g) was added to 180 mL of distilled water. The pH of the filtrate was measured with a pH meter (Orion 520A, Boston, USA). Acidity was determined by titration with 0.1 N of NaOH solution until pH of the juice reached 8.3, and expressed as the total acidity (%).

Total acidity (%) = [(mL of 0.1 N NaOH × 0.009)/weight of sample (g)] × 100

Enumeration of total cell number

The blended DLMK was diluted with 0.85% (w/v) of the sterile saline solution and then inoculated on a TGY (Trypton glucose yeast extracts) solid medium (Difco, USA) (10). The plate was duplicated and incubated at 30°C for 48 hr. The total cell numbers were counted as colony forming units (CFU/mL).

Antioxidative activity

Antioxidative activity in DLMK juice was determined by using the DPPH (α, α'-diphenyl-β-picrylhydrazyl) method of Blois (8). Five mL of the DPPH solution was added to 1 mL of DLMK juice and this solution was left to stand for 30 min. The absorbance of the resulting solution was measured at 528 nm with a spectrophotometer (Shimadzu UV-120A, Kyoto, Japan). Each sample was run four times and the average was calculated as % antioxidative activity.

Assay for ACE inhibitory activity

The angiotensin converting enzyme (ACE, peptidyldipeptide hydrolase, EC 3.4.15.1) inhibitory activity was assayed by the method described by Cushman and Cheung (11) with slight modification. The ACE and substrate for ACE : hippuryl-L-histidyl-L-leucine (Hip-His-Leu), were obtained from Sigma chemical Co.. The Hip-His-Leu was dissolved in 0.1 M of sodium borate buffer (pH 8.3). Then, 100 μL of 25 mM Hip-His-Leu solution was mixed with 50 μL DLMK juice and then preincubated for 10 min at 37°C. The reaction was initiated by adding 150 μL of ACE that was dissolved in sodium borate buffer (pH 8.3), and the mixture was incubated for 60 min at 37°C. The reaction was stopped by adding 250 μL of 1 N HCl. The hippuric acid was extracted with 1.5 mL of ethyl acetate. The extracts were centrifuged at 2,500 rpm for 10 min. The supernatant was dried and dissolved in 3 mL of 1 M NaCl. The absorbance level at 228 nm was measured to evaluate the degree of inhibition in the ACE activity. The extent of inhibitory ratio was calculated as follows : inhibitory ratio (%) = [(C-S)/(C-S')] × 100. where S is the absorbance of the sample, S' is the absorbance of control sample and C is the absorbance without sample.

RESULTS AND DISCUSSION

Changes of pH, acidity and total number of microbes

The pH or acidity has been proposed as a quality index to determine the degree of fermentation in DLMK. Changes in pH and acidity in DLMK during fermentation at 4, 10, 20 and 30°C are shown in Fig. 1 and Fig. 2. At 30°C, pH rapidly dropped as acidity increased. The optimum ripening pH (4.2 ~4.5) and acidity (0.6~0.8%) were reached within 10 days at 10°C, 6 days at 20°C and 2 days at 30°C in fermented DLMK. Decrease of pH and increase of acidity were in accordance with an increase of fermentation temperature. And it showed that fermentation temperature could be an important factor in DLMK storage. Many researchers have reported that

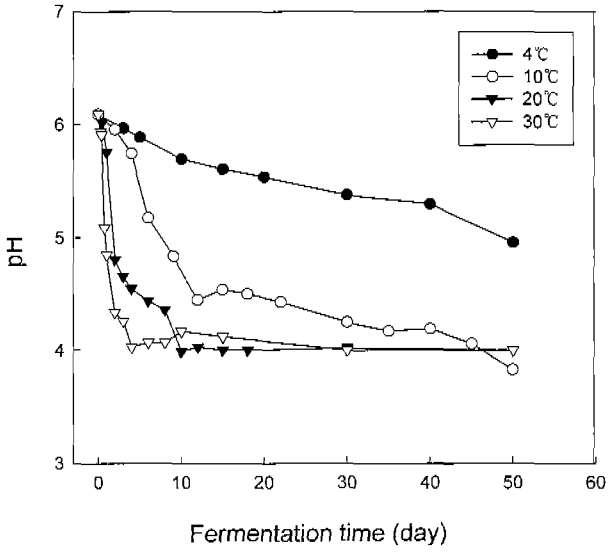

Fig. 1. Changes of pH in DLMK at various fermentation temperature.

