

ARTICLE

Open Access



# Fermentation profiling of rice wine produced by *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 newly isolated from Korean fermentation starter

Minjoo Kim and Jeong-Ah Seo\* 

## Abstract

The objective of this study was to determine the fermentation characteristics of rice wine produced by *koji* inoculated with *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 on moisturized wheat-bran and rice grain. We also compared rice wine samples produced in this study and three commercial *Makgeolli*. The alcohol content was about 12% higher in the rice wine samples fermented by wheat-bran *koji* than in the other samples. In all of the samples, the range of pH value was 3.8–4.6 and the total acid was below 0.5. The soluble solid content was highest in the rice wine sample prepared by the wheat-bran *R. oryzae* KJJ39 *koji* (15.5°Brix) and overall relatively higher in the samples with wheat-bran *koji* than rice *koji*. The content of reducing sugar was twofold higher in rice wine prepared by *koji* inoculated with *R. oryzae* KJJ39 than *A. oryzae* KSS2. The volatile acid content was higher in rice wine produced by the wheat-bran *A. oryzae* KSS2 *koji* than the others. In the analyses of five organic acids, *A. oryzae* KSS2 was found to produce more malic acid and fumaric acid while *R. oryzae* KJJ39 to do more citric acid, lactic acid and acetic acid. The rice wine sample prepared with the wheat-bran *A. oryzae* KSS2 *koji* contained much higher concentration of sucrose, maltose and amino acids. Comprehensively, the results of fermentation profiling suggest that both *A. oryzae* KSS2 and *R. oryzae* KJJ39 can be applied to the production of rice wine as a valuable fungal isolate for fermentation start.

**Keywords:** *Aspergillus oryzae* KSS2, Fermentation starter, Inoculation, Profiling wheat-bran *koji*, *Rhizopus oryzae* KJJ39, Rice *koji*, Rice wine

## Introduction

*Makgeolli* is a traditional Korean rice wine that has been consumed by Koreans for centuries. Korean rice wine is traditionally brewed using rice (as a starch) and *nuruk* (as a fermentation starter culture), and it involves a two-step fermentation process. The taste of *Makgeolli* is determined by a combination of four flavor profiles: sweet, sour, bitter, and astringent. Unlike other alcoholic liqueurs, *Makgeolli* is rich in nutrients, including (1) vitamin B, which is involved in human metabolism,

(2) acetylcholine, which boosts liver function, and (3) organic acids. *Makgeolli* also contains essential amino acids such as lysine, leucine, and arginine, as well as esters such as ethyl acetate, amyl acetate, and ethyl caproate, which are responsible for the sour taste of the rice wine [1–5]. Besides the nutritional and functional benefits of drinking *Makgeolli*, the presence of raw yeast gives it a unique taste when compared to other alcoholic beverages [4, 6, 7]. The quality of *Makgeolli* is usually determined by the alcohol contents, total acid contents, organic acid concentrations and flavor profile, and these factors vary depending on the production and storage conditions [8, 9].

\*Correspondence: sja815@ssu.ac.kr  
School of Systems Biomedical Science, Soongsil University, Seoul 06978, Republic of Korea

Traditional *nuruk* samples show significant variations of microbial composition that depend on the region, environment and process of production [10, 11]. The development and expansion of refined *nuruk* started in the 1970s, and this has allowed the production of alcohols of uniform quality [2, 6, 12, 13]. In order to support the increasing consumption and expanding market of *Makgeolli*, it is crucial to standardize the quality and production process through the standardization of the brewing conditions. It is also important to develop high-quality manufacturing technologies, and to maintain and improve the quality of the rice wine to meet the standards of present-day consumers.

Most of the previous studies on *Makgeolli* have focused on various topics including the alcohol contents, quality traits, alcohol fermentation [1, 6, 14, 15], sensory properties [2, 16–18] according to the processing method of starch and *nuruk* and the characteristics of volatile flavors of *Makgeolli* prepared with different types of *nuruk* [19–22]. Also, there have been a few studies about production of rice wine using a *koji* or modified *nuruk* inoculated with a single fungus [23–26]. In this study, to determine and compare the fermentation characteristics, we investigated the physiochemical properties of the rice wine samples produced by both wheat-*koji* and rice *koji* inoculated with *Aspergillus oryzae* KSS2 and *Rhizopus oryzae* KJJ39 which have been selected as high amylolytic enzyme producers in our previous study [11] and the commercial *Makgeolli* samples.

