Physicochemical changes in kimchi containing skate (*Raja kenojei*) pretreated with organic acids during fermentation

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Abstract The effects of supplementing kimchi with acetic acid (TSA)- and citric acid (TSC)-treated skate were investigated during the fermentation process. The chemical and microbiological properties of the product were also investigated. The quality parameters of kimchi were determined by measuring the pH, titratable acidity, reducing sugar content, volatile basic nitrogen (VBN), biogenic amines (BAs), and microbial analysis. During fermentation, the kimchi containing skate was observed to have a lower acidity than the control. Treatment of skate with an organic acid increased the acidity and gradually decreased the reducing sugar content during fermentation. Higher concentrations of organic acid resulted in a decrease in VBN and BAs in the TSA and TSC groups compared to those in the kimchi containing skate. The results suggest that supplementing kimchi with skate treated with organic acids affects the histamine content of the products.

Keywords: kimchi, skate, organic acid, fermentation, biogenic amine

Introduction

Kimchi is a traditional fermented food unique to Korea and is made through fermentation aging after adding subingredients and spices to cabbage, daikon radish, and other vegetables, along with salt and salted seafood. During the aging process, it becomes rich in organic acids and lactic acid bacteria, creating new aromatic components, and presents a unique taste through a combination of spicy, sour, salty, sweet, bitter, and refreshing umami flavors (1). Moreover, from a nutritional perspective, it is a low-calorie food with low sugar and fat contents. It is also known to possess many physiological functions such as prevention of hypertension, anticancer activity, and antioxidant effect owing to the substantial presence of bioactive ingredients such as vitamin C, β -carotene, and phenolic compounds (2).

Kimchi is generally a vegetarian food; however, seafood (salted or otherwise) is also used as a subingredient in many cases. A variety of methods have been reported to prepare seafood-added kimchi. Some of these methods include adding seafood during kimchi production (3); after cooking and freezing (4); after pickling in salt or salt water, followed by washing (5); after pickling in salt, followed by steaming (6); and after turning dried samples into powder form (1, 3). Seafood added by these methods is known to play a major role in enhancing the taste of kimchi while also increasing supplementary nutrients such as amino acids in the product (7). Some regions and segments of society have pursued enhancement of the nutritional

value and product diversification of kimchi by selectively adding various seafood materials and salted seafood. In a survey of homemakers on their preference for adding seafood in preparing kimchi, it was found that the reason for doing so was flavor enhancement and nutritional enrichment (8). Accordingly, as interest in the use of seafood as a subingredient in kimchi increases, various studies have examined seafood, which include warty sea squirt (1), skate (2), raw anchovy (3), octopus (6,9), squid (6,9), webfoot octopus (9), abalone (9), and sea cucumber (10), as kimchi additives. However, a personal aversion to adding seafood or salted seafood can also be seen because of the presence of a foreign taste, shortening of aging period, and defective pigmentation (8).

Total volatile basic-nitrogen (VBN) compounds in fish products contain mainly ammonia, trimethylamine (TMA), and dimethylamine, and the levels of VBN compounds increase with spoilage due to bacterial or enzymatic degradation. In particular, compounds contained in the meat of skate, which includes trimethylamine N-oxide (TMAO), are decomposed during aging to produce ammonia and TMA (11). The ammonia or TMA produced at such times creates strong, foreign flavors that irritate the nose and may not suit the preference of today's consumers. However, ammonia acts to neutralize gastric acid and inhibit the growth of harmful bacteria in the body (12). Skate also contains taurine, which plays an important role in growth and development, and anserine, which enhances the umami flavor and acts as a buffer for muscles. It is also rich in

essential fatty acids such as linoleic acid, linolenic acid, and arachidonic acid, which helps to reduce serum cholesterol levels and also enhances cognitive functions and brain growth and development. Moreover, skate cartilage is known as a health food since it has high levels of chondroitin, a mucopolysaccharide protein (13). As such, skate is rich in nutrients, but because of its pungent taste and unique aroma, it is highly preferred only by fans of this food, and there are difficulties in using it for the general public. Also, free amino acids produced from the decomposition of proteins contained in skate generate biogenic amines (BAs), which are formed from microbial decarboxylation reactions during the fermentation, aging, or storage processes. These BAs are essential metabolic regulators in the human body, but their excessive consumption can produce carcinogens, which can pose serious bodily harm (14). In recent times, BAs have been used as an index for measuring the degree of deterioration in foods. They are also being used as indicators of freshness in seafood and hygiene in protein foods. This indicates that the decomposition of proteins increases the BAs content, which is directly correlated with food quality (15). As the generation of BAs depends not only on the hygienic aspects of food but also on the production methods including fermentation techniques, many studies on the BA content analysis of fermented foods (15) and formation of BAs by microbes (14,16) have been reported.

