Changes in quality properties of kimchi based on the nitrogen content of fermented anchovy sauce, *Myeolchi Aekjeot*, during fermentation

Yun-Jeong Choi¹ · Hae-Won Lee¹ · Ji-Hee Yang¹ · Sung-Wook Hong¹ · Sung-Hee Park¹ · Mi-Ai Lee¹

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Abstract This study was conducted to examine the effects of the total nitrogen (TN) concentration in anchovy sauce added to kimchi on chemical properties during kimchi fermentation for 56 days. Kimchi was assigned to one of the following five treatments: CTL (No anchovy sauce addition), T-1 (TN 1.16% anchovy sauce), T-2 (TN 1.41% anchovy sauce), T-3 (TN 1.59% anchovy sauce), T-4 (TN 1.79% anchovy sauce). The quality characteristics of kimchi were determined by measuring the pH, acidity, reducing sugar, volatile basic nitrogen (VBN), amino nitrogen (AN), and microbial composition. During fermentation, the acidity of all treatment groups increased, whereas the reducing sugar content decreased gradually during fermentation. There were no significant differences in pH and acidity or reducing sugar content. The VBN and AN contents increased during fermentation from 15.65 to 31.21%. Overall, these results demonstrate that the TN concentration of anchovy mainly affects the VBN and AN levels in kimchi.

Keywords Kimchi · Anchovy sauce · Nitrogen · Glutamic acid · Fermentation

Introduction

Kimchi is a traditional Korean dish that is made of lactic acid-fermented kimchi cabbage or radish with salt and various spices, such as red pepper powder, scallion, garlic,

ginger, and jeotgal [1]. The main ingredient, Korean cabbage, is a major source of dietary fiber and vitamins, while the other ingredients, such as garlic, onion, garlic chives, ginger, and jeotgal, show anti-cancer effects [2]. Since the establishment of Codex international food standards in 2001, it was selected as one of the world's five healthiest foods by Health magazine of the United States in 2006 [3]. Kimchi is fermented by anaerobic halophilic lactic acid bacteria. During the fermentation process, lactic acid bacteria produce organic acids and bacteriocin, which suppress the growth of harmful bacteria and impart a unique flavor to kimchi [4]. Among the other ingredients, red pepper powder and jeotgal significantly affect the taste of kimchi [5]. Ginger and scallions do not have a remarkable effect on fermentation, while garlic, red pepper powder, and jeotgal have been found to accelerate the fermentation of kimchi [6].

Jeotgal is a traditional Korean food-item, prepared by long term fermentation and aging of muscles, intestines, or gonads of fish and shellfish, accompanied with a large amount of salt [7]. It can be directly consumed owing to its rich flavor and proper decomposition of proteins, carbohydrates, lipids, organic acids, and other substances [8]. Furthermore, jeotgal is rich in essential amino acids, such as lysine and threonine. It has various functional benefits, such as liver protection, appetite promotion, vitamin B supplementation, and digestion promotion by proteolytic enzymes [9]. Therefore, it has been used as an ingredient to prepare kimchi since ancient times. Now, jeotgal is mostly distributed as a solid-free anchovy sauce [10]. This anchovy sauce contains various amino acids and high amount of inosinic acid, which impart the umami taste [11]. Fish sauces are clear brown liquids with a salty taste and a distinctive fish flavor that are generally made by spontaneous long-term fermentation of salted anchovies

✉ Mi-Ai Lee
leemae@wikim.re.kr

¹ World Institute of Kimchi, Gwangju 61755, Republic of Korea

[12]. It has been used as kimchi seasoning, soy sauce substitute, and a flavour additive in various dishes, such as salads and pickles [13].

Studies on the quality of kimchi based on the presence and type of jeotgal [14], on qualities of commercial anchovy sauces and kimchi prepared from different commercial anchovy sauces [5], and on the characteristics of kimchi consisting of anchovy sauce from heat and non-heat treatments [7] have been previously conducted, but a study on the characteristics of kimchi based on the addition of anchovy sauce has not been done yet. In particular, there are few studies related to the nitrogen content of kimchi, which is one of the standard qualities of anchovy sauce. Thus, the present study was performed to investigate the effect of nitrogen content in anchovy sauce on kimchi fermentation. For this purpose, anchovy sauce with different nitrogen contents was added to kimchi, and the characteristics of kimchi were analyzed over the storage period.

Materials and methods

Experimental materials

Kimchi cabbages with a head mass of 2.5–3.0 kg were purchased from Seobu Agricultural and Fishery Market in Gwangju. Sun-dried salt (CJ Cheiljedang, Sinan, Korea), red pepper powder (Goesan Red Pepper Cooperative Joint Corporation, Goesan, Korea), four kinds of fermented anchovy sauces (A-1: Guldari Food Co., Asan, Korea; A-2: Shinan Saeujeot Inc., Shinan, Korea; A-3: Shingwang Food Co., Gimhae, Korea; A-4: Gomsoohbok Food Co., Buan, Korea), salted fermented shrimp (Yeonsu Food Co., Gwangju, Korea), and glutinous rice flour (Dami-food, Gwangju, Korea) were purchased from the market. The composition of anchovy sauce was 75% anchovy and 25% sun-dried salt. The other ingredients, such as radish, scallion, garlic, and ginger, were purchased from Seobu Agricultural and Fishery Market on the day before kimchi production. For experimental analysis, first grade analytical reagents were used.

Kimchi preparation

The salting process of kimchi cabbage for preparing kimchi is described below. Whole kimchi cabbages weighing 2.5–3.0 kg were purchased. After removing the inedible parts, the cabbages were cut in half and salted by mixing 0.139 kg sun-dried salt and 1.2 kg water per 1 kg cabbage. Salinity of the solution was 10%. The salting process was carried out for 16 h at 18 °C, followed by washing the

salted kimchi cabbage three times with running water and drying for 2 h at room temperature.

For prepared kimchi, radish, garlic, and ginger were ground using a blender (HR-1372, Philips, Guangdong, China) for seasoning, and green onions were cut into 1 cm lengths. The mixture ratio for kimchi was as follows: 85% salted-cabbage, 3.3% red pepper powder, 2.7% green onion, 2.5% garlic, 0.4% ginger, 1% anchovy sauce, 1.5% salted shrimp, and 3.6% glutinous rice paste. Kimchi was assigned to one of the following 5 treatments: CTL (control; added 22% saline solution instead of anchovy sauce), T-1 (added TN 1.16% anchovy sauce, A-1), T-2 (added TN 1.41% anchovy sauce, A-2), T-3 (added TN 1.59% anchovy sauce, A-3), T-4 (added TN 1.79% anchovy sauce, A-4), based on the nitrogen content of anchovy sauce. A quarter of each kimchi sample was separately packed in PE film (28 cm width × 38 cm length) and sealed using a vacuum packaging machine (AZC-070, INTRISE, Ansan, Korea). The packed kimchi was stored at 4 °C for 56 days in a refrigerator, and its characteristics were analyzed at intervals of 14 days.