## Materials and methods

### Preparation of commercial *Makgeolli* samples

The commercial *Makgeolli* samples were purchased from the shelf within 3 days on the market and stored at  $-80^{\circ}\text{C}$  until analyzed. The *Makgeolli* samples were made by three representative manufacturers, all using 100% rice without any food additives. The alcohol content of all samples was 6%, indicated on the label. Analyses have been triplicated with each sample of *Makgeolli*.

### Preparation of wheat-*koji* and rice *koji*

*Koji* was prepared as previously described with an exception of the amount of wheat-*koji* and rice used [13]. Briefly, 50 g of steamed rice was distributed in 250 ml polypropylene bottles covered with gauze (autoclaved at  $121^{\circ}\text{C}$  for 20 min) and inoculated with fungal spore suspension (about  $5 \times 10^5$  spores/g of rice). Wheat-*koji* was prepared with 50 g of wheat-*koji* (McSun, Dongawon) added to 30 ml of distilled water using the same method to make rice *koji*. The solid media prepared with wheat-*koji* and rice were incubated at  $30^{\circ}\text{C}$  for 24–72 h under a relative humidity of over 70%. The wheat-*koji* and rice *koji* were stored at  $-20^{\circ}\text{C}$  until used. For this study, non-glutinous rice (Gyeonggi Chucheon, Gyeonggi, Korea) was used and prepared. Wheat-*koji* and rice *koji* were prepared by inoculation of *A. oryzae* KSS2 and *R. oryzae* KJJ39 (Table 1).

### Rice wine fermentation

Rice wine fermentation was performed by the same methods as described [13]. Rice was soaked in water for 3 h, drained for 30 min, steamed for 90 min and cooled down for 20 min. The water content of the steamed rice was determined to be approximately 30% (w/w). Two hundred-fifty grams of steamed non-glutinous rice, 500 mL of water, 30 g of *koji* and  $1.0 \times 10^5$  cells/g mash of *Saccharomyces cerevisiae* (INRA7013, Fermevin, Denmark) were mixed for fermentation. After incubation at  $25 \pm 3^{\circ}\text{C}$  for 24 h, 250 g of steamed non-glutinous rice was added to the mixture and incubated under the same condition. For further fermentation, 500 g of steamed rice and 500 mL of water were added and the samples were incubated at  $25 \pm 3^{\circ}\text{C}$  for 7 days. All samples were stored at  $-80^{\circ}\text{C}$ . All of the fermentation process were carried out in triplicates for each type of *koji*.

**Table 1** Rice wine and *Makgeolli* samples used in this study

Sample	Description of sample
CA	Commercial <i>Makgeolli</i> A
CB	Commercial <i>Makgeolli</i> B
CC	Commercial <i>Makgeolli</i> C
AW	Rice wine produced by wheat- <i>koji</i> inoculated with <i>Aspergillus oryzae</i> KSS2
RW	Rice wine produced by wheat- <i>koji</i> inoculated with <i>Rhizopus oryzae</i> KJJ39
AR	Rice wine produced by rice <i>koji</i> inoculated with <i>Aspergillus oryzae</i> KSS2
RR	Rice wine produced by rice <i>koji</i> inoculated with <i>Rhizopus oryzae</i> KJJ39

## Rice wine analysis

### pH and total acid

The pH was measured using a pH meter (Orionstar A211, Thermo scientific, US). The total acid content was measured by titrating 10 mL of sample with 0.1 N NaOH solution until the pH became 8.2. The total acid content was calculated based on the amount of NaOH (mL) and then converted to acetic acid (%) [27].

### Volatile acidity and amino acids

The volatile acids were measured by taking 30 mL of the distillate used for alcohol contents analysis, titrating it with 0.01 N NaOH to pH 8.2–8.4 and then converting it to citric acid. The amino acid was determined by the titration method using phenolphthalein as the indicator. Ten mL of sample was first titrated with 0.1 N NaOH to pH 8.2. Subsequently, 5 mL of neutral formalin was added, and the solution was titrated again with 0.1 N NaOH to pH 9.2. The amino acid was calculated as a volume needed for titration after the addition of neutral formalin, and the molecular weight of glycine was used as a conversion value for calculation [27].