The present study produced skate kimchi using skate pretreated with organic acids to reduce the distinct smell of ammonia in skate and also to reduce the production of BAs. The chemical and microbiological quality parameters of kimchi with skate were analyzed during fermentation.

Materials and Methods

Experimental materials Korean cabbages and skates used in this study were purchased from the Seobu Agricultural and Fishery Market in Gwangju (Korea), with each head of the Korean cabbage weighing 2.5–3.0 kg. The subingredients required were purchased from various markets. These included red pepper powder (Jong Ga House, Seoul, Korea), anchovy sauce (Chung Jung Won, Seoul, Korea), salted shrimp (Hansung, Busan, Korea), sun-dried salt (Beksul, Seoul, Korea), sugar (Beksul), and rice powder (Dami Food, Gwangju, Korea). Other subingredients such as scallion, garlic, and ginger were purchased from the Seobu Agricultural and Fishery Market on the day before kimchi preparation.

Pretreatment of skate Skates were washed and trimmed and cut into pieces (2×1 cm) thereafter. Batches of these skate pieces were then immersed for 10 min each in baths containing 2% acetic acid (TSA-2), 4% acetic acid (TSA-4), 2% citric acid (TSC-2), and 4% citric acid (TSC-4), respectively, with the acid solution in each bath equivalent to 10 times the weight of the skate pieces immersed.

Subsequently, after washing the pieces in running water, the spices were added during kimchi preparation.

Kimchi preparation Korean cabbages were salted in the following manner to prepare both the regular kimchi as a control group and kimchi with skate groups with different pretreatments. Whole Korean cabbages weighing 2.5–3.0 kg were purchased, and after removing any inedible portions, they were cut in half and salted by a wet method. This process used a salt solution containing 0.139 kg of sun-dried salt and 1.2 kg of water per kg of cabbage. Here, the theoretical salt concentration in the solution was approximately 10%. The salting process was performed for 15 h at room temperature, following which the Korean cabbages were washed three times in running water and dried at room temperature. The kimchi was then prepared using salted cabbages with mixture ratios as follows: 74.95% salted Korean cabbage, 2.55% red pepper powder, 1.27% fermented anchovy sauce, 1.70% salted-fermented shrimp, 2.55% garlic, 0.51% ginger, 3.67% glutinous rice paste, 0.51% sugar, and 2.29% green onion. Control (CON) was produced without the addition of skate, while TS, TSA-2, TSA-4, TSC-2, and TSC-4 were produced with the addition of 10% of skate. The addition of Korean cabbage controlled the skate level; therefore, 84.95% salted Korean cabbage was added to CON. The prepared kimchi samples were stored for 56 days at 4°C. During fermentation, the quality characteristics of the kimchi were analyzed at 14-day intervals.

pH and acidity The pH was measured by directly placing a pH electrode into the samples that were made into a paste form using a blender. The titratable acidity of the samples was estimated using the equation given below. For this, exactly 1 g of each sample was made into a paste form using a blender, which was then diluted (approximately 100x) and filtered (Advantec No. 1; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). A fixed volume of this filtrate (20 mL) was titrated against a 0.01 N NaOH solution until a pH of 8.3 was reached.

$$\text{Titratable acidity (\%)} = \frac{A \times 0.0009 \times f \times D}{S} \times 100$$

A: Amount of 0.01 N NaOH solution consumed (in mL)

0.0009: Lactic acid (g) equivalent to 1 mL of 0.01 N NaOH

f: Titer value of 0.01 N NaOH solution

D: Dilution factor

S: Amount of sample used (g)

Reducing sugars The reducing sugar content of the kimchi samples was analyzed via a 3,5-dinitrosalicylic acid (DNS) method (17). For this, each sample in a paste form, made from grinding in a blender, was precisely weighed (1 g) and diluted appropriately (approximately 50x). These samples were then filtered (Advantec No. 1) and 3 mL of DNS reagent was added to 1 mL each of the filtrates. The mixtures were mixed thoroughly using a vortex mixer and then heated in a boiling water bath for 5 min. After cooling to room temperature, the

sample mixture was diluted with 16 mL of distilled water and its absorbance at 550 nm was measured using a UV-VIS spectrophotometer (UV1800; Shimadzu, Kyoto, Japan). The reducing sugar content of the samples was then calculated based on a predetermined glucose standard curve.

