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Effect of Low Temperature on the Qualities of Long-term Fermented Kimchi (Korean Pickled Cabbage)

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저온이 묵은지의 발효에 미치는 영향

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Abstract

Recently, many Koreans have started to consume aged kimchi fermented long-term at low temperatures. In the present study, the effect of low temperature (5℃) on pH, titratable acidity (TA), organic acid level, viable microbial cell count, amino acid concentration, and polygalacturonase activity (PG) during long-term fermentation (46 weeks) of kimchi, were evaluated. After 10 weeks of fermentation, kimchi had a pH of 4.1 and a TA of 1.0%, respectively after 46 weeks fermentation, these values were 3.9 and 1.3%, respectively. Lactic acid, the ratio of lactic acid to acetic acid, and the ratio of *Lactobacillus* species/*Leuconostoc* species in kimchi increased as fermentation progressed from 10 weeks to 46 weeks. However, total viable cell counts of aerobic bacteria, yeasts, *Lactobacillus* species, and *Leuconostoc* species, free amino acid levels, and PG decreased as the fermentation period was extended from 10 weeks to 46 weeks.

Key words : kimchi, temperature, long-term fermentation, *Leuconostoc mesenteroides, Lactobacillus plantarum*

Introduction

Kimchi is a traditional Korean fermented vegetables and has been developed to give vegetables an extended storage life (1-2). Kimchi is produced from the normal mixed biota of Chinese cabbage and other vegetables (3). Lactic acid bacteria (LAB) are the most typical microorganisms found in kimchi and therefore are one of the most extensively studied groups of microorganisms, of which *Leuconostoc mesenteroides* and *Lactobacillus plantarum* species are most common (1-3). *L. mesenteroides*, a dominant microbe at the early stage of kimchi fermentation, is a heterofermenter producing acetic acid, carbon dioxide, and lactic acid as the major products from fermentation; this species is most important for the kimchi fermentation. Homofermentative *L. plantarum* predominates at the later stage of kimchi fermentation, making overly acidic kimchi, which is unacceptable by consumers. Although *L. plantarum* is the species considered as probiotics that have a considerable safety record and have been widely exploited by the manufacturers in the milk industry, this bacterium is not welcome in kimchi fermentation (4-5).

Several trials for extending shelf-life of kimchi have been reported. The approach normally taken has involved using antimicrobial agents (6), ark shell powder (7), heat treatments (8), and a combination of N_2-packaging and heating with irradiation (9). Although these methods showed some positive effect in extending the shelf life of kimchi, chilled storage between 1 to 5℃ is generally acceptable to maintain the quality of kimchi (10). Based on this, special refrigerators,

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which are referred to as "kimchi-specialized refrigerators" have become typical equipment for fermentation and storage of kimchi. Although the use of kimchi refrigerators make possible for long-term storage of kimchi at low temperature, little information for aged-kimchi is available. The objective of this study was to investigate the effect of long-term fermentation at low temperature on qualities of aged-kimchi.

Materials and Methods

Kimchi preparation

For the preparation of kimchi, the following materials were used: Chinese cabbage 85.7%, hot pepper 5.6%, minced garlic 2.6%, salted shrimps 1.7%, green onion 1.7%, salted anchovies 1.3%, and minced ginger 1.3%. Whole Chinese cabbage was trimmed and divided into quarters and then brined for 2 hr in 20% NaCl solution (w/v). After removing some residual salt with tap water for 1 min, the Chinese cabbage was drained at room temperature for 2 hr. The sliced hot pepper, minced garlic, salted shrimps, green onion, salted anchovies, and minced ginger were mixed together in a metal bowl. About 300 g of kimchi was packed in plastic jars without headspace, capped and stored for up to 46 weeks at 5℃.