Analysis of pH and acidity

Kimchi was homogenized in a blender for measuring the pH and acidity. The solid contents were filtered through sterilized gauze (Sterile gauze No. 3, Soosung, Yangsan, Korea) and the filtrate was used for subsequent measurements. The pH and acidity were both measured using a pH meter (TitroLine easy, SI Analytics, Mainz, Germany). The pH was measured by directly placing a pH electrode of the pH meter in kimchi liquid. For measuring acidity by the Association of Official Analytical Chemists (AOAC) method [15], the amount of 0.1 N NaOH required to neutralize 10 mL of kimchi liquid to pH 8.3 was used. The acidity was calculated as percentage lactic acid by using the following formula:

$$\text{Acidity (\%)} = \frac{A \times f \times 0.009}{S} \times 100$$

where A is the titrated volume of the 0.1 N NaOH solution for the sample (mL), 0.009 is the lactic acid (g) equivalent to 1 mL of 0.1 N NaOH, *f* is the factor of 0.1 N NaOH solution, and S is the sample amount (mL)

Analysis of reducing sugars

The reducing sugar content of the kimchi samples were analyzed via a 3,5-dinitrosalicylic acid (DNS) method [16]. For this, each sample in a paste form, made from grinding in a blender, was precisely weighed (1 g) and diluted appropriately (approximately 50×). These 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.

Analysis of amino nitrogen (AN)

The AN content of the samples was measured in triplicate by using the formol method. In total, 2 g of each sample was suspended in distilled water to make a final volume of 100 mL and then sonicated for 30 min. Thereafter, 20 mL of this sample solution was added and mixed to 20 mL formalin solution, and then titrated to pH 8.3 with 0.1 N sodium hydroxide. The AN content was calculated as follows:

$$\text{AN content (mg\%)} = \frac{(A - B) \times 1.4 \times f \times D}{S} \times 100$$

where A is the titrated volume of 0.1 N sodium hydroxide consumed by the sample (mL), B is the titrated volume of 0.1 N sodium hydroxide consumed by the blank reagent (mL), 1.4 is the AN content value corresponding to mL of 0.1 N sodium hydroxide, f is the factor of 0.1 N sodium hydroxide, D is the dilution magnification, and S is the sample weight (g).

Analysis of volatile basic nitrogen (VBN)

The VBN content of the samples was measured in triplicate via the Conway microdiffusion method. Briefly, 5 g of each sample was suspended in distilled water to make a final volume of 100 mL and then sonicated for 10 min. After filtration, the sample filtrate was neutralized with 5% sulfuric acid, and 1 mL of this sample solution with 1 mL of saturated potassium carbonate was placed in the outer chamber of a Conway dish. Then, 1 mL of 0.01 N sulfuric acid was placed in the inner chamber of the Conway dish. The dishes were incubated at 25 °C for 60 min and then titrated with 0.01 N sodium hydroxide and Brunswik's reagent. The VBN content was calculated as follows:

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

where A is the titrated volume of 0.01 N sodium hydroxide consumed by the sample (mL), B is the titrated volume of 0.01 N sodium hydroxide consumed by the blank reagent (mL), 0.14 is the VBN content value corresponding to mL

of 0.01 N sodium hydroxide, f is the factor of 0.01 N sodium hydroxide, D is the dilution magnification, and S is the sample weight (g).

Analysis of free amino acids

The prepared kimchi samples were ground using a blender. Then, 10 g of it was diluted 50-fold, followed by sonication for 30 min. The samples were shaken for 2 h and refrigerated for 15 h. After filtration through a 0.45 µm membrane filter, the supernatant was used as a test solution for free amino acid analysis by HPLC. The area of the peak obtained after injecting 0.5 µL each of standard solution and test solution was determined. The free amino acids in each sample were quantitatively analyzed using a calibration curve. The following amino acids were analyzed: aspartic acid, glutamic acid, serine, histidine, glycine, threonine, alanine, arginine, GABA, taurine, tyrosine, valine, methionine, phenylalanine, isoleucine, leucine, lysine, proline, cystine, lysine, glutamine, asparagine, tryptophan, and hydroxy proline. The HPLC conditions for free amino acid analysis are given in Table 1.

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 (Bagmiser R400, Interscience, Saint Nom, France) for 1 min. The total viable bacteria determined by the PCA (Plate count agar, Difco, Maryland, USA) and incubated at 36 °C for 48 h. The lactic acid bacteria was determined by the MRS agar (*Lactobacilli* MRS agar, Difco) with 25 ppm BCP (bromocresol purple) indicator and incubated at 36 °C for 72 h. Colonies were counted the culture (acid producing bacteria) showing the total colony and yellow color reaction. Results were expressed as log CFU/g.

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 [17] to test for significant intersample differences ($p < 0.05$). The correlation analysis between chemical content of kimchi samples were performed using XLSTAT-Premium software package version 19.4 (Addinsoft, New York, USA).

Table 1 Conditions of HPLC analysis

Parameters	Conditions
HPLC system	Ultimate 3000
Column	VDSpher 100 C18-E (4.6 × 150 mm, 3.5 μm/VDS optilab, Germany)
Mobile phase	A: 40 mM sodium phosphate dibasic, pH 7.8 B: water/acetonitrile/methanol (10:45:45 v/v%)
Flow rate	1.5 mL/min
Detector	FL Detector 1260 FLD (Agilent, USA) : Emission 450 nm, Excitation 340 nm (OPA) : Emission 305 nm, Excitation 266 nm (FMOC) UV Detector (Thermo dionex, USA) : 338 nm
Oven temperature	40 °C

Results and discussions

Chemical properties of anchovy sauce

The pH, salinity, and total nitrogen (TN) of four anchovy sauces used in this study are indicated in Table 2 [18]. The pH of the anchovy sauces were in the range of 5.55–6.16 with A-1 exhibiting the lowest pH. The salinity was in a range of 22.97–23.69% with A-1 showing the highest value. The sauces possessed a TN content of 1.16–1.79% with a difference of about 0.2% between each other. According to Korean Industrial Standards [19], the quality standards of anchovy sauce include salinity below 25% and TN content above 1.2% (above 0.5 in the case of seasoned anchovy sauce). However, among the anchovy sauces used in the present study, the TN of A-1 was measured to be lower than the standard value. The nitrogen contents of foods are used for evaluation of food quality, and TN content is an approximate index of the protein content, an important nutritional factor [20].

