

Physicochemical and Microbial Properties of the Korean Traditional Rice Wine, *Makgeolli*, Supplemented with Banana during Fermentation

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ABSTRACT: The objective of the present study was to evaluate the physicochemical and microbial properties of the Korean traditional rice wine *Makgeolli*, supplemented with banana during 6 day fermentation. The alcohol contents of the control and banana *Makgeolli* were 17.0 and 16.5%, respectively. The pH values decreased while total acidity, total soluble solids, and color values increased throughout the fermentation process. An increase in microorganism counts throughout the 6-day fermentation period was noted in all samples. The major free sugar and organic acid detected in all samples were glucose and succinic acid, respectively. There were 39 volatile compounds detected in the control and banana *Makgeolli*. The major ester detected was ethyl acetate (20.037 and 22.604% for the control and banana *Makgeolli*, respectively). The major alcohol compounds detected were 3-methylbutanol (20.933%) and 3-methyl-1-butanol (34.325%) in the control. 2-methyl-1-propanol (22.289%) and 3-methyl-1-butanol (39.851%) were the highest alcohol compounds detected in the banana *Makgeolli*.

Keywords: *Makgeolli*, banana, fermentation, physicochemical properties, microbial properties

INTRODUCTION

Traditionally, Korean rice wine, or *Makgeolli*, has been brewed with *nuruk*, cooked rice, yeast, and some medicinal plants or herbs (1,2). *Nuruk* has been prepared by inoculating moistened ground wheat or rice with a mixture of airborne microorganisms, such as fungi, yeast, and a variety of other bacteria (3-7). The *nuruk* in the *Makgeolli* plays a role in breaking down the starch so the resulting sugar can be utilized by the yeast to create the *Makgeolli*. *Makgeolli* has been recorded in Chinese literature since the Koguryeo dynasty (8-10) and continues to be a part of Korea's culture. However, due to a lack of unique characteristics, inferior acceptability, and functionality, *Makgeolli* has been declining in popularity. Recently, different varieties of cocktail *Makgeolli*s were developed and many research groups have been studying how to improve the quality of *Makgeolli* through the study of its microbial activities, functional characteristics, utilization of raw materials, manufacture processes, extension of shelf life, and more (4,5,9,11,12).

Bananas are a group of monocot plants in the Musaceae

family that originated from Southern and Southeast Asia (13). Bananas are an excellent tropical fruit with an agreeable flavor and a high nutritional value. When consumed, the intake of sugars, fiber, vitamins, and minerals are high with very low contribution to fat intake (14). Overall bananas are not only a nutritious fruit for carbohydrates, vitamins, and minerals, but they also contain many secondary metabolites. Phenolics are a group of secondary metabolites in bananas which have been highlighted recently for their health benefits, such as treating diarrhea, promoting blood clotting and wound healing, and antimicrobial and anti-inflammatory activities (13). Bananas are the world's leading fruit crop considering their nutritional aspect, and economically the 5th most agricultural crop traded worldwide (15). However, no studies have reported evaluating the product quality and physicochemical properties of *Makgeolli* that incorporates banana. Thus, the objective of the present study was to investigate the physicochemical and microbial properties of *Makgeolli* supplemented with bananas during fermentation.

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MATERIALS AND METHODS

Materials

Rice was purchased from a local market (Yongin, Korea). *Nuruk* and yeast were obtained from Korea Enzyme, Co. (Hwaseong, Korea) and Saf-instant (Lesaffre, Marcq-en-Baroeul, France), respectively. Banana used in this study was purchased from E-mart (Yongin, Korea).

Preparation of the control and banana *Makgeolli*

The preparation of the *Makgeolli* (control) and banana *Makgeolli* is illustrated in Fig. 1. Two kg of rice was rinsed and soaked in tap water for 3 h. After soaking, the rice was then drained of water for 40 min and immediately steamed for 40 min. After the rice was steamed, the heat was turned off and left to sit for 20 min. The rice was quickly cooled by spreading it out thinly on an aluminum pan. Once cooled, the rice was placed in a 10 L glass bottle, along with the *nuruk* (80 g), yeast (28 g), and distilled water (3.5 L). For the banana *Makgeolli*, 400 g of bananas were peeled, grinded, and added to the mixture of rice, *nuruk*, yeast and distilled water (3.12 L). The top was secured with a plastic wrap and a rubber band and placed in a water bath that was set at 28°C and allowed to ferment for 6 days. The samples were filtered (Advantec 5B, Tokyo Roshi Kaisha, Ltd., Tokyo, Japan) before being tested.