Chemical analysis

The cabbage tissue and fermented kimchi juice were homogenized together using a mixer, and filtered through sterile gauze. The pH of the supernatant was measured using a pH meter (lstek Co., Seoul, Korea). For the determination of the titratable acidity (TA), 5 mL of the filtrate was titrated with 0.1 N NaOH and expressed as the quantity of lactic acid produced in kimchi. For determination of lactic and acetic acid, the filtrate was passed through Sulpeco C18 SEP-PAK filter catridges (Sulpeco Co., Bellafonte, PA, U.S.A.) to remove residual proteins, liquids and chromophores. After prewetting and sample addition, 20 μL of the filtrate eluted from the SEP-PAK catridges was injected directly into the HPLC. HPLC (Agilent Technologies Inc., Santa Clara, Calif., U.S.A.) was equipped with a Supelcogel C-610H (7.8 mm i.d. 30 cm) column (Sulpeco Co., Bellafonte, PA, U.S.A.). The mobile phase at a flow rate of 0.5 mL/min was composed of 0.1% phosphoric acid. The elutent was monitored with UV/VIS detector at 210 nm. To calculate the L(+)-lactic acid in kimchi, the lactate oxidase was used according to the suppliers instruction (Procedure No. 735) (Sigma, St. Louis. MO. U.S.A.). The lactate oxidase used in this procedure is specific for L(+)lactic acid. D(-)lactic acid is nonreactant. L(+)lactic acid is converted to pyruvate and hydrogen oxidase (H_2O_2) by lactate oxidase. In the presence of the H_2O_2 formed, peroxidase catalyzes the oxidase condensation of chromogen precursors to produce a colored dye with an absorption maximum at 540 nm (11). The increase in absorbance at 540 nm is directly proportional to lactate concentration in the sample. Amino acids concentration in raw ingredients, including Chinese cabbage, hot pepper, minced garlic, salted shrimps, green onion, salted anchovies, and minced ginger, was determined according to the published information (12). The amount of free amino acids in the kimchi was determined using an automatic amino acid analyzer (L-8800, Hitachi, Japan). This analysis was carried out in a ninhydrin flow rate of 0.33 mL/min at a column temperature of 50℃ and a 20 μL injection volume.

Microbial enumeration

After kimchi fermentation, 10-fold serial dilutions of cells were made and appropriate dilutions spread onto plates to enumerate viable cells. Plate count agar (Difco, St. Louis, MO., U.S.A.) was used for aerobic bacterial count. For enumeration of yeast, YPD (Difco) was used. For enumeration of *L. plantarum*, Rogosa SL agar (Difco) was used. For *L. mesenteroides*, phenylethyl alcohol agar (Difco) supplemented with 1% (w/v) sucrose was used. Cell numbers were counted in triplicate after incubation at 30℃ for 24~48 hr. Results of viable cell counts were presented as the average values of colony-forming units (CFU) per g of kimchi.

Measurement of polygalacturonase activity

The kimchi (300 g) was homogenized using a mixer and filtered through sterile gauze. These filterates were used as the source of polygalacturonase (PG, EC 3.2.1.15) activity. PG activity was determined by measurement of reducing sugar from polygalacturonic acid (from orange, Sigma, St. Louis. MO. U.S.A.) as described by Park *et al.* (13) at a wavelength of 520 nm in a UV-visible spectrophotometer (UV-1601, Shimadzu, Japan). One unit of PG activity is defined as the amount of enzyme required to release 1 mg of reducing D-galacturonic acid from polygalacturonic acid per 2 hr at pH 5.0 at 30℃.

Statistical analysis

Data were analyzed using one way analysis of variance at 95% level of significance (14). All experiments were replicated twice and all analyses were carried out in triplicates. The results presented are a mean of 6 observations±standard deviations (SD).

Results and Discussion

Changes in pH, titratable acidity (TA), and organic acid content of kimchi

The long-term fermentation of kimchi was investigated. Rather than designing experiments to cover a full possible fermentation periods, fixed term of fermentation periods (at 10 weeks and at 46 weeks) were chosen selectively. Since the kimchi consumers usually take kimchi aged between 2~4 weeks in fermentation, 10 weeks aged-kimchi was chosen for comparison. A 46 weeks aged-kimchi was also included for study since the kimchi consumers take aged-kimchi stored for 6~12 months. Table 1 shows the changes in the pH of kimchi during fermentation for 46 weeks. As fermentation proceeded, pH of the kimchi changed from 4.13 at 10 weeks to 3.85 at 46 weeks ($p<0.001$), while TA was increased from 0.99% at 10 weeks to 1.29% at 46 weeks, respectively ($P<0.001$) (Table 1). Lactic acid contents in kimchi were 0.69% at 10 weeks and 1.25% at 46 weeks. L(+) lactic acid content in kimchi were 0.20% at 10 weeks and 0.37% at 46 weeks. *Leuconostoc* species is a major lactic acid bacterium that is responsible for the production of D(-) lactic acid in fermented foods, whilst *Lactobacillus* species produce D(-) lactic and L(+) lactic acid (15). The concentrations of acetic acid were 0.28% (w/v) at 10 weeks and 0.30% (w/v) at 46 weeks (Table 2). The ratios of lactic acid to acetic acid in the kimchi were 2.48 at 10 weeks and 4.14 at 46 weeks.