Volatile basic nitrogen (VBN) The VBN content of the kimchi samples was measured via a trace diffusion method using a Conway unit (diffuser) (18). 10 g of the sample in a paste form made from grinding in a blender was precisely weighed and placed in a beaker. The paste was diluted with 100 mL of distilled water and sonicated for 10 min to achieve uniform mixing. The sample was then filtered (Advantec No. 1) and the filtrate was neutralized using a 5% H₂SO₄ solution. The resulting solution was made up to a certain volume (100 mL) with distilled water for use as the test solution. After placing 1 mL of the test solution and 1 mL of a saturated K₂CO₃ solution in the outer chamber of the diffuser, 1 mL of a 0.01 N H₂SO₄ solution was kept in the inner chamber. The lid of the diffuser was then closed and the system was allowed to sit at 25°C for 1 h. After opening the lid, a drop of the Brunswik reagent was added to the sulfuric acid solution inside the inner chamber, which was then titrated with a 0.01 N NaOH solution using a microburette. A separate blank test was performed on distilled water, and the final calculations were performed using the equation given below:

$$\text{Volatile basic nitrogen (mg\%)} = \frac{(B-A) \times 0.14 \times f \times D}{S} \times 100$$

A: Amount of 0.01 N NaOH solution consumed in the experiment (in mL)

B: Amount of 0.01 N NaOH solution consumed in the blank test (in mL)

0.14: Amount of nitrogen (mg) equivalent to 1 mL of 0.01 N NaOH

f: Titer value of 0.01 N NaOH solution

D: Dilution factor

S: Amount of sample used (g)

Biogenic amines (BAs) The standard product, putrescine (PUT) dihydrochloride (98%), cadaverine (CAD) (95%), spermidine (SPD), spermine (SPM) (97%), tryptamine (98%), histamine (HIS) dihydrochloride (99%), tyramine (TYR) hydrochloride (98%), 2-phenylethylamine (PHE) hydrochloride, serotonin hydrochloride, dopamine (DOP) hydrochloride, internal standard 1,7-diaminoheptane, and the derivatization reagent, dansyl chloride, used in BAs analysis were purchased from Sigma Corporation. After accurately weighing out 5 g of the sample, 50 mL of 0.1 N HCl was added to it, followed by 30 min of sonication. The sample was then filtered (Advantec No. 1) and 0.1 N HCl was added to make the volume 50 mL for use as the test solution. After placing 1 mL each of standard and test solutions in glass test tubes with a stopper, 100 µL of the internal standard solution (100 µg/mL) was added. This was followed by the addition of 0.5 mL of saturated sodium carbonate solution and 0.8 mL of 1%

Table 1. HPLC conditions for biogenic amines analysis

Items	Conditions
HPLC System	Agilent 1260 Infinity
Wavelength	254 nm
Column	Zorbax Extend-C18 (4.6×250 mm, 5 µm, Agilent)
Column temperature	40°C
Injection volume	10 µL
Flow rate	1.0 mL/min
Mobile phase	A: 100% Acetonitrile B: Water
Gradient	A:B=55:45, 0-10 min A:B=65:35, 10-20 min A:B=70:30, 20-23 min A:B=80:20, 23-27 min A:B=85:15, 27-30 min A:B=90:10, 30-40 min

dansyl chloride-acetone solution. The combined solution was mixed, stoppered, and heated to 45°C for 1 h. After derivatization, 0.5 mL of a 10% proline solution and 5 mL of ether were added to the derivatized standard and test solutions and shaken for approximately 10 min; the supernatant was thereafter enriched in nitrogen. Finally, 1 mL of acetonitrile was added to the solution and filtered to 0.45 µm, which was used for HPLC (1260 Infinity; Agilent, Santa Clara, CA, USA) analysis under the conditions given in Table 1. For the analysis, a modified version of the BAs analysis method from Lee *et al.* (19) was used.

Microbial analysis The sample was drawn aseptically and ground in a blender. After diluting the sample paste by 10-fold with 0.85% NaCl in a sterile filter bag, it was homogenized in a stomacher (Bagmixer R400; Interscience, Saint Nom, France) for 1 min. For the differential counting of lactic acid bacteria according to their various morphological characteristics, serially diluted samples were inoculated onto a medium produced by adding 0.2% BPB (bromophenol blue) to MRS agar (*Lactobacilli* MRS agar, Difco, Franklin Lakes, NJ, USA), following which a spreading culture method was applied for 72 h at 30°C. Here, colonies without a ring or showing dark blue coloration were counted as *Leuconostoc* spp., while colonies with dark blue ring in the center or having a light blue color throughout were counted as *Lactobacillus* spp. (20).

Statistical analyses The experimental results were expressed as means and standard deviations, derived from three repeated measurements. SPSS software package (Statistical Package for the Social Science, version 19, SPSS Inc., Chicago, IL, USA) was used to perform a two-way ANOVA-test for the determination of statistical significance, while Duncan's multiple range test was performed for post-hoc testing (21) to test for significant intersample differences ($p < 0.05$).