Changes in pH and acidity

Changes in pH and acidity of the kimchi samples prepared with anchovy sauces with varying nitrogen contents are shown in Table 3. Various organic acids were generated during the kimchi fermentation process through

decomposition of carbohydrates, the main component of Korean cabbage, resulting in unique fresh sour taste. Thus, pH and acidity can be regarded as key indicators of kimchi quality [21]. The changes in pH and acidity of kimchi during storage period showed a tendency of decreasing pH and increasing acidity as the aging progressed. This finding indicated normal fermentation conditions of kimchi. The pH measured immediately after kimchi production was in the range of 5.88–5.97, showing no significant differences between the treated groups. The pH decreased drastically with longer storage period, exhibiting a value of 4.30–4.37 on the 28 day of storage. On the 56 day of storage, a lower pH of 4.09–4.19 was observed without significant difference between the treated groups. Thus, addition of anchovy sauce and nitrogen content of anchovy sauce did not affect the pH of kimchi. These findings were consistent with previous study of Kim et al. [22], reporting no significant differences in pH on addition of anchovy sauce, as compared to the CTL. The acidity measured immediately after kimchi production was in the range of 0.35–0.41%, with the lowest value for the CTL group without anchovy sauce. The acidity increased rapidly with longer storage period. Acidity values of 0.60–0.67% and 0.83–0.97% were observed on the 14 day and the 28 day of storage, respectively. At this time, acidity of the CTL was the lowest, while acidity of T-4 was the highest. This finding confirmed that the acidity increased more rapidly with

Table 2 Chemical characterization of anchovy sauce

	A-1 ⁽¹⁾	A-2	A-3	A-4
pH	5.55 ± 0.05 ^{(2)A(3)}	5.64 ± 0.04 ^B	6.11 ± 0.03 ^C	6.16 ± 0.05 ^C
Salinity (%)	23.69 ± 0.52 ^B	23.30 ± 0.58 ^{AB}	22.31 ± 0.14 ^{AB}	22.97 ± 0.14 ^A
TN (%)	1.16 ± 0.02 ^A	1.41 ± 0.03 ^B	1.59 ± 0.02 ^C	1.79 ± 0.02 ^D

⁽¹⁾A-1: Anchovy sauce from Asan; A-2: Anchovy sauce from Shinan; A-3: Anchovy sauce from Gimhae; A-4: Anchovy sauce from Buan

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

⁽³⁾Mean sharing different letters in the same row (A–D) are significantly different ($p < 0.05$)

Table 3 Changes in pH, acidity, reducing sugar, and microbial properties of Kimchi during fermentation

	Fermentation period (days)	CTL ⁽¹⁾	T-1	T-2	T-3	T-4
pH	0	5.88 ± 0.04 ^{(2)Ae(3)}	5.88 ± 0.01 ^{Ae}	5.95 ± 0.02 ^{Be}	5.88 ± 0.01 ^{Ae}	5.97 ± 0.01 ^{Bd}
	14	4.74 ± 0.02 ^{Ad}	4.89 ± 0.02 ^{Dd}	4.82 ± 0.02 ^{Cd}	4.78 ± 0.01 ^{Bd}	4.77 ± 0.01 ^{ABc}
	28	4.30 ± 0.02 ^{Ac}	4.31 ± 0.01 ^{ABc}	4.33 ± 0.03 ^{Bc}	4.37 ± 0.01 ^{Cc}	4.32 ± 0.01 ^{ABb}
	42	4.24 ± 0.02 ^{Cb}	4.22 ± 0.01 ^{BCb}	4.19 ± 0.01 ^{ABb}	4.23 ± 0.01 ^{Cb}	4.18 ± 0.02 ^{Aa}
	56	4.17 ± 0.01 ^{Ba}	4.09 ± 0.01 ^{Aa}	4.08 ± 0.02 ^{Aa}	4.19 ± 0.01 ^{Ca}	4.18 ± 0.01 ^{BCa}
Acidity (%)	0	0.35 ± 0.01 ^{Aa}	0.38 ± 0.01 ^{Ba}	0.37 ± 0.01 ^{Ba}	0.41 ± 0.01 ^{Ca}	0.38 ± 0.02 ^{Ba}
	14	0.60 ± 0.02 ^{Ab}	0.61 ± 0.01 ^{Ab}	0.64 ± 0.01 ^{Bb}	0.65 ± 0.01 ^{Bb}	0.67 ± 0.01 ^{Cb}
	28	0.83 ± 0.01 ^{Ac}	0.94 ± 0.01 ^{Dc}	0.92 ± 0.01 ^{Cc}	0.87 ± 0.02 ^{Bc}	0.97 ± 0.01 ^{Dc}
	42	0.97 ± 0.01 ^{Bd}	0.98 ± 0.02 ^{Cd}	0.95 ± 0.01 ^{Ad}	1.01 ± 0.01 ^{Dd}	1.03 ± 0.01 ^{Ed}
	56	1.07 ± 0.03 ^{Ce}	1.06 ± 0.01 ^{Be}	1.08 ± 0.00 ^{Ce}	1.01 ± 0.00 ^{Ad}	1.11 ± 0.01 ^{De}
Reducing sugar (mg/g)	0	23.50 ± 0.10 ^{Ae}	27.61 ± 0.13 ^{Ee}	23.90 ± 0.26 ^{Be}	27.19 ± 0.26 ^{De}	24.88 ± 0.15 ^{Ce}
	14	16.75 ± 0.06 ^{Bd}	22.05 ± 0.22 ^{Ed}	20.31 ± 0.10 ^{Dd}	16.17 ± 0.16 ^{Ad}	17.99 ± 0.11 ^{Cd}
	28	10.44 ± 0.10 ^{Aa}	11.42 ± 0.05 ^{Bb}	9.79 ± 0.03 ^{Ab}	11.61 ± 0.08 ^{Bc}	13.41 ± 0.04 ^{Cc}
	42	11.52 ± 0.03 ^{Cc}	12.05 ± 0.16 ^{Dc}	10.52 ± 0.15 ^{Ac}	11.07 ± 0.04 ^{Bb}	12.55 ± 0.04 ^{Eb}
	56	10.78 ± 0.04 ^{Eb}	10.11 ± 0.08 ^{Ca}	7.48 ± 0.01 ^{Aa}	9.29 ± 0.06 ^{Ba}	10.24 ± 0.11 ^{Da}
Total viable bacteria (log CFU/g)	0	6.52 ± 0.05 ^{Da}	6.11 ± 0.08 ^{Aa}	6.41 ± 0.01 ^{CDa}	6.27 ± 0.03 ^{Ba}	6.34 ± 0.01 ^{BCa}
	14	8.74 ± 0.03 ^{Be}	8.56 ± 0.02 ^{Ae}	8.74 ± 0.04 ^{Be}	8.60 ± 0.02 ^{Ae}	8.72 ± 0.05 ^{Bd}
	28	7.97 ± 0.04 ^{BCd}	8.12 ± 0.01 ^{Dd}	8.01 ± 0.03 ^{Cd}	7.94 ± 0.01 ^{ABd}	7.90 ± 0.03 ^{Ac}
	42	7.39 ± 0.02 ^{Ab}	7.94 ± 0.03 ^{Cc}	7.73 ± 0.03 ^{Bc}	7.77 ± 0.02 ^{Bc}	7.98 ± 0.02 ^{Cc}
	56	7.71 ± 0.01 ^{Cc}	7.82 ± 0.03 ^{Db}	7.55 ± 0.06 ^{Bb}	7.41 ± 0.01 ^{Ab}	7.54 ± 0.05 ^{Bb}
Lactic acid bacteria (log CFU/g)	0	6.33 ± 0.01 ^{Da}	6.10 ± 0.01 ^{Aa}	6.30 ± 0.01 ^{Da}	6.16 ± 0.02 ^{Ba}	6.21 ± 0.03 ^{Ca}
	14	8.71 ± 0.05 ^{ABcd}	8.73 ± 0.03 ^{BCe}	8.76 ± 0.02 ^{Ce}	8.59 ± 0.09 ^{Ad}	8.61 ± 0.05 ^{ABe}
	28	7.89 ± 0.03 ^{ABc}	8.10 ± 0.01 ^{Cd}	7.96 ± 0.05 ^{Bd}	7.93 ± 0.01 ^{Bb}	7.84 ± 0.03 ^{Ac}
	42	7.70 ± 0.04 ^{Ab}	7.91 ± 0.01 ^{Cc}	7.69 ± 0.02 ^{Ac}	7.84 ± 0.03 ^{Bc}	7.98 ± 0.02 ^{Dd}
	56	7.67 ± 0.05 ^{Cb}	7.68 ± 0.02 ^{Cb}	7.50 ± 0.01 ^{Bb}	7.40 ± 0.01 ^{Ac}	7.52 ± 0.06 ^{Bb}