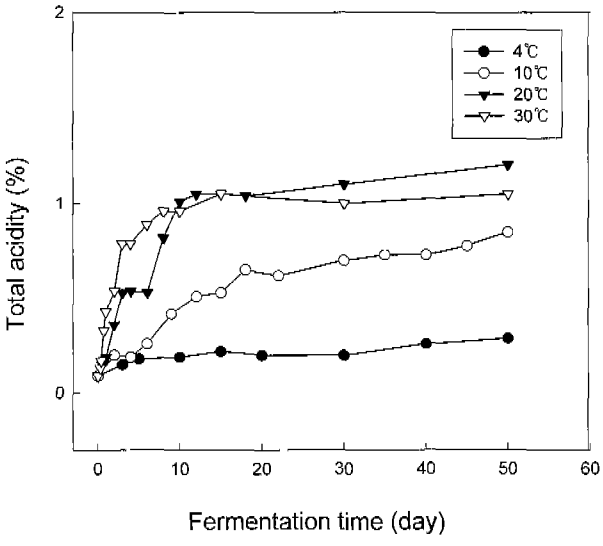

Fig. 2. Changes of total acidity in DLMK at various fermentation temperature.

Chinese cabbage *kimchi* would ripen optimally at a pH of 4.2~4.5 and acidity of 0.6~0.8 (1,2) and it could be eaten after 4 days at fermentation temperature of 17°C (12). The fermentation rate of chinese cabbage *kimchi* was more rapid than that of the LMK. The reason for this is probably due to some antimicrobial activity by allylisothiocyanate in the leaf mustard (13).

Fermentation of kimchi occurred mainly by microorganism. Therefore, we examined the changes of microbes in DLMK at various fermentation temperature. Changes of the total number of microbes in DLMK are given in Fig. 3. Overall, the number of total microbes were increased until reaching the optimum ripening period and after that, the numbers were slowly decreased. In comparison with the fermentation temperature, DLMK of high temperature (20~30°C) showed a higher number of total microbes (10^8 CFU/mL) than that of the low temperature (4~10°C). In case of 4°C fermentation, the DLMK was maintained in a level of 10^6 CFU/mL. Many researchers have reported that lactic acid bacteria would play a crucial role in improving taste and preserving food, advancing digestion by particle disjointing of protein, controlling intestinal microflora, absorbing promotion of calcium, declining operation for cholesterol in the blood stream and most importantly it has an effect of possibly preventing cancer (14,15). According to these effects, microorganism in DLMK may affect various physiological activities.

Physiological activity in DLMK juice

The antioxidative activity in DLMK juice measured by DPPH was shown in Fig. 4. The activity increased in the following order : 4°C < 10°C < 20°C < 30°C in fermented DL MK and optimally ripened DLMK showed much stronger activities than others. In particular, juice 5-day fermented DLMK at 30°C showed 83% of antioxidative activity. of ac Song et al. (7) along with Kim (5) reported that LMK had

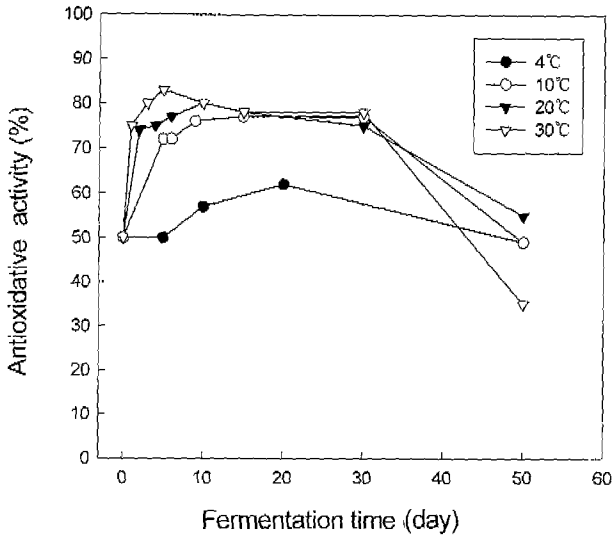

Fig. 4. Changes of antioxidative activity in DLMK at various fermentation temperature.

an antioxidative activity, and that the optimally ripened LMK had a higher activity when compared to fresh LMK.

