Biogenic amine production of makgeollis with controlled alcohol concentrations

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Abstract In this study, we examined the effects of controlling the alcohol concentration of non-sterilized makgeolli on the levels of biogenic amines (BAs) as an indicator of hygiene. Makgeollis were prepared at four different alcohol concentrations (5.73–13.17%) and stored at 20 °C for 30 days. Makgeollis with low alcohol contents (5.73–8.07%) showed significant variations in alcohol and BA concentrations, volatile acid contents, pH, total acidity, and lactic acid bacteria counts under these conditions. In contrast, makgeollis with alcohol contents of 11.47% or more showed no significant changes. In low-alcohol makgeolli, the produced BA was mostly tyramine, which increased dramatically on day 20 of storage in makgeolli containing 5.73% alcohol and on day 30 in makgeolli containing 8.07% alcohol. These results indicated that makgeolli could be stored for long periods at high temperatures, such as those during summer, by controlling the alcohol concentration.

Keywords Non-sterilized makgeolli · Biogenic amine · Lactic acid bacteria · Alcohol concentration · Storage temperature

Introduction

Many Koreans consumed makgeolli because of its various health benefits, including high nutrient content and functionality, with low alcohol content, unlike other alcoholic beverages (Lee et al., 2010). However, makgeolli can be contaminated by bacteria in raw materials or by non-hygienic equipment, environmental factors, or manipulation during production. These contaminated bacteria and spontaneously emerging bacteria during fermentation, including lactic acid bacteria (LAB), can act as harmful factors negatively affecting human health, particularly if the makgeolli is stored at an inappropriate temperature (Jeong et al., 2013; Kim et al., 2011, 2014; Kwak et al., 2014).

Biogenic amines (BAs) are produced by substrate-specific amino acid decarboxylase enzymes and are nitrogenous compounds that have roles in maintaining pH homeostasis in acidic environments. BAs are mainly produced by yeast or LAB during fermentation of foods and beverages (Anli and Bayram, 2009; Doeun et al., 2017). At low concentrations, BAs are metabolized in the human gut to physiologically less active forms by monoamine oxidase and diamine oxidase. These metabolic products then play many critical biological roles in the nervous system and have also been shown to regulate body temperature and blood pressure. However, consumption of foods or beverages containing high amounts of these compounds can have undesirable effects (Anli and Bayram, 2009; Beneduce et al., 2010; Silla Santos, 1996). Moreover, the toxic

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effects of BAs are potentiated in the presence of alcohol, acetaldehyde, and other amines (Souza et al., 2005).

The existence of BAs in foods has traditionally been used as a hygiene indicator because BAs are formed by contaminated microorganisms during the wine-making process (Beneduce et al., 2010; Souza et al., 2005). Importantly, BA accumulation in wine is affected by several factors, such as pH, temperature, amino acid precursors, ethanol concentrations, and sulfur dioxide, thereby influencing the growth of microorganisms (Anli and Bayram, 2009; Fleet, 2003; Smit et al., 2012).

We previously showed that the number and diversity of LAB in non-sterilized makgeolli are markedly increased upon storage at 20 °C as compared with that at 4 °C; additionally, generation of BAs is associated with changes in LAB populations (Kim et al., 2011). In addition, BAs are produced at high levels by inoculation with *Lactobacillus plantarum* strains isolated from makgeolli stored at 20 °C for 21 days, but high levels of BAs are not present in fresh makgeolli (Kwak et al., 2014). This result suggests that large amounts of amino acid are released via proteolysis of raw materials and by self-degradation of many microorganisms during high-temperature storage of makgeolli and that these amino acids are used as a substrate for producing BA (Kim and Han, 2012).