### Measurement of soluble-solid and reducing sugar content

Soluble-solid content was measured using a digital refractometer (HI 96801, Hanna Instruments Inc, USA) and recorded in brix units. Reducing sugar was measured using the dinitrosalicylic acid (DNS) method [28]. Briefly, 1 mL of DNS reagent was added to 1 mL of sample and the mixture was heated in a water bath for 10 min. The solution was then cooled down at room temperature and 3 mL of distilled water was added. The absorbance was measured at 550 nm using a spectrophotometer (MULTISKAN Go, Thermo Scientific, USA). The reducing sugar (%) was determined based on a glucose standard curve.

### Measurement of alcohol, organic acid, free sugar and amino acid

Sample preparation for analyses was followed by the previous report [4]. The *Makgeolli* samples were prepared by centrifugation at 1000 rpm for 10 min. The supernatant was passed through a Sep-pack C18 cartridge (Waters Co., Milford, MA, USA), followed by filtration using a membrane filter (0.45  $\mu$ m, Advantec MFS, Inc, Tokyo, Japan) and then analyzed by HPLC (Ultimate3000, Thermo Dinex, Japan). An Aminex HPX-87H column (300 mm  $\times$  10 mm, Bio-Rad, USA) was used to analyze the organic acids and alcohol content in the samples, using 20 mM H<sub>2</sub>SO<sub>4</sub> (pH 2.7) as the mobile phase, a flow rate of 0.5 mL/min and an injection volume of 10  $\mu$ L. Analysis was performed using an

RI (ERC, Refractor MAX 520, Japan) and UV detector (210 nm). Analysis of free sugars was performed with a Sugar-pak (300 mm  $\times$  6.5 mm, Waters) column under 70 °C by heating, and using water as the mobile phase, 0.5 mL/min as the flow rate and 10  $\mu$ L as the injection volume. The detector used was Shodex RI-101 (Shodex, Japan). For amino acid analysis, an Inno C18 column (150 mm  $\times$  4.6 mm, Younginbiochrom, Korea) was used with the following conditions: a column temperature of 40 °C, flow rate of 1.5 mL/min, injection volume of 0.5  $\mu$ L and a mobile phase of 40 mM sodium phosphate (pH 7) and distilled water/acetonitrile/methanol (10:45:45, v/v/v %) used in a gradient. Analysis was performed using an Agilent 1260 Infinity fluorescence detector (Agilent, USA).

### Statistical analysis

Data processing was performed using the KoreaPlus Statistics (embedded on SPSS Statistics25) on Windows to evaluate statistical differences in the principle components of all samples.

## Results and discussion

### Alcohol production

The alcohol content is a major factor that impacts the quality of *Makgeolli* along with the degree of fermentation. During the fermentation of *Makgeolli*, ethanol is produced through the degradation of starch by the microorganisms present in *nuruk*. This means that the alcohol content increases with the duration of fermentation, and the air bubbles caused by the formation of carbon dioxide during the process can act as visual indicators of the degree of fermentation [29]. The alcohol contents of CA, CB, and CC were 6.0, 5.3, and 4.8%, respectively, which showed a difference up to 1.8% from the product label indicated on (Table 2). The rice wine samples produced by *koji* showed higher alcohol contents than commercial ones, ranging from 12.3–12.6% for AW and RW and 9.9–10.2% for AR and RR. In previous studies, a few isolates of *A. oryzae* and *R. oryzae* originated from Korean fermentation starters had been tested for making *koji* and producing rice wine [13, 25]. In those cases, alcohol contents produced by new fungal isolates ranged from 10 to 14%. In this study, the results showed that alcohol production was similar to the previous analyses, and we found that wheat-bran *koji* produced more alcohol than rice *koji* regardless of the fungal strains.