Fig. 5 shows the ACE inhibitory activity (% ratio) of DLMK at various fermentation temperature. Angiotensin converting enzyme (ACE, peptidyldipeptide hydrolase, EC 3.4. 15.1) converts angiotensin I into angiotensin II by cleaving the C-terminal dipeptide (His-Leu) of angiotensin I. And it also inactivates bradykinin which lowers blood pressure. The ACE inhibitory activity in fermented DLMK for 10 days at 30°C was the highest activity (62%) than others (4, 10 and 20°C). The maximum rate of ACE inhibitory activity in fermented DLMK at 4, 10 and 20°C were shown as 28%, 46% and 60%, respectively. Patterns from Fig. 4 and Fig. 5 were similar together. According to the progress of fermentation, the physiological activity gradually increased. The maximum

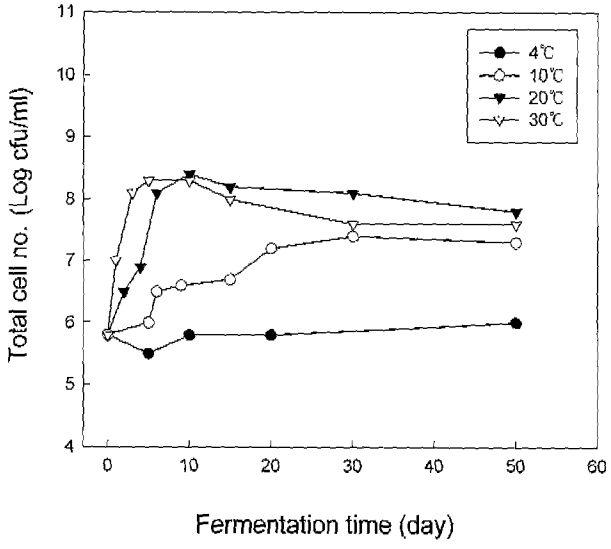

Fig. 3. Changes of total cell number of microbes in DLMK at various fermentation temperature.

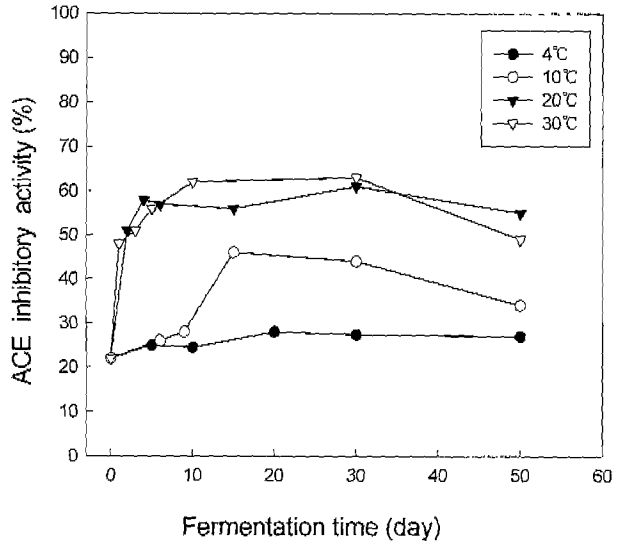

Fig. 5. Changes of ACE inhibitory activity in DLMK at various fermentation temperature.

ratio of activity appeared at an optimum ripened period (pH 4.2~4.5, acidity 0.6~0.8%). In fact, physiological activity varied with the fermentation progress. Hence, this fact also indicated that physiological activity related substance may be varied with the fermentation progress, and this variation could directly affect the physiological activity. In this study, the physiological activity in over ripened DLMK was higher than that of the fresh DLMK. We made an assumption that physiological activity related substance of the optimally ripened DLMK might be maintained until an over ripened DLMK was attained. And this pattern of physiological activity was similar to changes of microbes number in DLMK. Probably, this substance of physiological activity may be produced by microorganisms in the DLMK fermentation progress.