Makgeolli contains 15–18% alcohol at the end of the main fermentation process, and the alcohol concentration (6.69–13.5%) of commercial makgeolli varies greatly (Jung et al., 2014). The produced makgeolli should be refrigerated at below 10 °C during distribution. However, when the cold chain is destroyed during the distribution and storage process in the summer, microorganisms in raw makgeolli can rapidly proliferate and produce BAs (Kim et al., 2011). In our previous studies, in which raw makgeolli was stored at 4 °C, 10 °C, and 20 °C, the physicochemical changes were found to be dependent on temperature; four types of makgeolli stored at 4 °C showed little physicochemical changes, including BA production, during storage for 30 days (Kim et al., 2011; Kim and Han, 2012).

However, the effects of alcohol concentration on BA accumulation in makgeolli are unknown. Determination of the range of alcohol concentrations that can inhibit BA accumulation will facilitate the production of non-sterilized makgeolli with an improved shelf life. Accordingly, in this study, we prepared makgeolli containing various alcohol concentrations and investigated the relationships between alcohol concentrations and BA accumulation.

Materials and methods

Materials and reagents

BA standards (cadaverine, histamine, phenylethylamine, putrescine, tryptamine, tyramine, spermidine, and spermine) and 4-chloro-3,5-dinitrobenzotrifluoride (CNBF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile (Thermo Fisher Scientific, Waltham, MA, USA) and acetic acid (Showa, Tokyo, Japan) were used as analytical eluents. All other chemicals were from Sigma-Aldrich.

Makgeolli sample preparation

Makgeolli mash was prepared from 10 kg steamed nonglutinous Odae cultivar rice (Cheolwon, Korea) as a raw material, 300 g nuruk (Gaeryang-nuruk, 1000 sp/g; Korea Enzyme, Hwaseong, Korea) as a saccharogenic agent, 7 g *Saccharomyces cerevisiae* (La Parisienne S.I. Lesaffre Co., Marcq-en-Baroeul, France) as fermenting yeast, and 25 L tap water. Nuruk, a Korean mold bran, was prepared by inoculating moistened ground wheat or rice with various airborne microorganisms, including fungi, yeasts, and bacteria (Jeong et al., 2013). Fermentation proceeded at 25 °C for 5 days. After fermentation, the makgeolli mash was transferred to a filter cloth and roughly squeezed, leaving behind the lees. The alcohol concentration of the mash was 13%. The mash was diluted with water to make an original makgeolli of 6.55%. In general, the alcohol concentration of makgeolli could be controlled by manipulating the degree of dilution after fermentation of the makgeolli mash. However, the purpose of this study was to examine differences in BA production solely according to the alcohol concentration of makgeolli; other factors, such as the concentration of solid components in makgeolli, had to be kept as constant as possible. Therefore, instead of using the traditional method, the makgeolli in this study was titrated with water or 95% purified ethanol (Duksan Pure Chemicals, Ansan, Korea) as shown in Table 1. Four types of makgeolli with different alcohol concentrations (6, 8, 10, and 12%) were produced. For the calculation for alcohol adjustment, for example, 6% makgeolli in a 3 L volume was produced using $3 \times (6/7) \cong 2.57$ L of 7% original makgeolli and 0.43 L of water. However, the actual initial alcohol concentrations measured after the adjustments were 5.73% (M1), 8.07% (M2), 11.43% (M3), and 13.17% (M4). Samples were stored in 750 mL bottles at 20 °C and monitored for 30 days.

Table 1 Preparation of makgeolli samples with different alcohol concentrations

Samples	Desired alcohol conc. (%)	Original makgeolli ¹ (mL)	Added water (mL)	Refined spirits ² (mL)	Measured alcohol conc. (%)	Total volume (mL)
M1	6	2748.09	251.91	0	5.73	3000
M2	8	2950.82	0	49.18	8.07	3000
M3	10	2882.98	0	117.02	11.43	3000
M4	12	2815.15	0	184.85	13.17	3000

¹The original makgeolli was fermented at 25 °C for 5 days, and the final alcohol concentration was 6.55%