Alcohol content

The alcohol contents of the *Makgeolli*s were taken using the procedures adapted from Woo et al. (16). Briefly, 100 mL of the sample was run through a distiller until around 70 mL was collected. The collected sample set to 100 mL with distilled water and the alcohol content (%) was measured using an alcohol hydrometer. The alcohol-temperature correction table was used with the sample's alcohol content and temperature.

pH, total acidity, and total soluble solid content

The pH was measured using an Orion 3 Star pH Benchtop (Thermo Electron Co., Beverly, MA, USA). After measuring the pH, 10 mL of the sample combined with the indicator Bromothymol blue and neutral red and titrated with a 0.1 N NaOH solution. The amount of

NaOH (mL) was then converted to tartaric acid (16). The total soluble solid content was measured using a digital refractometer (Model Dr-103L, Bellingham+Stanley Ltd., Tumblebridge Wells, UK) and measured in Brix (°Bx).

Color

The color values were obtained using the Tri-Stimulus Colorimeter (Model JC801, Color Techno System Co., Tokyo, Japan), which was calibrated with the original value from a standard plate (L=98.48, a=0.14, and b=0.41). The L (lightness), a (redness), and b (yellowness) values were obtained.

Total viable cells, lactic acid bacteria, and yeast

Total viable cells, lactic acid bacteria, and yeast counts were measured according to the Korean Food and Drug Association (17). The standard plate count was used on the pour plate method for each sample. The samples were diluted in a 10 fold dilution with sterile saline (0.85% NaCl). In the total viable cell count, 1 mL of the diluted samples were mixed with 20 mL of PCA (plate count agar; Difco™, Lawrence, KA, USA) and incubated at 37°C for 48 h. In the lactic acid bacteria count, 1 mL of the diluted samples were mixed with 20 mL of BCP (bromocresol purple agar, Eiken Chemical, Tokyo, Japan) and incubated at 37°C for 72 h. In the yeast count, 1 mL of the diluted samples were mixed with 20 mL of PDA (potato dextrose agar; Difco™) and incubated at 25°C for 120 h. Each dilution was duplicated and the values were averaged. The number of microorganisms was counted by colony forming units (CFU).

Free sugar

Free sugar contents were obtained using the method adapted from Kerem et al. (18). The samples were filtered through a 0.2 µm membrane filter and analyzed by HPLC system (JASCO Co., Tokyo, Japan). Using the external standard method, the standards, fructose, glucose, sucrose, and maltose, were used and the curve was quantified. The HPLC conditions were set as follows: the flow rate through the carbohydrate high performance column (4.0 µm, 4.6×250 mm, Waters, Milford, MA, USA) was 1.4 mL/min, the column temperature

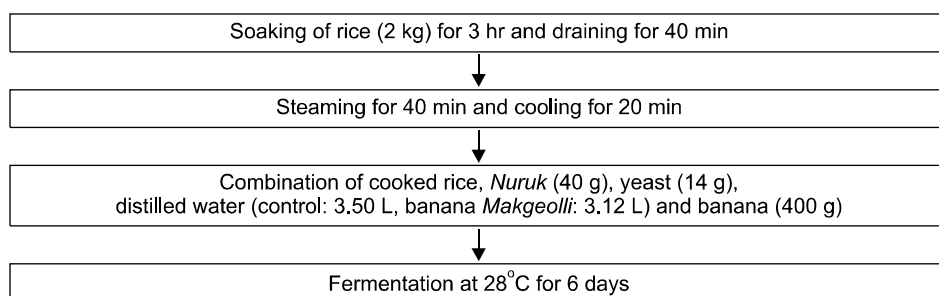


Fig. 1. Procedure for the control and banana *Makgeolli*.

was set at 35°C, the mobile phase was 75% acetonitrile, and the detector was RI-930 detector (JASCO Co.).