Results and Discussions

Changes in pH and acidity during fermentation Changes in pH observed over 14-day increments in kimchi samples with various pretreatments of skate during 56 days of storage at 4°C, are as shown in Fig. 1. As kimchi fermented, a tendency of decreasing pH and increasing acidity was seen, which was consistent with previous reports on the fermentation pattern of kimchi (22). After its preparation, kimchi showed a pH range of 5.75–6.10 in the initial stages, with the TSA-4 group showing the lowest pH and the TS treatment showing the highest pH ($p < 0.05$). Moreover, higher concentrations of organic acids resulted in lower pH, with the TSA groups showing lower pH than the TSC groups. These findings were consistent with previous results, which reported that aged skate treated with organic acids showed lower pH values than skate in the control group, and as the concentrations of TSC and TSA increased, the pH tended to decrease (23). A drastic decrease was seen in all the test groups on the 14th day, and the values in the TS group was lower than the treated groups. Similar to the early fermentation periods, the pH was lower in the TSA groups than in the TSC groups; however, in general, the pH appeared to be higher with higher concentrations of organic acids. On the 28th day of storage, the TS group again showed the lowest pH value of 4.12, while other treated groups showed a pH range of 4.48–4.81. Just as on the 14th day, pH was lower in the TSA groups than in the TSC groups, and again, the pH was higher with higher concentrations of organic acids. A decrease in the pH was seen in all the treated groups even after 42 days of storage. The observation about groups with skate having higher pH than the group without skate was consistent with the findings from a previous study, which reported that the rate of decrease in pH for kimchi with added seafood was slower than regular kimchi. This phenomenon was attributed to a buffering effect of proteins contained in the skate (3).

Changes in acidity observed in 14-day increments from kimchi with various pretreatments of skate during 56 days of storage at 4°C as shown in Fig. 1. The acidity measured immediately after preparing the kimchi showed a range of 0.35–0.47, which increased rapidly on the 14th day. On the 28th day, the acidity measurements showed a range of 0.98–1.21, with the TS group showing the highest value, while the other treated groups did not show significant differences among each other. On the 42nd day of storage, the TSA groups showed the highest acidity, while the TSC groups showed values similar to the TS group. On the 56th day of storage, acidity in the CON group was the lowest at a value of 1.58, while groups with skate showed no significant differences among each other. This is believed to be due to the high production of organic acids from a proliferation of lactic acid bacteria. Results from the present study show that in comparison to the CON group, the groups with skate tended to have slower changes in pH and faster increases in acidity. These results are consistent with the findings from previous studies reported in the literature (3,5). Moreover, in the case of groups with skate, aging

Fig. 1. Changes in pH (A) and titratable acidity (B) of kimchi containing skate (*Raja kenojei*) treated with organic acids during fermentation for 56 days at 4°C. ●, CON: control (without additives); ○, TS: treatment with skate; ▼, TSA-2: skate treated with 2% acetic acid; △, TSA-4: skate treated with 4% acetic acid; ■, TSC-2: skate treated with 2% citric acid; □, TSC-4: skate treated with 4% citric acid

progressed slower with higher concentrations of organic acid and more so in the groups treated with citric acid than acetic acid, reaching the optimum ripening period at a later time. In general, the optimum ripening for kimchi with the best taste requires a pH range of 4.2–4.6 and acidity range of 0.5–0.75%. This optimum ripening period depends on various factors such as the main and sub-ingredients used and the aging temperature (24).

Changes in reducing sugar Changes in the reducing sugar present in kimchi with various pretreatments of skate are shown in Table 2. The reducing sugar content measured immediately after preparing the kimchi showed a range of 24.04–27.45 mg/g. However, the values decreased drastically on the 14th day of storage. This period corresponded to the period wherein the acidity of kimchi increased rapidly (25). The reducing sugar content continued to decrease up to 56th day of storage, and groups with skate showed a lower reducing sugar content than the CON group. These findings were consistent with the results from a study by Ryu *et al.* (3) who reported that the

Table 2. Changes in volatile basic nitrogen of kimchi containing skate (*Raja kenojei*) treated with organic acids during fermentation