²Ethanol concentration of 95%

Chemical properties of makgeolli samples after fermentation

The pH of makgeolli samples was measured using an Orion 3 star pH meter (Thermo Fisher Scientific). Total acid concentrations were converted from titratable acidity (as acetic acid), which was measured by adding 10 mL sample to 50 mL deionized water and titrating with 0.1 N NaOH to pH 8.3 (Jung et al., 2014). The alcohol concentration of each sample was determined using an alcoholmeter (Model 211-DK-12; Daekwang Industries, Hwaseong, Korea) after water distillation according to the Analysis Regulation for Alcohol Beverages protocol (NTS, 2009). The specific gravity of the ethanol–water mixture was measured at 15 °C using an alcoholmeter. Measurements were calibrated to alcohol content (% v/v) according to the Alcohol-Temperature Conversion Table (NTS, 2009). The amount of volatile acid was determined using the methods recommended by the Analysis Regulation for Alcoholic Beverages (NTS, 2009).

Total viable LAB counts

Makgeolli samples were spread on 1% (w/v) de Man-Rogosa Sharpe (MRS) agar (BD, Le Pont-de-Claix, France) and incubated at 37 °C for 20 h, after which viable LAB were counted.

Detection of BAs during makgeolli storage

Makgeolli samples (1 mL) were centrifuged at 13,000 rpm for 5 min, and the supernatant (0.2 mL) was stored at – 20 °C after passage through a 0.2 µm cellulose acetate filter. For the derivatization reaction, 100 µL CNBF methanol solution (18.5 mM) was added to a 1 mL vial containing 200 µL mixed BAs or supernatant containing 300 µL H₃BO₃–Na₂B₄O₇ buffer (pH 9.5). Each solution was diluted to 1 mL with water and allowed to react at 65 °C for 30 min. The reaction was terminated by adding 10 µL of 2 M HCl. BA content in the makgeolli samples

was analyzed by HPLC (Younglin, Anyang, Korea) equipped with an ultraviolet detector at 254 nm (Kim et al., 2011). Chromatographic separation was performed on a reversed-phase Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA) with a 1 mL flow rate at 35 °C. Acetonitrile (solution A) and HAc–NaAc buffer (0.1 M, pH 6.2; solution B) were used as gradient elution solutions. The linear gradient was programmed starting from 70% of eluent A to reach 100% by 22 min. Each sample was assayed in triplicate.

Statistical analysis

Statistical analyses were carried out using SPSS v.12.0 software (SPSS Inc., Chicago, IL, USA). Values are expressed as means ± standard deviations. Differences between mean values were compared by two-way analysis of variance followed by Duncan's multiple range test, with *P* values of less than 0.05 considered significant. Pearson's correlation analysis was also performed.

Results and discussion

Changes in alcohol content during storage

Four types of makgeolli, with alcohol concentrations of 5.73% (M1), 8.07% (M2), 11.43% (M3), and 13.17% (M4), were prepared by diluting the original liquor with a high alcohol concentration. The changes in alcohol concentration were examined by storing these makgeolli samples at 20 °C (Table 2). After 30 days of storage, the alcohol concentration of M1 decreased significantly from 5.73 to 4.03%, and that of M2 decreased from 8.07 to 7.03%. However, there was no change in the alcohol concentration of M3, which had a high initial alcohol concentration, and alcohol concentration was significantly increased from 13.17 to 14.03% in M4. In contrast, when the four types of