Table 1. Changes in pH and titratable acidity (TA) of kimchi during fermentation at 5℃ for 46 weeks

Atributes	Fermentation period	
	10 weeks	46 weeks
pH	4.13±0.11[1)]	3.85±0.06[**]
TA(%)	0.99±0.02	1.29±0.06[**]

1)Values are Mean ± S.D., n=6.
Means are significantly different with the week 10 kimchi based on the one way analysis of variance (ANOVA).
**p<0.001.

With kimchi refrigerators, long-term storage of kimchi is possible. The effect of long-term fermentation at low temperature on qualities of aged-kimchi was investigated. kimchi gaves its best flavour and texture at pH between 4.1 to 4.5 (16). Jung and Jeon (10) suggested that the pH decrease in kimchi was affected by fermentation temperatures and optimum pH should be 4.2~4.5 at 2℃ and 4.10~4.51 at 5℃. The pH range of kimchi were 3.7 (at 52 weeks) ~ 4.2 (at 10 weeks) when fermented at -1℃, 4.1 (at 24 weeks) ~ 4.3 (at 7 weeks) at 0~2℃, and 3.5 (at 9 weeks) ~ 4.1 (at 24 weeks) at 4~5℃(10, 17-18). According to the above suggestion, pH of kimchi at 10 weeks was within this range, but the pH of kimchi at 46 weeks was too acidic (Table 1). The pH drop found in kimchi during extended kimchi fermentation for 46 weeks could be explained by the continuous growth of microbes. TA and pH are considered as the major quality attributes of kimchi because it has the characteristic sour and taste (19). Ku *et al.* (16) reported that commercially available kimchi showed a TA ranging from 0.28% to 1.0%. It takes more fermentation period to reach this acidity range when kimchi fermentation occurred at 1~5℃, compared to that at 20℃. The TA range of kimchi were 0.5% (at 10 weeks) ~ 1.38% (at 52 weeks) at -1℃, 0.9% (at 24 weeks) ~ 1.25% (at 24 weeks) at 0~2℃, and 0.6% (at 9 weeks) ~ 1.25% (at 60 days) at 4~5 ℃(10, 17-18). Jung and Jeon (10) reported that kimchi fermentation at 4~5℃ gave TA from 1.0% at 10 weeks to 1.4% at 24 weeks. Acid production has been used as useful index for evaluating kimchi fermentation. The acidic components in kimchi are lactic, acetic, succinic, malic, and citric acids. Among them, malic and citric acids are released from the vegetable. But other acids (acetic, lactic and succinic acids) may produce during kimchi fermentation (20). In the present study, lactic acid was the major organic acid during the kimchi fermentation (Table 2). The production rates of lactic acid and acetic acid have a close relationship with the distribution of LAB in kimchi. *L. mesenteroides* generates acetic acid and lactic acid, while *L. plantarum* produces lactic acid (4). It was reported that the ratio of lactic acid to acetic acid in the kimchi on the 14~21 day of fermentation ranged between 1.1~11.6(7, 21-22). According to Yoo *et al.* (18), the ratio of lactic acid

Table 2. Concentration(g/100 g) of acetic and lactic acids in kimchi during fermentation at 5℃ for 46 weeks

Production	Fermentation period	
	10 weeks	46 weeks
Lactic acid (LA)	0.69	1.25
L(+) lactic acid	0.20	0.37
Acetic acid (AA)	0.28	0.30
LA/AA[1)]	2.48	4.14

1)Ratio of lactic acid to acetic acid.

to acetic acid obtained from kimchi fermented at -1℃, were 2.50 at 10 weeks and 2.54 at 30 weeks. From data presented in Table 2, it could be concluded that growth of homofermentative LAB was less affected than that of heterofermentative LAB during long-term kimchi fermentation.

Enumeration of viable cell and polygalacturonase (PG) activity in kimchi

In order to determine the effect of long-term kimchi fermentation on microbes, enumeration of aerobic bacteria, yeast, *Leuconostoc* species and *Lactobacillus* species were carried out using selective media (Table 3). Total bacterial cells of kimchi decreased from 287 to 3 ($1x10^5$ CFU/g of kimchi) over a 36 weeks from 10 to 46 weeks ($p<0.001$). In the same period, yeast cells of kimchi decreased from 119 to 4 ($1x10^5$ CFU/g of kimchi) ($p<0.001$). *Lactobacillus* species in kimchi decreased from 417 to 48 ($1x10^5$ CFU/g of kimchi) over a 36 weeks from 10 to 46 weeks ($p<0.001$). In the same time, numbers of *Leuconostoc* species in the kimchi changed from 29 to 0.15 ($1x10^5$ CFU/g of kimchi) ($p<0.001$). Enumeration of cells was also expressed as percentage ratio of *Lactobacillus* species/*Leuconostoc* species to enable comparison between 10 weeks aged-kimchi and 46 weeks aged-kimchi (Table 3). The ratios of *Lactobacillus* species/*Leuconostoc* species were 14 at 10 weeks and 314 at 46 weeks. In case of PG activity, enzyme activity in the weeks 46 kimchi was far lower than that of weeks 10 kimchi ($p<0.001$) (Table 4).