⁽¹⁾CTL (control; added 22% saline solution instead of anchovy sauce), T-1 (added TN 1.16% anchovy sauce, A-1), T-2 (added TN 1.41% anchovy sauce, A-2), T-3 (added TN 1.59% anchovy sauce, A-3), T-4 (added TN 1.79% anchovy sauce, A-4)

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

⁽³⁾Mean sharing different letters in the same row (A–E) and column (a–e) are significantly different ($p < 0.05$)

higher nitrogen content of anchovy sauce, resulting in faster aging of kimchi. Thereafter, the acidity of kimchi continued to increase, showing a range of 1.01–1.11% on the 56 day of storage. The highest value of 1.11% was observed in T-4 containing the anchovy sauce with high nitrogen content. During storage period of kimchi, the CTL sample without anchovy sauce showed low acidity, while the T-4 sample containing the anchovy sauce with the highest nitrogen content showed high acidity. This finding was consistent with previous reports by No et al. [6], which showed that addition of anchovy sauce accelerated kimchi fermentation, and the nitrogen content of anchovy sauce affected kimchi fermentation.

Changes in reducing sugar

Changes in reducing sugars content in the kimchi samples prepared with different nitrogen contents of the anchovy sauces are shown in Table 3. Microbes use reducing sugars as nutrients, decomposing them into lactic acid, alcohol, and CO₂ to produce several components. These components mutually balance each other, imparting the unique taste and flavor to kimchi. In addition, reducing sugar content can be used as an indicator of kimchi ripening, microbial growth, and flavor change [23]. The reducing sugar content measured immediately after kimchi production was 23.50–27.61 mg/g, with the lowest value for the CTL. On the 14 day of storage, the reducing sugar content of T-3 rapidly reduced to 16.17 mg/g, and T-1 exhibited a smaller decrease in its sugar content (to 22.05 mg/g) than

the other treated groups. Thereafter, it decreased rapidly until the 28 day of storage to 9.79–13.41 mg/g. As the storage period increased, the reducing sugar content continued to decrease and reached 7.48–10.78 mg/g on the 56 day of storage. As a result, the nitrogen content of the anchovy sauce added during kimchi production did not affect the content of reducing sugar during fermentation. In case of commercial anchovy sauce, the microbial community changed during fermentation [12], and the anchovy source used in this experiment also showed differences in microbial community [18]. Thus, reducing sugar content during kimchi fermentation can affect the sugar content and the microbial community because the ability to use sugar varies depending on the microorganism. Therefore, it is considered that it does not affect the reducing sugar according to the nitrogen content of anchovy sauce.

Changes in levels of volatile basic nitrogen

Volatile basic nitrogen (VBN) is a key indicator of freshness of fish and shellfish. It is not only closely related with flavor, but can also be used as an indicator of abnormal fermentation, such as putrefaction. Although VBN is not an objective quality indicator, it is generally used as an index component in fermentation studies [24]. Changes in VBN contents of the kimchi samples prepared with different nitrogen contents of anchovy sauces are shown in Fig. 1. The VBN contents measured immediately after kimchi production were in the range of 15.51–18.74 mg% without significant differences between the treated groups. A slight increase was observed as the fermentation progressed, exhibiting values between 22.71 and 26.76 mg% on 28 day of fermentation. On the 42 day of fermentation, the VBN contents were in the range of 24.96–29.04 mg% with the lowest value for the CTL and the highest value for T-4, which was prepared using anchovy sauce with the highest nitrogen content. On the 56 day of storage, the values ranged between 27.26 and 31.21 mg% without significant differences between the treated groups. The highest value was observed for the T-4 sample. The increase in VBN content can be associated with the presence of weakly basic substances, such as TMA (trimethylamine), produced from the reduction of TMAO (tetramethylammonium oxalate) and oxidation of phospholipids during storage, as well as with ammonia nitrogen generated from protein decomposition by proliferating bacteria. Among the methods used to evaluate freshness of fish and shellfish, measuring levels of substances that are absent or present only in trace amounts in fresh meat and increase with decline in freshness as indicators is the most representative method [25]. It was also reported that VBN is closely related with fishy smell [26]. Thus, the VBN content of kimchi was highly