Chemical properties	Fermentation period (days)	CON ¹⁾	TS	TSA-2	TSA-4	TSC-2	TSC-4
Reducing sugar (mg/g)	0	26.64±0.10 ^{2)(Ca3)}	26.75±0.30 ^{Ba}	26.80±0.16 ^{Ba}	24.04±0.37 ^{Da}	27.40±0.34 ^{Aa}	27.45±0.18 ^{Aa}
	14	18.13±0.13 ^{Cb}	10.79±0.14 ^{Eb}	16.79±0.27 ^{Db}	22.89±0.61 ^{Ab}	16.34±0.16 ^{Db}	20.20±0.46 ^{Bb}
	28	16.00±0.06 ^{Ac}	8.77±0.05 ^{Ec}	10.23±0.06 ^{Cc}	13.07±0.48 ^{Bc}	9.46±0.19 ^{Dc}	10.08±0.08 ^{Cc}
	42	14.10±0.10 ^{Ad}	6.06±0.02 ^{Ee}	6.53±0.06 ^{Dd}	11.46±0.19 ^{Bd}	6.58±0.10 ^{Dd}	8.59±0.21 ^{Cd}
	56	11.47±0.33 ^{Ae}	6.47±0.14 ^{Cd}	6.04±0.02 ^{Be}	9.97±0.29 ^{Be}	5.55±0.07 ^{Ee}	9.76±0.23 ^{Bc}
Volatile basic nitrogen (mg%)	0	21.35±1.04 ^{Dc}	40.84±1.58 ^{Ad}	33.96±1.78 ^{Bd}	36.13±0.32 ^{Bd}	29.97±1.28 ^{Ce}	30.37±1.34 ^{Cc}
	14	20.83±0.10 ^{Fc}	70.05±0.08 ^{Ac}	45.85±0.34 ^{Dc}	33.48±1.29 ^{Ed}	51.41±0.59 ^{Cd}	62.71±2.10 ^{Bb}
	28	24.76±3.01 ^{Eb}	69.70±0.32 ^{Ac}	52.63±3.77 ^{Cb}	40.02±1.17 ^{Dc}	59.74±3.66 ^{Bc}	64.86±3.26 ^{Ab}
	42	28.56±0.97 ^{Ea}	76.08±2.01 ^{Ab}	56.25±5.35 ^{Cab}	41.59±0.69 ^{Db}	65.57±0.70 ^{Bb}	65.01±0.36 ^{Bb}
	56	24.90±0.53 ^{Fb}	81.55±0.69 ^{Aa}	58.86±0.83 ^{Da}	45.08±0.48 ^{Ea}	70.72±0.46 ^{Ba}	68.48±0.42 ^{Ca}

¹⁾CON, control (without additives); TS, treatment with skate; TSA-2, skate treated with 2% acetic acid; TSA-4, skate treated with 4% acetic acid; TSC-2, skate treated with 2% citric acid; TSC-4, skate treated with 4% citric acid

²⁾All values are mean±SD of the three replicates.

³⁾Means sharing different letters in the same row (A-E) and column (a-d) are significantly different ($p<0.05$).

addition of raw anchovy to kimchi results in a higher initial reducing sugar content than not adding it, but was depleted quickly as aging progressed. Moreover, although the groups with skate did not show major differences based on organic acids, considering having higher concentrations of organic acids resulted in higher reducing sugar content than having lower concentrations, it is believed that aging of kimchi progressed slowly. It is believed that the phenomenon of decrease in reducing sugar was due to the sugars being transformed to lactic acid, acetic acid, alcohol, carbon dioxide, and other substances from microbial activities during the fermentation process as the microbes decompose the sugars in kimchi for use as an energy source (26).

Changes in volatile basic nitrogen Changes in the VBN of kimchi with various pretreatments of skate are shown in Table 2. The VBN measured immediately after preparing the kimchi showed a range of 21.35–40.84 mg%, with the CON group showing the lowest value and the TS group showing the highest value. The CON group showed virtually no changes with storage time, while the groups with skate showed a rapid increase on the 14th day. The VBN values increased on the 28th day as well, and in comparison to the TS group, the TSA-4 group showed the lowest value. The groups with skate showed a tendency of increasing VBN on the 56th day as well, and among the treated groups, the highest value was seen for the TS group (81.55 mg%), while the lowest value was seen for the TSA-4 group (45.08 mg%).

An increase in VBN can be attributed to basic substance such as TMA, which is produced from the oxidation of phospholipids and reduction of TMAO, along with the ammonia and nitrogen produced from the decomposition of proteins by proliferating bacteria. Therefore, VBN is generated from decomposition of proteins by microbes, and among the methods for assessing freshness in fish and shellfish, VBN is the most representative method of using substances that were present in very small amounts in fresh meat but are produced and

increased according to the decline in freshness as an indicator (27). In addition, VBN has been reported to be closely associated with fishy smell (28), and Lee *et al.* (29) reported the reason for the increase in the VBN contents during fermentation, which increased the nonprotein-nitrogenous compounds such as free amino acids, nucleotides, amines, ammonia, and creatine by cutting of the protein chain and unique taste and flavor and attribute to formation of off-odor. Accordingly, it is believed that pretreating the skate with organic acids before their addition to kimchi can eliminate the distinct ammonia smell of skate by suppressing protein deterioration during fermentation and aging of the kimchi. According to the findings of the present study, treating skate with TSA was more effective than treating it with TSC, and protein deterioration was suppressed with increasing concentrations of acid.