Table 3. Changes in aerobic bacteria, yeast, and two lactic acid bacteria cells(CFU/g) in kimchi during fermentation at 5℃ for 46 weeks

Bacteria	Fermentation period	
	10 weeks	46 weeks
Aerobic bacteria	$2.87x10^7±1.32x10^7$[1)]	$3.07x10^5±1.90x10^{5**}$
Yeast	$1.19x10^7±8.34x10^6$	$4.07x10^5±2.02x10^{5**}$
Lactobacillus spp.	$4.17x10^7±1.42x10^7$	$4.81x10^6±8.20x10^{5**}$
Leuconostoc spp.	$2.92x10^6±1.22x10^6$	$1.53x10^4±7.60x10^{3**}$
Lactobacillus/*Leuconostoc*	14	314

1)Values are Mean ± S.D., n=6.
Means are significantly different with the week 10 kimchi based on the one way analysis of variance (ANOVA).
**p<0.001.

Result from enumeration of viable cell agrees with previous reports on the changes of microorganism in kimchi, in where extended kimchi fermentation caused decrease in cell viability (18). Low viability in kimchi during long-term fermentation may be due to the high acidity found in Table 1 and Table 2. *Leuconostoc* species was more sensitive to growth inhibition at long-term fermentation than *Lactobacillus* species, as reported by Mheen and Kwon (5) in where they found when the *L. mesenteroides* decreased during long-term kimchi fermentation, the population of *L. plantarum* became dominant. Lim *et al.* (23) found the effect of temperature on kimchi fermentation. They found that the *Lactobacillus* species/*Leuconostoc* species of kimchi were 0.51 at 5℃ and 4.35 at 15℃ and 25℃, respectively. Based on these observations, they suggest that a fermentation temperature at 5℃ gave better conditions for producing more flavorful kimchi. PG is the enzyme in decreasing the quality of kimchi by softening the tissue (21). By the same authors, it was reported that PG activity was constant throughout the fermentation period for 21 days at 10℃. In the current study, PG activity in the 46 weeks aged-kimchi was lower than that of the 10 weeks aged-kimchi ($p<0.001$). This may be due to the low cell viability seen in Table 3, in where viability of aerobic bacteric and yeast in the weeks 46 kimchi were far lower than those of weeks 10 kimchi.

Table 4. Changes in polygalacturonase (PG) activity(unit[1)]/g) of *kimchi* during fermentation at 5℃ for 46 weeks

	Fermentation period	
	10 weeks	46 weeks
Polygalacturonase acitivity	5.85±1.08[2)]	$3.40±0.37^{**}$

1)One unit of PG activity is defined as the amount of enzyme required to release 1 mg of reducing D-galacturonic acid from polygalacturonic acid per 2 hr at pH 5.0 at 30℃.
2)Values are Mean ± S.D., n=6
Means are significantly different with the week 10 kimchi based on the one way analysis of variance (ANOVA).
**p<0.001

Changes in free amino acids

As seen in Table 5, 35 different types of amino acids and related compounds were found in aged-kimchi. The concentration of free amino acids and their related compounds were 1,232 mg and 1,018 mg, respectively per 100 g of 10 weeks aged-kimchi and 46 weeks aged-kimchi (Table 5). Overall, total free amino acid in kimchi was higher at week 10 than at week 46. After 10 weeks in kimchi fermentation, glutamic acid which confers a sour taste was the dominant amino acid, and alanine and serine followed (Table 5). Similar trends were also seen in the 46 weeks aged-kimchi, however, arginine, histidine, and ornithine were low in the 46 weeks aged-kimchi.