Table 2 Changes in alcohol content (%) of makgeolli during storage at 20 °C

Samples	Storage period (days)					
	0	5	10	15	20	30
M1	5.73 ± 0.06 ^{1D2a3}	5.17 ± 0.15 ^{Db}	4.73 ± 0.25 ^{Dc}	3.50 ± 0.20 ^{De}	5.00 ± 0.20 ^{Dbc}	4.03 ± 0.15 ^{Dd}
M2	8.07 ± 0.21 ^{Ca}	8.40 ± 0.00 ^{Ca}	8.30 ± 0.10 ^{Ca}	7.43 ± 0.21 ^{Cb}	8.10 ± 0.10 ^{Ca}	7.03 ± 0.55 ^{Cb}
M3	11.43 ± 0.35 ^{Bb}	11.60 ± 0.00 ^{Bab}	11.97 ± 0.12 ^{Ba}	11.73 ± 0.31 ^{Bab}	10.30 ± 0.10 ^{Bc}	11.57 ± 0.35 ^{Bab}
M4	13.17 ± 0.21 ^{Ac}	13.73 ± 0.12 ^{ABb}	14.07 ± 0.12 ^{Aa}	13.60 ± 0.00 ^{Ab}	13.17 ± 0.21 ^{Ac}	14.03 ± 0.12 ^{Aa}

¹Alcohol content (%). Values represent means ± standard deviations (n = 3)

²Means in a column with different superscripts are statistically significant at the 5% significance level, as determined by Duncan's multiple range test

³Means in a row with different superscripts are statistically significant at the 5% significance level, as determined by Duncan's multiple range test

makgeollis were stored at 4 °C, only M1 showed a slight decrease in alcohol concentration (data not shown).

Similar to wine, makgeollis are ripened and fermented by interactions among yeasts, fungi, and bacteria, including LAB and acetic acid bacteria (Fleet, 2003). Non-sterilized makgeolli contains living microorganisms that can alter alcohol content via oxidation of ethanol to acetaldehyde and acetic acid during storage (Lonvaud-Funel, 1996). The temperature of 20 °C is too high to store non-sterilized makgeolli due to the growth of microorganisms, such as acetic acid bacteria, and at this temperature, microorganisms can change the alcohol concentration during storage. Alcohol oxidation by the growth of acetic acid bacteria may be accelerated at high temperatures and low alcohol concentrations. Therefore, for M1 and M2 makgeollis having low alcohol concentrations, alcohol decomposition would be accelerated by the actions of microorganisms, such as acetic acid bacteria, which were not inhibited by alcohol.

Changes in pH and total acid content during storage

The initial pH of makgeollis ranged from 3.50 to 3.69; the pH value of M1 was significantly lower than that of the other makgeollis. Greater increases in pH were observed for M1 and M2 than for M3 and M4 after 30 days of storage (Fig. 1A). Despite the increase in LAB in M1 and M2, the pH of M1 and M2 increased during the storage period. This result could be explained by the observations that the LAB can generate amines for themselves to adapt to the acid and that these materials are alkaline (Kim et al., 2012).

The total acid concentration was also affected by storage temperature. The greatest effect was observed in M1; total acid, which was 0.20% at the initial storage of experiment, increased rapidly to 0.29% at day 15 but decreased to 0.17% at day 20. On the other hand, M2 showed the highest

acid concentration of 0.23% at day 20, but decreased slightly at day 30. In contrast, the concentrations of M3 and M4, which had the highest alcohol concentrations, increased slightly throughout the 30 day period (Fig. 1B).

The increase in the total acidity of makgeolli could be explained by proliferation of the LAB over time (Fig. 1D). The total acid contents in makgeolli mostly originate from the processing of nuruk or raw materials during fermentation (Kim et al., 2012), and all organic acids in makgeolli are generated by the activities of yeast and LAB (Jeon and Lee, 2011; Lee and Shim, 2010). Thus, changes in pH and total acid content during storage reflect the levels of contamination during fermentation. In general, the pH of makgeolli decreases slightly during this process (Song et al., 2006).

Our results indicated that an increase in total acid content did not always correspond to pH reduction. Notably, the decomposition of residual protein in makgeolli is assumed to increase amino acid contents via yeast autolysis, functioning as a buffer during storage (Kim and Han, 2012). Additionally, the BA produced from the makgeolli causes the pH to be recovered by consuming protons and releasing amines and carbon dioxide during the decarboxylation of the precursor amino acid, thereby increasing the survival rate of LAB under acidic stress conditions (Spano and Massa, 2006).