Changes in biogenic amines Biogenic amines (BAs) are nitrogenous organic bases produced in foods because of decarboxylation of amino acids or transamination of aldehydes and ketones (30). The most important BAs are HIS, TYR, tryptamine (TRP), PUT, and CAD, which are formed from the free amino acids histidine, tyrosine, tryptophan, ornithine, and lysine, respectively (30).

The present study prepared kimchi using skate with various pretreatments and analyzed the changes in BA levels during storage (Table 3) and chromatogram of BAs standard mix showed in Fig 2. All BAs were detected in all the samples except for TYR, serotonin, SPM, which were not detected in all samples in this study. A pattern of increase was seen in all treated groups during the storage period. In general, storage of skate kimchi resulted in a significant increase in BA levels during fermentation. The TS group containing skate showed the highest level of BAs and the maximum levels of PUT, CAD, HIS, and TYR were 59.75, 53.73, 90.22, and 70.69 mg/kg, respectively. The HIS level of kimchi in this study was in the range of 9.46–14.74 mg/kg in initial fermentation. The HIS content in the CON group immediately after preparing the kimchi was 9.46 mg/kg, which was

Table 3. Changes in biogenic amines contents of kimchi containing skate (*Raja kenojei*) treated with organic acids during fermentation

Biogenic amines ¹⁾ (mg/kg)	Fermentation period (days)	CON ²⁾	TS	TSA-2	TSA-4	TSC-2	TSC-4
TRY	0	ND ³⁾	ND	ND	ND	ND	ND
	28	ND	ND	ND	ND	ND	ND
	56	ND	ND	ND	ND	ND	ND
PHE	0	4.80±0.25 ^{4)ba5)}	4.26±0.30 ^{Bc}	5.94±0.58 ^{Aa}	3.18±0.16 ^{Cb}	2.74±0.20 ^{Cb}	2.56±0.19 ^{Dc}
	28	4.39±0.24 ^{Ba}	5.96±0.49 ^{BAb}	2.64±0.16 ^{Cb}	2.66±0.69 ^{Cb}	5.61±0.32 ^{Aa}	5.34±0.16 ^{Ab}
	56	5.08±0.56 ^{Ca}	7.39±0.35 ^{Aa}	5.88±0.57 ^{Bca}	6.00±0.49 ^{Bca}	5.02±0.50 ^{Ca}	6.85±0.95 ^{ABa}
PUT	0	7.01±0.21 ^{Bc}	8.00±0.16 ^{Ac}	7.15±0.55 ^{Bc}	8.13±0.29 ^{Aa}	7.40±4.23 ^{Bc}	7.40±0.31 ^{Bb}
	28	19.26±0.02 ^{Cb}	35.91±0.72 ^{Ab}	30.22±1.02 ^{Ba}	8.16±0.51 ^{Db}	29.83±0.26 ^{Bb}	34.25±2.61 ^{Aa}
	56	55.45±3.57 ^{Ba}	59.75±0.61 ^{Aa}	26.00±0.39 ^{Db}	10.05±0.55 ^{Ea}	47.48±2.98 ^{Ca}	31.74±1.23 ^{Da}
CAD	0	5.05±0.14 ^{Ec}	5.68±0.15 ^{Dc}	6.67±0.20 ^{Bc}	6.02±0.20 ^{Ca}	6.51±0.05 ^{Bc}	7.09±0.24 ^{Ac}
	28	5.49±0.15 ^{Eb}	51.41±1.69 ^{Bb}	28.44±0.45 ^{Da}	3.55±0.52 ^{Eb}	61.73±1.83 ^{Aa}	39.29±1.19 ^{Ca}
	56	11.59±0.08 ^{Da}	53.73±0.47 ^{Aa}	21.17±0.16 ^{Cb}	3.44±0.20 ^{Eb}	53.47±1.15 ^{Ab}	34.72±1.24 ^{Bb}
HIS	0	9.46±0.45 ^{Dc}	11.28±0.48 ^{Cc}	14.74±0.93 ^{Ac}	12.90±0.53 ^{Bb}	12.17±0.25 ^{Bc}	12.17±0.25 ^{Bcc}
	28	38.83±2.23 ^{Ab}	20.48±0.80 ^{Bb}	39.80±1.40 ^{Ab}	15.38±0.75 ^{Cb}	20.89±0.56 ^{Bb}	13.83±0.77 ^{Cb}
	56	49.03±0.54 ^{Ca}	90.22±1.31 ^{Aa}	62.00±0.82 ^{Ba}	48.22±2.29 ^{Ca}	28.22±0.84 ^{Da}	16.18±0.29 ^{Ea}
SER	0	ND	ND	ND	ND	ND	ND
	28	ND	ND	ND	ND	ND	ND
	56	ND	ND	ND	ND	ND	ND
TYR	0	2.57±0.17 ^{Cc}	3.01±0.08 ^{Bc}	3.63±0.22 ^{Ac}	2.97±0.08 ^{Bb}	3.14±0.10 ^{Bc}	3.89±0.33 ^{Ab}
	28	16.19±0.30 ^{Cb}	35.95±0.19 ^{Ab}	11.75±0.81 ^{Db}	5.08±0.68 ^{Ea}	35.60±0.74 ^{Aa}	25.80±0.62 ^{Ba}
	56	18.67±0.79 ^{Da}	70.69±1.93 ^{Aa}	14.17±0.26 ^{Ea}	5.66±0.29 ^{Fa}	32.79±0.81 ^{Bb}	25.58±0.31 ^{Ca}
SPD	0	12.71±0.34 ^{Ab}	10.48±0.67 ^{Da}	11.38±0.78 ^{BcDa}	10.80±0.41 ^{CDa}	12.12±0.42 ^{ABa}	11.58±0.14 ^{Bca}
	28	16.35±0.15 ^{Aa}	9.06±0.12 ^{Cb}	8.97±0.11 ^{Cc}	11.99±0.91 ^{Ba}	8.71±0.33 ^{Cb}	9.08±0.17 ^{Cb}
	56	12.06±1.88 ^{Ab}	10.03±0.24 ^{Ba}	9.87±0.32 ^{Bb}	11.85±0.85 ^{Aa}	8.90±0.04 ^{Bb}	9.95±0.27 ^{Bb}
DOP	0	32.63±0.38 ^{Ab}	26.63±2.76 ^{Bca}	29.00±0.37 ^{Ba}	24.67±1.39 ^{Db}	28.26±2.45 ^B	30.01±2.44 ^{AB}
	28	38.08±4.35 ^{Ab}	27.76±1.74 ^{Ba}	26.27±4.10 ^{Ba}	28.59±1.19 ^{Ba}	ND	ND
	56	48.42±2.20 ^{Aa}	27.82±0.10 ^{Ba}	25.09±1.16 ^{Ca}	ND	ND	ND
SPM	0	ND	ND	ND	ND	ND	ND
	28	ND	ND	ND	ND	ND	ND
	56	ND	ND	ND	ND	ND	ND