The typical flavor of kimchi depends not only organic acid,

Table 5. Changes in free amino acids and related compounds (mg/100 g of kimchi) in kimchi during fermentation at 5℃ for 46 weeks

Amino acid	Before fermentation[1)]	Fermentation period	
		10 weeks	46 weeks
Isoleucine	65.2	38.2	27.3
Leucine	102.4	54.0	49.6
Lysine	108.4	51.6	48.7
Methionine	33.2	14.8	14.7
Cystine	23.7	0.5	1.1
Phenylalanine	59.1	38.8	34.5
Tyrosine	47.8	29.1	13.2
Trptophane	32.8	ND[2)]	ND
Threonine	64.1	45.1	36.7
Valine	77.2	58.6	46.7
Arginine	146.7	24.8	1.0
Histidine	37.1	21.5	ND
Alaline	97.4	119.1	121.7
Aspartic acid	178.2	64.5	10.8
Glutamic acid	403.2	291.4	241.2
Glycine	77.2	40.0	35.1
Proline	56.9	22.8	19.7
Serine	69.2	64.9	45.2
Phosphoserine		6.8	6.5
Taurine		7.2	6.1
Phosphoethanolamine		37.0	34.7
Urea		6.9	ND
α-amino adipic acid		2.2	1.0
Sarcosine		ND	ND
Citruline		7.5	6.2
α-amino-n-butyric acid		1.7	3.2
Cystathionine		3.1	1.3
β-alanine		5.6	6.8
β-amino-n-isobutyric acid		1.2	0.4
γ-amino-n-butyric acid		49.3	127.2
Ethanolamine		5.8	4.9
Hydroxy lysine		0.2	ND
Ornithine		54.7	12.1
1-methyl histidine		10.5	3.3
Anserine		6.0	2.6
Carnosine		1.2	2.1
Hydroxy proline		1.0	ND
Total amino acid	(1,680.0)[3)]	1,231.6 (979.7)	1,018.2 (747.2)

[1)]Information is from Food composition table (12). Where in blank, information is not available.
[2)]ND: not detected.
[3)]Numbers in parenthesis indicate sum of 18 amino acids from isoleucine to serine.

carbon dioxide and seasonings, but also free amino acids. In the present study, 85.7% of the kimchi is occupied by Chinese cabbage that contains 663 mg (12) of total amino acid in 100 g of raw vegetable. Among seven ingredients, two components (Chinese cabbage and salted anchovies) made up 68% (1,148 mg/100 g of kimchi) of the total amino acid concentration (1,680 mg/100 g of kimchi) in raw materials (analyses of amino acid concentration in seven raw materials were based on the report) (12). Glutamic acid is the major amino acid in both raw materials prior to fermentation and aged-kimchi. Cho and Rhee (24) reported that kimchi containing the salted anchovies gave the high level of lysine, methionine, isoleucine, leucine, and phenylalanine. In the same paper, authors demonstrated that flavor of kimchi appears to be better in the case of kimchi containing large amounts of lysine, aspartic acid, glutamic acid, valine, methionine, leucine, and isoleucine. Some of amino acids (alanine, glycine, serine and threonine) confer a sweet taste. Leucine, phenylalanine, and valine confer a bitter taste (24). Amino acids are produced by breaking down protein sources in vegetables and condiments, but some of amino acids (lysine, leucine) can be produced during fermentation.

Conclusion

In this study, the effect of long-term kimchi fermentation (46 weeks) at 5℃ on the pH, titratable acidity, organic acid, viable cell counts, amino acid, and PG activity were evaluated. Kimchi fermented for 10 weeks at 5℃ is acceptable, whereas kimchi fermented for 46 weeks at 5℃ is too sour. However, comparing sauerkraut to kimchi obtained from 46 weeks, sauerkraut fermentation is routinely completed at the pH 3.3~3.8 and TA>1.5% (25), whereas kimchi fermented for 46 weeks at 5℃ gave the final pH 3.85 and TA 1.29%. Therefore, kimchi fermentation for 46 weeks at 5℃ might be acceptable too. However, more work needs to be performed on the biological safety of long-term kimchi fermentation.

요 약

최근, 저온 장시간 발효된 묵은지에 대한 소비가 증가하고 있다. 본 논문에서는, 저온(5℃)에서 46주 동안 발효된 묵은지의 pH, 산도, 유기산, 생균수, 아미노산, polygalacturonase (PG) 활성 등을 분석하였다. 10주 발효한 김치에서는 pH 4.1, 산도 0.99%를 나타낸 반면, 46주 발효한 김치에서는 pH 3.9, 산도 1.29%를 보였다. 한편, 10주에서 46주로 발효 기간이 증가하면서, 젖산, 젖산/초산비, *Lactobacillus* species /*Leuconostoc* species비 등은 증가한 반면, 호기성 세균, 효모, *Lactobacillus* species, *Leuconostoc* species, 유리아미노산, PG 효소 활성은 감소하였다.

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