The relationship of physiological activity and cell number

We observed that the relationship of cell number, antioxidative activity and ACE inhibitory activity. Fig. 6 shows the relation between antioxidative activity and cell number in DLMK at various fermentation temperature. The relation between ACE inhibitory activity and cell number of DLMK is given in Fig. 7. Fig. 6 and 7 show excellent correlation existing between cell number and physiological activity in fermentation time indicated the maximum physiological activity. Our results showed that the DLMK at high fermentation temperature had a more stronger physiological activity than that of low fermentation temperature. In addition, the DLMK at high fermentation temperature showed an increased physiological activity and microorganisms in a short period. In contrast, DLMK that was fermented at high temperature (30 °C) possessed poor texture and a bad odor. Nevertheless, it seemed that microorganisms in a fermenting DLMK might produce any substance which had some physiological activity.

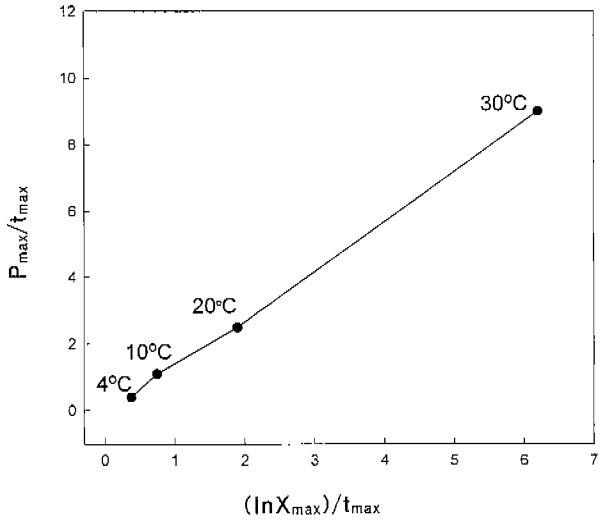

Fig. 6. The relationship between antioxidative activity and cell number at various fermentation temperature. t_{max} : time reached to maximal antioxidative activity, P_{max} : maximal antioxidative activity at various temperature, X_{max} : cell number at maximal antioxidative activity.

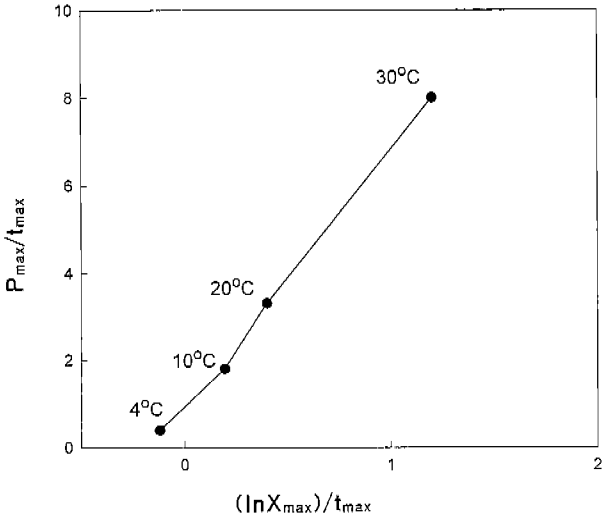

Fig. 7. The relationship between ACE inhibitory activity and cell number at various fermentation temperature. t_{max} : time reached to maximal ACE inhibitory activity, P_{max} : maximal ACE inhibitory activity at various temperature, X_{max} : cell number at maximal ACE inhibitory activity.

Consequently, cell number was significantly related to the physiological activity of DLMK. Thereby, in order to obtain high quality and physiologically active DLMK, it could be beneficial to develope of fermentation method for obtaining a high number of cell at low temperature.

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