Organic acid

Organic acid contents were obtained using the method adapted from Kerem et al. (18). The samples were filtered through a 0.2 µm membrane filter and analyzed by HPLC system (JASCO Co.). Using the external standard method, the standards, oxalic acid, citric acid, malic acid, succinic acid, formic acid, and acetic acid, were used and the curve was quantified. The HPLC conditions were set as follows: the flow rate through the Supelcogel C-610H column (9 µm, 7.8×300 mm, Sigma, St. Louis, MO, USA) was 0.5 mL/min, the column temperature was set at 30°C, the mobile phase was 0.1% phosphoric acid, and the detector was the multi-wavelength detector (MD-2010 Plus, JASCO Co.).

Volatile compounds

Octanal (15 µL) was used as the standard for the analysis of volatile compounds, and 20 mL of the samples were obtained in a 240 mL screw amber bottle (Sigma-Aldrich Co.). The samples were placed in a 50°C dry oven for 10 min and the volatile compounds were collected. The volatile compounds were analyzed using the GC-MS

(GCMS-QP2010, Shimadzu, Kyoto, Japan), and the condition at which they were analyzed were as follows: GC was run with AT1 column (60×0.25 mm, Shimadzu), the rate of temperature increase was 8°C/min between 5°C to 120°C, 12°C/min to 180°C, 15°C/min to 230°C, the carrier gas used was N₂, and the injection temperature was 200°C while the detection temperature was 250°C.

Statistical analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) test. Significance was defined at the 5% level.

Table 1. Alcohol contents of the control and banana *Makgeolli* on day 6 of the fermentation period

Sample	Alcohol contents (%)
Control	17.0
Banana <i>Makgeolli</i>	16.5

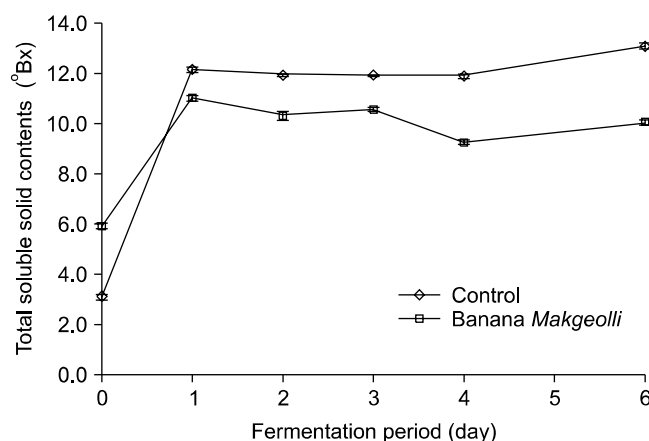
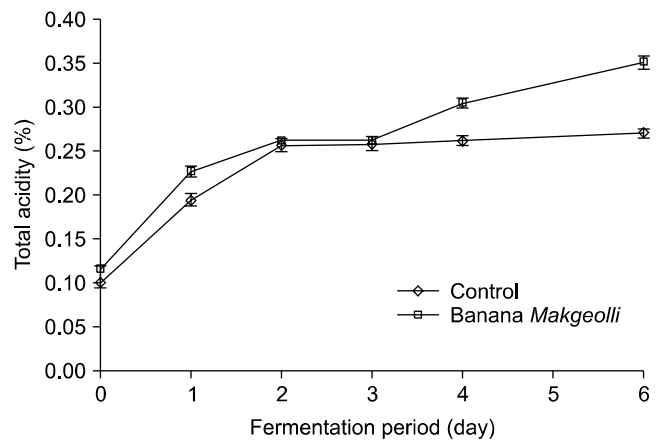
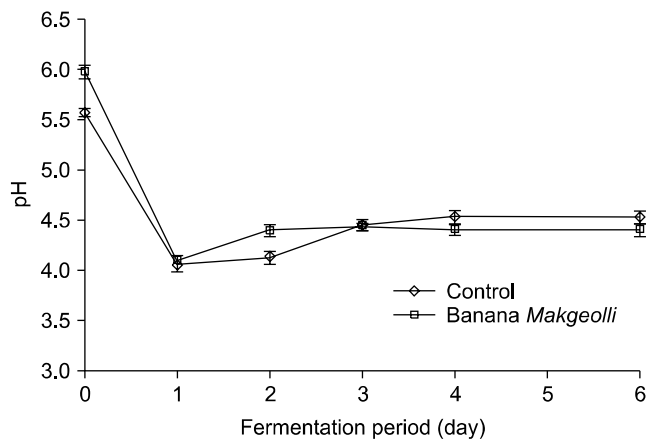