¹⁾CAD, cadaverine; SPD, spermidine; SPM, spermine; TRY, tryptamine; HIS, histamine; TYR, tyramine; PHE, α -phenylethylamine; SER, serotonin; DOP, dopamine; PUT, putrescine

²⁾CON, control (without additives); TS, treatment with skate; TSA-2, skate treated with 2% acetic acid; TSA-4, skate treated with 4% acetic acid; TSC-2, skate treated with 2% citric acid; TSC-4, skate treated with 4% citric acid

³⁾ND, not detected

⁴⁾All values are mean±SD of the three replicates.

⁵⁾Means sharing different letters in the same row (A-E) and column (a-d) are significantly different ($p<0.05$).

the lowest, and an increase in HIS levels was seen during storage of samples in all the treated groups. While the TS group showed a rapid increase to an elevated value of 90.22 mg/kg by day 56, the TSA groups showed HIS content in the range of 23.55–62.00 mg/kg, which indicated that organic acid pretreatment of skate suppressed HIS production during fermentation. Higher concentrations of organic acid resulted in greater suppression of HIS production. During fermentation, HIS production was lower in the TSC groups than in the TSA groups and CON group. Moreover, addition of organic acid influences the HIS production in normal control which added just anchovy sauce and salted fermented shrimp. In this study, there was a significant ($p<0.05$) increase in the TYR level from day 0 to day 56 of storage for both treated groups and CON group.

Compared with TS, the treated groups showed significant ($p<0.05$) changes in TYR content. The concentration of TYR was lower in the TSA and TSC groups with a reduction of approximately 92% and 64% from the TS, respectively. Compared to the TS, treated groups (TSA and TSC) showed significantly reduced ($p<0.05$) concentrations of BAs, specifically TYR and HIS.

Formation of BAs in fermented foods has been attributed to the decarboxylase activity of bacteria involved in fermentation (31). Several lactic acid bacteria (LAB) species have been reported to produce BAs in fermented food (32), and HIS- and TYR-producing *Lactobacillus* strains have been isolated from kimchi (33). Jeotgal, a traditional high-salt fermented seafood, which is sometimes added to kimchi to enhance flavor and support bacterial growth, has been

Fig. 2. Chromatogram of biogenic amines standard mix. TRY, tryptamine; PHE, α -phenylethylamine; PUT, putrescine; CAD, cadaverine; HIS, histamine; ISTD, internal standard; SER, serotonin; TYR, tyramine; SPD, spermidine; DOP, dopamine; SPM, spermine

regarded as a potential source of BAs (34). In general, monoamine and diamine oxidases that degrade BAs are present in the small intestines to serve as a detoxification system; however, because excessive consumption of amines prevents enzymatic actions, reduction measures are needed (35). Recently, BAs reduction techniques for fermented agricultural and fishery products, including fermented sauces, have been studied (16). Kang (36) reported the analysis of HIS content in kimchi with heat-treated and non-heat-treated fish sauce.