Changes in volatile acid concentrations

Changes in the volatile acid concentrations of makgeollis stored at 20 °C were also similar to those of total acids. In other words, the makgeollis with high alcohol concentrations, i.e., M3 and M4, showed only minor changes during the storage period of 30 days, whereas the volatile acid concentration of M1 increased rapidly from 31.53 to 63.67 ppm at the beginning to 567.53 ppm on day 15, followed by a sharp decrease on day 20 (Fig. 1C).

Fig. 1 Changes in the pH (A), total acids (B), volatile acids (C), and lactic acid bacteria (D) of makgeolli during storage at 20 °C. M1, M2, M3, and M4 had initial alcohol concentrations of 5.73, 8.07, 11.43, and 13.17%, respectively

Most volatile compounds (e.g., fuel alcohols, acetate esters, and ethyl esters produced from rice through yeast metabolism), when present in excess, generate an off flavor in alcoholic beverages (Kang et al., 2014). Additionally, acetic acids, ethanol metabolites of oxidation by acetic acid bacteria, can spoil foods and alcoholic beverages (Raspor and Goranovic, 2008). The presence of volatile compounds in makgeolli is determined by the amount of acetic acids in makgeolli distillate, which is an indicator of acidic putrefaction. Makgeolli may contain airborne and nuruk-derived acetic acid bacteria (Lee et al., 2009). In this study, a negative correlation was observed between the volatile acid concentration and alcohol content ($P < 0.01$). In contrast, the yeast count in makgeolli stored at 20 °C was unaffected by alcohol concentration (data not shown). These results suggested that the alcohol level of low-alcohol makgeolli decreased during storage because the alcohol was turned into acetic acid by acetic acid bacteria (Table 2 and Fig. 1C).

Changes in viable LAB counts

Changes in LAB according to the storage period of makgeolli also varied according to the initial alcohol concentration. In M1, the LAB began to increase rapidly from day 5 of the storage period, reaching a peak value on day 20 and then decreasing again. In M2, LAB began to increase rapidly from day 10 and continued to increase gradually until day 30 afterwards. In contrast, in M3 and M4, the LAB tended to decrease slightly until day 20, but then increased again, still remaining lower than the initial LAB value. LAB counts of M1, M2, M3, and M4 at day 20 were 1.4×10^8 , 7.2×10^7 , 7.8×10^6 , and 3.0×10^6 CFU/mL, respectively. Thus, LAB growth was completely inhibited at alcohol concentrations greater than 11%, even after storage at a relatively high temperature (Fig. 1D).

LAB such as *Lactobacillus hilgardii*, *Lactobacillus brevis*, and *Leuconostoc oenos* are tolerant to ethanol concentrations greater than 10%, whereas in most other bacteria, growth is inhibited in a dose-dependent manner at

ethanol concentrations of 1–10% (Couto et al., 1996; Mazzoli et al., 2009; Moreno-Arribas and Lonvaud-Funel, 1999; Rollan et al., 1995). Histamine production in *Lactobacillus hilgardii* ISE 5211, isolated from Italian red wine, was shown to be altered by ethanol when the cells were cultured in histidine-supplemented MRS medium (Moreno-Arribas and Lonvaud-Funel, 1999). Bacterial growth and histamine production were inhibited at ethanol concentrations greater than 13% but were unaffected by concentrations less than 9%. However, histamine continuously accumulated, even without bacterial growth, at an ethanol concentration of 11%. Moreover, cell suspensions and extracts of *Lactobacillus brevis* IOEB 9809 and *Lactobacillus brevis* ATCC 367, both of which are potent tyramine producers isolated from wine, had no effect on tyrosine decarboxylase activity at ethanol concentrations of 0–10%. However, activity was slightly decreased in cell suspensions containing 12% ethanol, which is the average concentration of wine (Moreno-Arribas and Lonvaud-Funel, 1999). These findings indicated that the inhibitory effects of ethanol on LAB in wine were similar to those in makgeolli with different ethanol concentrations.