Fig. 2. pH values, total acidity values and total soluble solid contents of the control and banana *Makgeolli* throughout the 6-day fermentation period.

RESULTS AND DISCUSSION

Alcohol content, pH, total acidity, and total soluble solid content

The alcohol contents of the control and banana *Makgeolli* on the final day of the fermentation period were 17 and 16.5%, respectively (Table 1). These values fall within the range of alcohol content given by Jin et al. of 15~18% (19). Alcohol content is one of the factors that affect the quality of *Takju* and can also be used to show the degree of fermentation throughout the fermentation process (20). Starch is converted to glucose by hydrolysis throughout the fermentation process. Glucose is then used by yeast to produce alcohol and CO₂. Therefore, formation of bubbles can also be seen throughout the process of fermentation.

The pH, total acidity, and total soluble solid contents of the control and banana *Makgeolli* can be seen in Fig. 2. The pH values of the control and banana *Makgeolli* were similar throughout the 6 day fermentation period. A sharp decrease in pH on day 1 of the fermentation was noted, which then slightly increased to a pH of around 4.4. The final pH values of the control and banana *Makgeolli* fall within the range of 3.4 to 4.5 (1,8,19).

In the analysis of total acidity, all the samples sharply increased on day 1 of the fermentation period and slowly increased to a final value of 0.26 and 0.35% for the control and banana *Makgeolli*, respectively. Production of organic acids by microorganisms affect the total acidity of

the *Makgeolli*, and the change of total acidity is a factor for knowing that compounds are changing in the *Makgeolli* (20). A previous study showed the combination of total acidity and volatile compounds affect the quality of flavor and shelf life of *Makgeolli*s (21).

Due to the addition of bananas, the banana *Makgeolli* had a higher initial total soluble content than the control. A sharp increase in soluble contents was observed on day 1 of the fermentation period, and the final total soluble contents of the control and banana *Makgeolli* were 13.2 and 10.1 °Bx, respectively. Due to the sharp changes on day 1 in the pH, total acidity, and total soluble contents, we speculated that most of the microbial activities occur on the first day of fermentation.

Color

Table 2 shows the values of the color analysis. When compared to the banana *Makgeolli*, the control had a higher L value after the final fermentation day. The banana *Makgeolli* had the higher a and b values than the control after the fermentation period. Also, the L, a, and b values increased throughout the fermentation process. Kim et al. (20), Lee et al. (22) and Park et al. (23) also reported similar results.

Microbial changes

The microbial cell counts for the total viable cells, lactic acid bacteria, and yeast can be seen in Table 3. The final total viable cells, lactic acid bacteria, and yeast counts for the control and banana *Makgeolli* were 2.88×10^7 and 3.57×10^7 , 1.50×10^6 and 1.84×10^6 , and 1.90×10^7 and 2.17×10^7 CFU/mL, respectively, with an increase of all microorganisms in all samples throughout the fermentation period. Seo et al. (8) and Rhee et al. (24) also reported an increase of total microorganisms throughout the fermentation process.

Free sugar

The free sugar contents of the control and banana *Makgeolli* are displayed in Table 4. The major free sugar detected in both the control and banana *Makgeolli* was glucose. Glucose significantly decreased after the 6 day fermentation period. Glucose plays an important role in

Table 2. Color values (L, a and b) of the control and banana *Makgeolli* throughout the 6 day fermentation period

Sample	Fermentation period (day)	Color		
		L	a	b
Control	0	64.29	0.12	0.22
	1	66.40	0.11	3.30
	3	68.66	0.67	7.50
	4	68.93	0.62	8.30
	6	69.01	0.79	9.70
	Banana <i>Makgeolli</i>	0	59.85	1.18
1		63.07	1.43	9.84
3		65.63	1.91	9.98
4		65.84	1.87	10.62
6		65.22	2.10	11.85