Fig. 1 Changes in VBN and AN of Kimchi during fermentation. CTL (control; added 22% saline solution instead of anchovy sauce), T-1 (added TN 1.16% anchovy sauce, A-1), T-2 (added TN 1.41% anchovy sauce, A-2), T-3 (added TN 1.59% anchovy sauce, A-3), T-4 (added TN 1.79% anchovy sauce, A-4). Mean sharing different letters in the same storage period (A–D) are significantly different ($p < 0.05$)

associated with the nitrogen content of anchovy sauce added during kimchi production.

Changes in amino nitrogen contents

Amino nitrogen (AN) is the chemical form of nitrogen present as a free amino group, which can be used as an indicator of food quality, related with the total amount of substances having terminal amino group. High AN content refers to high amount of amino acids produced from decomposition of proteins in a specimen to low molecular weight peptides by microbes or autolytic enzymes [27]. Changes in AN contents of the kimchi samples prepared with anchovy sauces containing different levels of nitrogen are shown in Fig. 1. The AN contents measured immediately after kimchi production were in the range of 89.24–113.39 mg%. The CTL showed the lowest AN content, and the other treated groups showed similar values. On the 14th day of storage, the values ranged between 103.51 and 125.58 mg% with the CTL showing the lowest content and T-4 showing the highest content. On the 28 day of storage, the values ranged between 124.43 and

146.96 mg%. Higher the AN of the added anchovy sauce, higher the AN content. According to Jang et al. [24], the AN content was higher with increasing TN content of anchovy sauce. Consistently, the AN content of kimchi in the present study was also affected by the TN contents of the anchovy sauces used during kimchi production. On the 56 day of storage, the AN content was in the range of 138.15–151.17 mg% without significant differences between the treated groups. The highest AN content was observed for T-4.

Changes in microbial contents

Changes in microbiological properties during storage of the kimchi samples prepared with different nitrogen contents of anchovy sauces are shown in Table 3. For microbes that have direct effects on pH and acidity, the total microbial count increased rapidly as the aging progressed. Thereafter, the inhibition of proliferation of microbes reduced because of the produced acid [22]. The results of this study also indicated a rapid increase in microbial growth, followed by a decrease over increasing storage period. The total microbial count at initial stage ranged between 6.11 and 6.52 log CFU/g with the CTL showing the highest value. On the 14 day of storage, the value was in the range of 8.56–8.74 log CFU/g, indicating a rapid increase in all the treated groups. On the 28 day of storage, the values of all treated groups were in the range of 7.90–8.12 log CFU/g, which decreased thereafter. At this time, the treated T-4 group showed the lowest value. As the storage period increased, the total microbial count continued to decrease. On the 56 day of storage, it showed a value of 7.41–7.82 log CFU/g. The initial count of lactic acid bacteria was 6.10–6.33 log CFU/g. Similarly, as in the case of total microbial count, the CTL showed the highest value. Thereafter, it drastically increased to 8.59–8.76 log CFU/g on 14 day of storage. As the storage period increased, the count of lactic acid bacteria slowly decreased. It ranged between 7.84 and 8.10 log CFU/g on the 28 day of storage and between 7.40 and 7.68 log CFU/g on the 56 day of storage. No et al. [6] reported that the jeotgal added groups required shorter time to reach the optimum value of total microbial count, as compared to the CTL group, confirming the effect of jeotgal on the proliferation of microbes. Nevertheless, no significant difference in bacterial count was observed between the CTL and the treated groups added with anchovy sauces in the present study. Moreover, the nitrogen content of anchovy sauce did not affect the proliferation of microbes.

Changes in free amino acid contents

The results of free amino acid analysis of the kimchi samples prepared according to nitrogen contents of the anchovy sauces are given in Table 4. The free amino acids present in kimchi affected not only taste, but also the proliferation of lactic acid bacteria and activities of related enzymes, which are the most important factors in kimchi fermentation [28]. In the present study, twenty-two free amino acids were detected. Among them, glutamine accounted for about 40% of the total free amino acids. Its concentration was found to be significantly higher than that of the other amino acids, and its content in the anchovy sauce-added groups was higher, as compared to that in the CTL. An increase was observed in the total amino acid content of the CTL, while a decrease was observed in those of the anchovy sauce-added groups during the storage period and there was no significant difference between the treatment groups. There was almost no difference between the contents of the treated groups in the late period of fermentation except T-4. Aspartic acid and glutamic acid are amino acids with sour taste [29]. The experimental results confirmed higher contents of aspartic acid and glutamic acid with longer storage period, while the CTL exhibited a lower content compared to the other treated groups with anchovy sauce. This finding was consistent with the results reported by Cho et al. [30]. In particular, the sour and umami glutamic acid is a free amino acid mainly present in anchovy sauce. A higher initial content was observed from the anchovy sauce added groups compared to the CTL. As storage period increased, all treated groups showed an increase in glutamic acid content. The increase of glutamic acid may be influenced by the anchovy sauce, but it is considered that it affects the microbial community in kimchi of each treatment group. Previous research reported that the major dominant bacteria of the anchovy sauce were of the genera *Halanaerobium*, *Lactobacillus*, *Leuconostoc*, and *Tetragenococcus* [18]. Therefore, the increment of glutamic acid may be different depending on the microbial community of kimchi. The arginine content measured immediately after kimchi production was 48.15–62.59 mg/100 g. The high content of arginine was initially observed, which decreased drastically to 1.21–1.61 mg/100 g on the 56 day of storage. The asparagine, serine, alanine, and GABA contents of the CTL increased with longer storage period, while those of the groups with anchovy sauce, it remained constant. Since the jeotgal used during kimchi production contains a high amount of free amino acids, the total free amino acids contents measured immediately after kimchi production were in a range of 834.69–1075.05 mg/100 g. The CTL showed the lowest content, while there were no significant differences between the treated groups with anchovy