Therefore, LAB growth at a concentration of 11% or less also decreases depending on the alcohol concentration.

BA production

We previously reported that storage temperature is critical for BA production in makgeolli (Kim et al., 2011). Here, we found that alcohol concentration, in addition to storage temperature, was an important determinant of BA production in makgeolli. The total amounts of BAs in M3 and M4 during the storage period varied in narrow ranges, from 30.3 to 38.5 mg/L and 30.1 to 40.2 mg/L, respectively. Similar changes were observed at 4 °C (29.0–40.8 mg/L) throughout the entire storage period of 30 days (data not shown). However, the total amounts in M1 and M2 at 20 °C increased sharply on days 20 and 30, respectively, with the highest amounts observed on day 30 (491.1 and 301.8 mg/L, respectively; Table 3). Therefore, these findings demonstrated that the production of BA of makgeolli was completely inhibited by alcohol concentrations of 11.47% or more.

Table 3 Biogenic amine production in makgeolli with different alcohol contents during storage at 20 °C

Samples	Storage period (days)	Biogenic amine concentration (mg/L)					
		Tyramine	Putrescine	Cadaverine	Phenyl-ethylamine	Spermidine	Total BA
M1	0	7.64 ± 1.63 ^{c12}	20.34 ± 0.38 ^c	10.65 ± 0.50 ^{bc}	ND ³	ND	38.64 ± 3.55 ^c
	5	5.62 ± 0.22 ^c	22.16 ± 0.30 ^b	9.48 ± 0.30 ^c	ND	ND	37.25 ± 1.16 ^c
	10	5.42 ± 0.66 ^c	24.10 ± 0.22 ^a	10.25 ± 0.90 ^{bc}	ND	ND	39.77 ± 1.30 ^c
	20	321.60 ± 1.69 ^b	ND	24.04 ± 0.21 ^a	5.14 ± 5.14 ^b	3.89 ± 0.96 ^a	354.66 ± 3.21 ^b
	30	448.16 ± 39.26 ^a	ND	12.57 ± 1.05 ^b	26.07 ± 0.66 ^a	4.25 ± 0.22 ^a	491.05 ± 57.63 ^a
M2	0	8.78 ± 0.16 ^c	18.74 ± 0.17 ^a	9.73 ± 0.79 ^b	ND	ND	37.24 ± 1.14 ^b
	5	4.59 ± 0.05 ^d	18.15 ± 1.12 ^a	8.69 ± 0.05 ^b	ND	ND	31.43 ± 1.73 ^b
	10	4.44 ± 0.41 ^d	21.40 ± 1.6 ^a	10.04 ± 0.13 ^b	ND	ND	35.88 ± 6.20 ^b
	20	11.94 ± 0.22 ^b	19.96 ± 0.41 ^a	8.59 ± 0.23 ^b	ND	1.25 ± 1.25	41.73 ± 1.21 ^b
	30	280.48 ± 1.27 ^a	ND	13.51 ± 0.63 ^a	4.57 ± 4.57	3.26 ± 0.52	301.81 ± 8.41 ^a
M3	0	8.29 ± 0.85	18.21 ± 1.55 ^b	9.34 ± 0.33 ^{ab}	ND	ND	35.84 ± 3.86
	5	5.42 ± 0.23	23.45 ± 0.45 ^a	9.67 ± 0.21 ^b	ND	ND	38.54 ± 1.25
	10	4.58 ± 0.16	21.92 ± 0.21 ^a	9.87 ± 0.26 ^b	ND	ND	36.37 ± 1.79
	20	6.46 ± 0.66	16.41 ± 0.85 ^b	7.47 ± 0.64 ^c	ND	ND	30.34 ± 3.05
	30	5.25 ± 1.61	17.64 ± 0.95 ^b	7.90 ± 0.50 ^{bc}	ND	ND	30.78 ± 1.63
M4	0	8.56 ± 0.14 ^a	18.84 ± 0.01	9.11 ± 0.08	ND	ND	36.51 ± 0.11
	5	4.10 ± 1.26 ^c	19.12 ± 5.22	7.90 ± 1.37	ND	ND	31.11 ± 11.09
	10	4.64 ± 0.33 ^{bc}	25.49 ± 0.88	10.05 ± 0.12	ND	ND	40.17 ± 3.78
	20	6.17 ± 0.41 ^{abc}	16.36 ± 1.33	7.62 ± 0.45	ND	ND	30.15 ± 3.10
	30	6.64 ± 0.54 ^{ab}	18.91 ± 2.87	8.51 ± 0.53	ND	ND	34.06 ± 5.59