Table 3. Microbial cell counts of the control and banana *Makgeolli* throughout the 6 day fermentation period (Unit: CFU/mL)

Sample	Microorganism	Fermentation period (day)			
		0	1	3	6
Control	Total viable cells	1.23×10^5	2.50×10^6	4.24×10^6	2.88×10^7
	Lactic acid bacteria	1.10×10^5	1.70×10^5	1.15×10^6	1.50×10^6
	Yeasts	1.26×10^5	2.38×10^6	1.56×10^7	1.90×10^7
Banana <i>Makgeolli</i>	Total viable cells	3.36×10^5	4.50×10^6	5.67×10^6	3.57×10^7
	Lactic acid bacteria	2.02×10^5	3.50×10^5	1.30×10^6	1.84×10^6
	Yeasts	3.74×10^5	4.86×10^6	1.67×10^7	2.17×10^7

Table 4. Free sugar contents of the control and banana *Makgeollis* on day 1 and 6 of the fermentation period

Sample	Storage period (day)	Fructose	Glucose	Sucrose	Maltose	Lactose
Control	1	ND ¹⁾	49.855 ^a	0.114 ^c	0.834 ^c	0.326 ^b
	6	0.103 ^c	29.831 ^c	0.679 ^a	1.686 ^a	ND
Banana <i>Makgeolli</i>	1	0.140 ^a	38.74 ^b	nd	0.723 ^d	0.285 ^b
	6	0.119 ^b	25.060 ^d	0.535 ^b	1.477 ^b	1.021 ^a

Units: mg/mL.

Values with different letters within the same column differ significantly ($P < 0.05$).¹⁾ND: not detected.**Table 5.** Organic acid contents of the control and banana *Makgeollis* on day 1 and 6 of the fermentation period

Sample	Storage period (day)	Oxalic acid	Citric acid	Tartaric acid	Malic acid	Succinic acid	Lactic acid	Acetic acid
Control	1	ND ¹⁾	0.155 ^c	0.037 ^c	0.174 ^d	7.698 ^d	0.082 ^c	1.256 ^d
	6	0.003 ^a	0.127 ^d	0.035 ^c	0.201 ^c	24.587 ^b	0.440 ^a	1.696 ^c
Banana <i>Makgeolli</i>	1	0.002 ^a	0.660 ^a	0.096 ^b	0.315 ^b	9.118 ^c	0.057 ^c	2.626 ^b
	6	0.002 ^a	0.374 ^b	0.105 ^a	0.362 ^a	25.698 ^a	0.373 ^b	2.860 ^a

Units: mg/mL.

Values with different letters within the same column differ significantly ($P < 0.05$).¹⁾ND: not detected.

fermentation as its production from starch allows for the production of alcohol in *Makgeolli*. Glucose is formed through the hydrolysis of starch by amylases in the *nuruk*. Therefore, through α -amylase, glucose increases in *Makgeolli*. Glucose was also found to be a major sugar in a study by Park et al. (23). Fructose, glucose and maltose were found to be the major reducing sugars used by yeast to create alcohol, and the reduction of these sugars was found to give *Makgeolli* its particular taste (19).

Organic acid

Malic, succinic, lactic, and acetic acids were significantly elevated throughout the fermentation period in all the samples (Table 5). Banana *Makgeolli* had a significant increase in tartaric acid as well. Succinic acid contents were highest in both the control and banana *Makgeolli*. Jeon et al. (3) suggests that the production of organic acid may be dependent on several factors, including *nuruk* fermentation conditions, or other circumstances. Rhee et al. (24) explained that the suppression of lactic acid may be due to the high colony forming unit of yeast in the initial stage of the brewing, which may suppress the acid forming bacteria but allowed the high content of succinic acid.