Table 4 Changes in free amino acid of Kimchi during fermentation

	Fermentation period (days)	CTL ⁽¹⁾	T-1				T-2				T-3				T-4			
			(mg/100 g)															
Aspartic acid	0	22.91 ± 0.48 ^{(2)Aa(3)}	31.90 ± 1.68 ^{Ca}	29.55 ± 0.44 ^{Ba}	38.07 ± 0.61 ^{Da}	29.27 ± 0.23 ^{Ba}												
	28	30.29 ± 0.87 ^{Ab}	42.82 ± 1.41 ^{Db}	38.11 ± 0.97 ^{Cb}	34.64 ± 0.10 ^{Bb}	41.14 ± 1.84 ^{CDb}												
	56	36.05 ± 0.75 ^{Ac}	34.79 ± 1.38 ^{Aa}	39.10 ± 0.51 ^{Bb}	42.61 ± 0.50 ^{Cc}	45.48 ± 0.96 ^{Dc}												
Glutamic acid	0	35.58 ± 0.70 ^{Aa}	44.17 ± 2.11 ^{Ba}	47.85 ± 0.65 ^{Ca}	54.55 ± 0.79 ^{Db}	48.35 ± 0.11 ^{Ca}												
	28	42.70 ± 1.19 ^{Ab}	54.33 ± 1.55 ^{Cb}	52.59 ± 1.10 ^{Cb}	48.32 ± 0.06 ^{Ba}	46.84 ± 1.91 ^{Ba}												
	56	56.83 ± 1.31 ^{Bc}	51.82 ± 2.04 ^{Ab}	64.83 ± 0.69 ^{Cc}	60.21 ± 0.78 ^{Bc}	66.40 ± 1.36 ^{Cb}												
Asparagine	0	71.70 ± 1.50 ^{Aa}	76.97 ± 3.65 ^{Ab}	75.98 ± 0.76 ^{Ab}	83.38 ± 1.93 ^{Bc}	72.00 ± 0.13 ^{Ab}												
	28	72.08 ± 1.90 ^{Bab}	74.19 ± 1.74 ^{Bab}	74.41 ± 1.08 ^{Bb}	66.30 ± 0.50 ^{Aa}	63.08 ± 2.15 ^{Aa}												
	56	77.49 ± 1.70 ^{Cb}	65.93 ± 2.42 ^{Aa}	70.27 ± 0.66 ^{Ba}	70.97 ± 0.70 ^{Bb}	62.73 ± 1.12 ^{Aa}												
Serine	0	27.34 ± 0.15 ^{Aa}	37.03 ± 1.86 ^{Cb}	32.27 ± 0.27 ^{Ba}	38.04 ± 1.09 ^{Cb}	33.88 ± 0.02 ^{Ba}												
	28	31.63 ± 0.93 ^{Ab}	37.09 ± 0.82 ^{Bb}	34.48 ± 0.29 ^{Bb}	29.74 ± 0.10 ^{Aa}	36.61 ± 1.19 ^{Bbc}												
	56	40.12 ± 0.82 ^{Cc}	31.75 ± 1.28 ^{Aa}	38.39 ± 0.38 ^{Cc}	38.67 ± 0.37 ^{Cb}	36.19 ± 0.60 ^{Bb}												
Glutamine	0	344.24 ± 1.45 ^{Aa}	456.20 ± 22.67 ^{Cc}	463.03 ± 5.90 ^{Cc}	408.09 ± 10.66 ^{Bb}	470.37 ± 0.18 ^{Cc}												
	28	381.91 ± 9.53 ^{Bb}	384.36 ± 8.55 ^{Bb}	409.77 ± 5.38 ^{Cb}	342.12 ± 0.32 ^{Aa}	388.26 ± 13.08 ^{BCb}												
	56	382.29 ± 8.37 ^{Cb}	313.29 ± 11.68 ^{Aa}	353.78 ± 3.17 ^{Ba}	357.20 ± 3.62 ^{Ba}	353.52 ± 6.35 ^{Ba}												
Histidine	0	14.84 ± 0.09 ^{Aa}	16.71 ± 0.78 ^{Ba}	17.91 ± 0.47 ^{Ca}	18.52 ± 0.14 ^{Ca}	18.87 ± 0.19 ^{Ca}												
	28	29.60 ± 0.27 ^{ABb}	33.02 ± 0.93 ^{Bb}	42.12 ± 0.80 ^{Cb}	28.40 ± 0.55 ^{Ab}	42.41 ± 2.69 ^{Cc}												
	56	34.24 ± 0.68 ^{Bc}	31.30 ± 1.43 ^{Ab}	40.59 ± 0.41 ^{Db}	37.01 ± 0.52 ^{Cc}	34.14 ± 0.46 ^{Bb}												
Glycine	0	12.33 ± 0.05 ^{Aa}	15.91 ± 0.78 ^{Ba}	16.29 ± 0.15 ^{Ba}	19.01 ± 0.69 ^{Ca}	17.55 ± 0.20 ^{Ca}												
	28	16.88 ± 0.51 ^{Ab}	22.24 ± 0.31 ^{Cb}	21.44 ± 0.00 ^{Cb}	19.79 ± 0.15 ^{Ba}	23.24 ± 0.57 ^{Cb}												
	56	23.29 ± 0.51 ^{Bc}	21.39 ± 0.91 ^{Ab}	24.77 ± 0.36 ^{Cc}	25.43 ± 0.31 ^{Cb}	27.57 ± 0.58 ^{Dc}												
Threonine	0	20.66 ± 0.12 ^{Aa}	21.71 ± 1.03 ^{ABa}	23.12 ± 0.30 ^{BCa}	22.96 ± 0.69 ^{BCa}	23.40 ± 0.11 ^{Ca}												
	28	24.50 ± 0.71 ^{Ab}	27.91 ± 0.89 ^{Bb}	28.72 ± 0.75 ^{Bb}	26.01 ± 0.07 ^{Ab}	25.63 ± 0.88 ^{Ab}												
	56	30.03 ± 0.56 ^{Bc}	29.88 ± 1.07 ^{Bb}	30.73 ± 0.24 ^{Bc}	28.10 ± 0.45 ^{Ac}	30.78 ± 0.44 ^{Bc}												
Arginine	0	54.60 ± 0.66 ^{Bb}	54.66 ± 2.62 ^{Bb}	52.12 ± 0.54 ^{Bb}	62.59 ± 1.45 ^{Cb}	48.15 ± 0.02 ^{Ab}												
	28	1.23 ± 0.03 ^{Aa}	1.57 ± 0.13 ^{Ba}	1.49 ± 0.05 ^{Ba}	1.14 ± 0.03 ^{Aa}	1.53 ± 0.12 ^{Ba}												
	56	1.47 ± 0.03 ^{Ba}	1.21 ± 0.09 ^{Aa}	1.41 ± 0.05 ^{Ba}	1.45 ± 0.05 ^{Ba}	1.61 ± 0.00 ^{Ca}												
Alanine	0	62.70 ± 0.27 ^{Aa}	81.40 ± 4.05 ^{Bb}	91.03 ± 1.33 ^{CDb}	86.51 ± 2.49 ^{BCb}	95.46 ± 0.37 ^{Dc}												
	28	71.62 ± 1.82 ^{Ab}	87.36 ± 1.88 ^{Db}	81.65 ± 0.72 ^{Ca}	77.43 ± 0.17 ^{Ba}	81.31 ± 2.12 ^{BCa}												
	56	93.28 ± 1.96 ^{Cc}	70.86 ± 2.87 ^{Aa}	94.94 ± 0.88 ^{Cc}	85.11 ± 1.35 ^{Bb}	88.57 ± 1.42 ^{Bb}												
Taurine	0	5.15 ± 0.03 ^{Ab}	6.52 ± 0.56 ^{Bb}	6.47 ± 0.09 ^{Bb}	8.45 ± 0.17 ^{Cb}	6.81 ± 0.12 ^{Ba}												
	28	4.47 ± 0.01 ^{Aa}	6.04 ± 0.06 ^{Bab}	6.18 ± 0.28 ^{BCb}	5.94 ± 0.23 ^{Ba}	6.78 ± 0.40 ^{Ca}												
	56	4.51 ± 0.03 ^{Aa}	5.31 ± 0.24 ^{Ba}	5.54 ± 0.02 ^{Ba}	6.09 ± 0.26 ^{Ca}	8.28 ± 0.25 ^{Db}												
GABA	0	43.20 ± 0.23 ^{Aa}	56.20 ± 3.33 ^{Cb}	46.47 ± 0.39 ^{ABa}	59.09 ± 2.61 ^{Cc}	48.52 ± 0.26 ^{Ba}												
	28	47.48 ± 1.06 ^{Bb}	54.30 ± 0.89 ^{Cab}	54.28 ± 0.23 ^{Cc}	43.96 ± 0.17 ^{Aa}	67.99 ± 1.44 ^{Db}												
	56	57.50 ± 1.20 ^{Cc}	48.55 ± 2.11 ^{Aa}	52.46 ± 0.61 ^{Bb}	54.08 ± 0.46 ^{Bb}	48.17 ± 0.74 ^{Aa}												