¹Values represent means ± standard deviations (n = 3)

²Means in a column with different superscripts are significantly different at the 5% significance level, as determined by Duncan's multiple range test

³Not detected

In the four makgeolli samples with different alcohol concentrations, three commonly detected BAs were tyramine, putrescine, and cadaverine. Phenylethylamine and spermidine were detected only in makgeolli with low alcohol concentrations stored for more than 20 days. The most important BAs in foods and beverages are histamine, tyramine, putrescine, cadaverine, and β -phenylethylamine, which are products of the decarboxylation of histidine, tyrosine, ornithine, lysine, and β -phenylalanine, respectively (Doeun et al., 2017). BA production has also been shown to be closely associated with the availability of LAB amino acid decarboxylase and the presence of amino acid precursors in foods and alcoholic beverages (Doeun et al., 2017; Mazzoli et al., 2009; Moreno-Arribas and Lonvaud-Funel, 1999; Silla Santos, 1996). In this study, the major BA produced in low alcoholic makgeolli was tyramine, accounting for 91.2% (M1) and 92.9% (M2) of total BA produced on day 30 of storage (Table 3). The tyrosine content of rice protein is rather low compared with the contents of other amino acids (Hibino et al., 1989). Therefore, the high tyramine concentration in low-alcohol makgeolli was not due to tyrosine content. In contrast, we previously reported that when the commercial makgeolli was stored at 20 °C, the BAs that were produced were mainly putrescine and cadaverine (Kim et al., 2011). Arena et al. (2008) reported that the amounts and types of BAs formed are influenced strongly by the food composition, the presence of microorganisms with amino acid decarboxylases, and other parameters that allow bacterial growth during food processing and storage. Therefore, BA production by LAB may be controlled at various factors involved in makgeolli fermentation and storage.

The alcohol concentration in M1 and our previously reported makgeolli samples (Kim et al., 2011) in this study was similar (about 6%), and the increasing pattern of LAB counts was also similar (Table 3 and Fig. 1D). However, the differences in the production of individual BAs may be due to differences between the LAB population and the amino acid precursor contained in the makgeolli. We have found that BA production showed distinctively different characteristics when three different strains of *Lactobacillus plantarum*, isolated from makgeolli that had been stored long term at 20 °C, was cultured in different media. Furthermore, when these strains were cultured in makgeolli medium supplemented with pyridoxal phosphate at 20 °C for 21 days and then sterilized, all strains produced excessive amounts of putrescine, tyramine, histamine, and cadaverine (Kwak et al., 2014).

In conclusion, we found that alcohol concentration affected the final quality of makgeolli. Makgeolli is made by diluting the alcohol concentration to an appropriate level at the end of production. By adjusting the initial alcohol concentration to 11.47% or higher, makgeolli can

be stored for up to 30 days without affecting the quality or increasing hygiene-associated risks due to suppression of LAB proliferation and BA production, even at a relatively high storage temperature of 20 °C. These findings can be applied to the development of nonsterilized makgeolli products in which quality can be preserved even under high storage temperatures (e.g., during the summer season).

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