Volatile compounds

The relative peak areas of volatile compounds of the control and the banana *Makgeolli* are shown in Table 6. A total of 37 volatile compounds were detected in the control and the banana *Makgeolli*. The major volatile compounds in the samples were esters and alcohols, which was also reported in studies by Lee et al. (1) and Rhee et al. (24). Lee et al. (1) reported that all the major compo-

nents seem to be the production of yeast fermentation. The major ester compound detected was ethyl acetate. The amount detected for the control and the banana *Makgeolli* was 20.037 and 22.604%, respectively. Ethyl acetate is a major volatile compound that gives *Makgeolli* its fruity taste (25). Kwon et al. (26) reported that the major esters found were (E)-cinnamaldehyde, ethyl linoleate, ethyl linolenate, ethyl caprate, and ethyl palmitate. The differences of compounds may be due to the differences in ingredients and yeasts used. The major alcohol compounds in the control were 3-methylbutanol (22.469%) and 3-methyl-1-butanol (34.325%). For the banana *Makgeolli*, the major alcohol compounds were 2-methyl-1-propanol (23.150%) and 3-methyl-1-butanol (39.851%). Acetaldehyde was also detected in the samples. In general, acetaldehyde provokes the smell and was also detected in Korean traditional soju and *Chonju* (24). Han et al. (27) analyzed 9 different alcohols, excluding ethanol, and found that iso-amyl alcohol and iso-butyl alcohol were the main alcohols detected. Lee et al. (28) studied the effects of different yeasts on 8 different alcohols; 4 out of 5 *Makgeollis* were observed to have iso-amyl alcohol as its major alcohol (28). The differences of volatile compounds detected may be due to the differences in yeast and ingredients. Han et al. (27) stated that a greater amount of 2-methyl-1-propanol than 3-methyl-1-butanol in *Makgeolli* resulted in a low quality *Makgeolli*.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

Table 6. Volatile compounds of the control and banana *Makgeolli* on day 6 of the fermentation period

No	RI ¹⁾	Compounds	Sample	
			Control	Banana <i>Makgeolli</i>
1	362	Acetaldehyde	0.572	0.844
2	367	Methanol	0.165	0.053
3	373	Ethanol	3.050	0.667
4	600	Hexane	4.338	0.046
5	603	1-Propanol	4.208	0.211
6	623	Ethyl acetate	20.037	22.604
7	650	3-Methylbutanal	22.469	0.097
8	670	2-Methyl-1-propanol	0.057	23.150
9	676	Acetic acid	0.005	0.007
10	699	1-Butanol	0.018	0.226
11	727	Propanoic acid, ethyl ester	0.386	0.719
12	730	Acetic acid, propyl ester	0.076	0.092
13	770	3-Methyl-1-butanol	34.325	39.851
14	775	Ethyl isobutyrate	0.928	1.135
15	786	Acetic acid, 2-methylpropyl ester	0.325	0.433
16	794	3-Methyl-1-hexanol	0.274	0.581
17	795	Hexanal	0.274	0.581
18	799	Isobutyric acid	0.096	0.244
19	801	2,4-Dimethyl-hexane	0.094	0.171
20	806	Butanoic acid, ethyl ester	0.530	0.821
21	831	2,4-Dimethyl-heptane	0.004	0.000
22	860	Ethyl 2-methyl butyrate	0.571	0.577
23	864	3-Ethoxy-1-propanol	0.019	0.571
24	870	4-Methyl octane	0.658	0.228
25	885	2-Methyl butyl acetate	0.278	0.219
26	901	Pentanoic acid, ethyl ester	0.028	0.017
27	961	Benzaldehyde	0.240	0.129
28	996	Octanal	5.052	5.222
29	1,046	1,8-Cineole	0.009	0.000
30	1,061	3-Ethyl-3-methyl heptane	0.001	0.001
31	1,068	3,8-Dimethyl decane	0.010	0.002
32	1,100	Undecane	0.033	0.021
33	1,107	3,8-Dimethyl-undecane	0.020	0.012
34	1,124	Phenethyl alcohol	0.161	0.000
35	1,195	Octanoic acid, ethyl ester	0.606	0.428
36	1,799	Tetradecanoic acid, ethyl ester	0.014	0.020
37	1,995	Hexadecanoic acid, ethyl ester	0.065	0.020
		Total	100	100

Units: Peak area (%).

¹⁾RI: Retention index.

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