Table 4 continued

	Fermentation period (days)	CTL ⁽¹⁾	T-1				T-2		T-3		T-4	
			(mg/100 g)									
Tyrosine	0	10.87 ± 0.32 ^{Aa}	12.08 ± 0.49 ^{Ba}	10.94 ± 0.06 ^{Aa}	13.14 ± 0.18 ^{Ba}	10.90 ± 0.06 ^{Aa}						
	28	13.62 ± 0.32 ^{Ab}	14.96 ± 0.24 ^{BCb}	14.43 ± 0.23 ^{ABb}	13.70 ± 0.06 ^{Aa}	15.48 ± 0.56 ^{Cb}						
	56	17.80 ± 0.37 ^{Cc}	15.63 ± 0.63 ^{Ab}	16.54 ± 0.13 ^{ABc}	17.49 ± 0.24 ^{BCb}	17.28 ± 0.27 ^{BCc}						
Valine	0	19.22 ± 0.27 ^{Aa}	24.96 ± 1.24 ^{Ba}	24.79 ± 0.26 ^{Ba}	30.04 ± 0.80 ^{Ca}	24.61 ± 0.09 ^{Ba}						
	28	24.58 ± 0.79 ^{Ab}	29.63 ± 0.72 ^{Bb}	29.62 ± 0.02 ^{Bb}	29.12 ± 0.22 ^{Ba}	30.40 ± 0.99 ^{Bb}						
	56	28.37 ± 0.09 ^{Ac}	28.82 ± 1.42 ^{Ab}	31.86 ± 0.48 ^{Bc}	33.50 ± 0.67 ^{Bb}	33.45 ± 0.50 ^{Bc}						
Methionine	0	4.40 ± 0.08 ^{Aa}	6.39 ± 0.39 ^{Ba}	6.09 ± 0.09 ^{Ba}	7.59 ± 0.19 ^{Ca}	6.30 ± 0.02 ^{Ba}						
	28	6.18 ± 0.25 ^{Ab}	8.51 ± 0.16 ^{CDb}	8.27 ± 0.04 ^{BCb}	7.87 ± 0.10 ^{Ba}	8.91 ± 0.17 ^{Db}						
	56	8.42 ± 0.09 ^{Ac}	8.42 ± 0.45 ^{Ab}	9.19 ± 0.04 ^{Bc}	10.21 ± 0.26 ^{Cb}	10.84 ± 0.14 ^{Cc}						
Tryptophane	0	4.19 ± 0.14 ^{Aa}	4.71 ± 0.13 ^{Ba}	4.18 ± 0.18 ^{Aa}	6.24 ± 0.03 ^{Cc}	4.54 ± 0.21 ^{ABa}						
	28	6.17 ± 0.26 ^{ABb}	6.92 ± 0.36 ^{ABb}	7.29 ± 0.11 ^{ABb}	5.90 ± 0.15 ^{Ab}	7.63 ± 1.18 ^{Bb}						
	56	5.40 ± 0.41 ^{Ab}	5.53 ± 0.46 ^{Aa}	6.95 ± 0.69 ^{Bb}	5.58 ± 0.05 ^{Aa}	5.07 ± 0.00 ^{Aa}						
Phenylalanine	0	12.44 ± 0.40 ^{Aa}	16.01 ± 0.69 ^{Ba}	15.44 ± 0.18 ^{Ba}	18.81 ± 0.38 ^{Cb}	15.05 ± 0.00 ^{Ba}						
	28	15.45 ± 0.12 ^{Ab}	19.11 ± 0.36 ^{Cb}	18.51 ± 0.17 ^{Cb}	17.40 ± 0.10 ^{Ba}	19.28 ± 0.56 ^{Cb}						
	56	20.48 ± 0.43 ^{Bc}	19.25 ± 0.71 ^{Ab}	20.78 ± 0.25 ^{Bc}	22.57 ± 0.36 ^{Cc}	22.61 ± 0.31 ^{Cc}						
Isoleucine	0	13.69 ± 0.28 ^{Aa}	17.53 ± 1.01 ^{Ba}	17.99 ± 0.22 ^{Ba}	19.61 ± 0.36 ^{Cb}	17.31 ± 0.03 ^{Ba}						
	28	15.65 ± 0.13 ^{Ab}	19.75 ± 0.33 ^{Ca}	20.33 ± 0.19 ^{Cb}	18.59 ± 0.05 ^{Ba}	19.54 ± 0.62 ^{Cb}						
	56	20.35 ± 0.44 ^{Ac}	19.47 ± 0.75 ^{Aa}	21.75 ± 0.30 ^{Bc}	21.74 ± 0.28 ^{Bc}	22.52 ± 0.36 ^{Bc}						
Leucine	0	16.91 ± 0.62 ^{Aa}	23.26 ± 1.04 ^{Ca}	21.06 ± 0.26 ^{Ba}	25.48 ± 0.51 ^{Da}	21.68 ± 0.03 ^{Ba}						
	28	23.65 ± 0.51 ^{Ab}	30.49 ± 0.66 ^{Cb}	28.83 ± 0.22 ^{Bb}	27.54 ± 0.11 ^{Bb}	31.31 ± 1.08 ^{Cb}						
	56	32.06 ± 0.65 ^{ABc}	31.11 ± 1.25 ^{Ab}	33.66 ± 0.36 ^{BCc}	35.30 ± 0.56 ^{CDc}	35.61 ± 0.47 ^{Dc}						
Lysine	0	22.61 ± 0.43 ^{Aa}	30.22 ± 1.78 ^{Aa}	29.51 ± 0.09 ^{Aa}	36.01 ± 1.00 ^{Ca}	30.64 ± 0.09 ^{Aa}						
	28	26.39 ± 0.62 ^{Ab}	34.48 ± 0.92 ^{Ba}	33.93 ± 0.50 ^{Bb}	34.07 ± 0.19 ^{Ba}	35.92 ± 0.07 ^{Cb}						
	56	34.17 ± 0.78 ^{Ac}	34.93 ± 1.50 ^{Aa}	35.52 ± 0.97 ^{Bb}	39.72 ± 0.40 ^{Cb}	41.07 ± 0.84 ^{Cc}						
Proline	0	15.11 ± 3.58 ^{Aa}	18.50 ± 3.18 ^{Aa}	17.52 ± 0.90 ^{Aa}	18.86 ± 7.77 ^{Aa}	15.96 ± 4.60 ^{Aa}						
	28	16.92 ± 1.58 ^{Aa}	17.76 ± 2.96 ^{Aa}	17.61 ± 3.01 ^{Aa}	18.56 ± 0.05 ^{Aa}	15.86 ± 6.76 ^{Aa}						
	56	20.18 ± 1.45 ^{Aa}	18.88 ± 2.87 ^{Aa}	20.27 ± 2.74 ^{Aa}	24.17 ± 1.72 ^{Aa}	24.31 ± 3.94 ^{Aa}						
Total amino acid	0	834.69 ± 3.95 ^{Aa}	1023.04 ± 12.66 ^{Ba}	1042.11 ± 6.68 ^{Ba}	1040.04 ± 15.02 ^{Ba}	1054.62 ± 11.46 ^{Ba}						
	28	902.99 ± 22.90 ^{Ab}	1006.84 ± 19.82 ^{Ba}	1024.07 ± 9.85 ^{Ba}	1030.53 ± 35.71 ^{Ba}	1049.14 ± 29.73 ^{Ba}						
	56	974.34 ± 48.26 ^{Ab}	1015.13 ± 9.26 ^{ABa}	1033.35 ± 15.71 ^{ABa}	1027.19 ± 13.38 ^{ABa}	1041.20 ± 15.13 ^{Ba}						