Changes in microbial activities Changes in *Leuconostoc* spp. and *Lactobacillus* spp. during storage of kimchi with pretreated skate are as shown in Fig. 3. MRS agar (with BPB) changes color within a range from pH 3 to 5, it is useful for detection of the pH change produced during fermentation of LAB. During fermentation, LAB produced various colonies including light blue, small, and flat colonies of *Leuconostoc* spp., white with blue center and convex colonies of *Lactobacillus* spp. (37). Fermentation patterns of *Leuconostoc* spp. showed a bacterial content of 5.43–6.33 log CFU/g immediately after kimchi preparation with the untreated skate group (TS) showing the lowest content. The TSC-4 group showed the lowest value among the groups treated with organic acid. The bacterial content continued to increase with progression of storage period to reach a range of 7.36–8.29 log CFU/g on the 28th day of storage, after which, all treated groups began to show a decrease to show a range of 6.17–7.14 log CFU/g on the 56th day of storage. Fermentation patterns of *Lactobacillus* spp. were the same as that of *Leuconostoc* spp. A range of 5.78–6.71 log CFU/g was seen immediately after kimchi preparation with the TS group showing the highest value among groups with skate, while the TSC-4 group showed the lowest value among all groups treated with organic acid. The bacterial content continued to increase to reach 8.59–8.98 log CFU/g on the 28th day; however, there were no significant differences between the treated groups. On the 42nd day,

the content began to decrease in all treated groups, just as in the case of *Leuconostoc* spp., and on the 56th day, a range of 8.36–8.76 log CFU/g was observed. On examining the fermentation patterns of *Leuconostoc* spp. and *Lactobacillus* spp. during storage of kimchi with pretreated skate, it was shown that the fermentation period at which *Leuconostoc* spp. and *Lactobacillus* spp. reached their maximum TBC was the same, but as fermentation time increased, the *Leuconostoc* spp. count appeared lower than the *Lactobacillus* spp. count, which was consistent with a previous study result (38), which reported that *Leuconostoc* spp. showed slightly lower TBC than *Lactobacillus* spp. in each treatment group (36). Moreover, both *Leuconostoc* spp. and *Lactobacillus* spp. showed a decrease after reaching their maximum growth at longer storage times, which was due to the activities of lactic acid bacteria being suppressed by acids produced during fermentation of kimchi. This was in addition to the proliferation of microbes being limited from a decrease in reducing sugars that serve as a carbon source (9).

In the present study, immediately after kimchi preparation, the lactic acid bacteria count was lower in groups with higher concentrations of added organic acid. This was more so in the TSC groups than the TSA groups. Based on these results, it could be inferred that using skate treated with organic acid as an additive could have a controlling effect on microbes in the initial stage. Moreover, it is believed that the reason for the groups with added skate showing greater production of lactic acid bacteria than the group without skate was due to the proteins contained in skate, which serve as a nutritional source for lactic acid bacteria.

In conclusion, high levels of VBN and BAs have been detected during the fermentation process in commercially available kimchi, and as such, kimchi with skate was prepared by pretreating the skate with organic acids to reduce the harmful elements. Our results showed a decrease in pH and increase in acidity during kimchi fermentation, which was consistent with previously reported kimchi

Fig. 3. Changes in *Leuconostoc* spp. (A) and *Lactobacillus* spp. (B) of kimchi containing skate (*Raja kenoei*) treated with organic acids during fermentation. ●, CON: control (without additives); ○, TS: treatment with skate; ▼, TSA-2: skate treated with 2% acetic acid; △, TSA-4: skate treated with 4% acetic acid; ■, TSC-2: skate treated with 2% citric acid; □, TSC-4: skate treated with 4% citric acid

fermentation patterns. VBN tended to be higher in the groups with skate than the group without skate, and among the groups treated with organic acids, the groups treated with citric acid showed lower values than the groups treated with acetic acid. BAs contents also tended to be lower in the groups treated with organic acid. Moreover, having higher concentrations of organic acids and specifically a treatment with citric acid rather than acetic acid, were shown to be effective in controlling microbial activities. Therefore, it is believed that the findings in the present study can be used as data foundation for further studies on the pretreatment of seafood, which could be added to kimchi.

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