⁽¹⁾CTL (control; added 22% saline solution instead of anchovy sauce), T-1 (added TN 1.16% anchovy sauce, A-1), T-2 (added TN 1.41% anchovy sauce, A-2), T-3 (added TN 1.59% anchovy sauce, A-3), T-4 (added TN 1.79% anchovy sauce, A-4)

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

⁽³⁾Mean sharing different letters in the same row (A–D) and column (a–c) are significantly different ($p < 0.05$)

Table 5 Correlation coefficient between chemical components of kimchi

Variables	TN	pH	Acidity	VBN	AN	Glutamic acid
TN	1					
pH	0.490	1				
Acidity	0.273	− 0.224	1			
VBN	0.786**	0.192	0.564*	1		
AN	0.700**	0.734**	0.345	0.546*	1	
Glutamic acid	0.786**	0.259	0.649*	0.795*	0.714*	1

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

saucers. The total free amino acid content of the CTL tended to increase with increasing storage period. Groups treated with anchovy sauce initially showed a decrease in the total free amino acid from T-3, and then it tended to increase with increasing storage period. Meanwhile, a decrease was observed in the amino acid content of T-1 from preparation to the 56 day of storage. This finding confirmed that free amino acids were initially used as nutrient sources for proliferation of lactic acid bacteria during fermentation, and proteins were thereafter decomposed as the activity of lactic acid bacteria increased, producing free amino acids [31].

Correlation coefficient between each variables

The Pearson correlation coefficient between TN% of anchovy sauce and pH, acidity, VBN, AN, glutamic acid of kimchi are shown in Table 5. There were highly significant positive correlations between TN of anchovy sauce and VBN (0.786) and AN (0.700), glutamic acid (0.786) of kimchi. Also, there were highly significant positive correlations between VBN and glutamic acid (0.795) in kimchi. Lee et al. [32] and Cho et al. [33] reported that the correlation coefficient between glutamic acid and AN content were higher correlation positive. There are observed that the AN content was higher with increasing glutamic acid content of kimchi.

In conclusion, kimchi was prepared using anchovy saucers with different nitrogen content. Comparison of physico-chemical characteristics showed that there were no significant pH differences between the treated groups during storage. However, the CTL exhibited lower acidity compared to the treated groups. In addition, T-4 sample, which contained anchovy sauce with the highest nitrogen content, showed a slower decline in reducing sugars content compared to the other treated groups. The VBN and AN contents of the CTL were the lowest during the storage period, while those of T-4 increased over the storage period. Thus, the nitrogen content of the anchovy sauce added during kimchi preparation affected the nitrogen contents of samples. Free amino acid analysis showed that glutamine accounted for about 40% of the total free amino acids. A

trend of increasing glutamic acid concentration with increasing nitrogen content of anchovy saucers, which affected the umami taste, was confirmed.

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