### pH and total acidity

The pH value is greatly influenced by the types and concentrations of organic acids and other acid-based substances, and it is an important indicator of the progress

**Table 2** Fermentation characteristics of rice wine prepared with *Aspergillus oryzae* KSS2 (AW and AR) and *Rhizopus oryzae* KJJ39 (RW and RR) and commercial *Makgeolli* (CA, CB and CC)

Sample	Alcohol (%)	pH	Acidity (%)	Soluble-solid content (°Brix)	Reducing sugar (%)	Volatile acid (ppm)	Amino acid (%)
CA	6.0±0.5	3.86±0.09	0.18±0.03	3.73±0.06	0.29±0.00	30.8±0.70	0.06±0.01
CB	5.3±0.2	3.94±0.07	0.22±0.03	3.67±0.21	0.28±0.00	24.8±0.70	0.08±0.01
CC	4.8±0.6	4.59±0.05	0.26±0.01	11.10±0.92	0.73±0.01	38.8±0.70	0.09±0.00
AW	12.3±0.5	4.39±0.08	0.36±0.05	13.13±1.31	0.29±0.00	60.4±0.70	0.26±0.04
RW	12.6±0.6	4.22±0.25	0.33±0.14	15.47±1.62	0.76±0.03	51.2±3.02	0.10±0.01
AR	9.9±5.1	4.24±0.13	0.27±0.06	9.27±0.31	0.32±0.04	44.0±6.90	0.25±0.04
RR	10.2±0.4	3.99±0.15	0.38±0.04	12.03±2.10	0.67±0.03	34.4±3.90	0.08±0.00

of fermentation and the production of alcohol in *Makgeolli* [29]. The pH values of CA, CB, and CC were in the range of 3.9–4.6, while the ones of AW, RW, AR and RR ranged from 4.0 to 4.4 (Table 2). All of the rice wine samples had pH values within the range established by the Liquor Tax Act (pH 3.8–4.4). Acidity is an important factor that affects the flavor and preservation of *Makgeolli* [30]. Excessive acidity indicates that abnormal fermentation has occurred, which makes the product undrinkable due to the acidic taste [31]. On the other hand, the product will be tasteless if the acidity is too low [32]. In the present study, the total acidity was 0.18–0.26% for CA, CB and CC and 0.27–0.38% for the samples prepared with wheat-bran *koji* and rice *koji* (Table 2). The Liquor Tax Act dictates that the optimal acidity of *Makgeolli* is less than 0.5%, indicating that all of the samples analyzed in this study had acceptable acidity.

#### Soluble-solid and reducing sugar contents

The soluble-solids content of *Makgeolli* greatly affects its sweetness. The CC sample contained a soluble-solid content of 11.10°Brix, which was about twofold higher than those of CA and CB which were 3.73 and 3.66°Brix, respectively (Table 2). Rice wine prepared with wheat-bran *koji* (AW and RW) showed soluble-solids contents of 13.13 and 15.47°Brix respectively, while the samples prepared with rice *koji* (AR and RR) had soluble-solids contents of 9.27 and 12.03°Brix, respectively. Furthermore, rice wine prepared with wheat-bran *koji* and rice *koji* showed threefold higher soluble-solid content than that of CA, which was supplemented with iso-malto-oligosaccharide during its industrial production. A previous study reported that the soluble-solids contents of other commercial *Makgeolli* were in the range of 2.9–4.7°Brix [4] less than the ones of the commercial samples used in this work (3.7–11.1°Brix). Their study concluded that differences in soluble-solids content are probably due to differences in the types and quantity of raw material used,

the fermentation starter, and the conditions of fermentation. In the present study, wheat-bran *koji* was more effective as a fermentation starter than rice *koji* for both fungal strains used.

Glucose, fructose, and maltose are reducing sugars. During the fermentation of *Makgeolli*, amylase digests starch into smaller carbohydrates, eventually breaking it down into glucose molecules. Glucose is an important component used as the substrate for alcohol fermentation that greatly impacts the acidity, taste, and alcohol content of *Makgeolli* [29]. In this study, the reducing-sugars content of CA and CB was 0.29%, while that of CC was more than double, at 0.73% (Table 2). The rice wine samples inoculated with *A. oryzae* KSS2 (AW and AR) showed reducing-sugars contents of 0.29% and 0.32%, respectively, while those inoculated with *R. oryzae* KJJ39 showed significantly higher values of 0.76% (RW) and 0.67% (RR). The results suggest that *R. oryzae* KJJ39 was better than *A. oryzae* in production of reducing sugars.

#### Volatile acid concentration and amino acid ratio

Acetic acid is the predominant volatile acid in *Makgeolli*, but in excessive amounts it imparts an unpleasant odor similar to that of vinegar. This indicates the need to control the amount of acetic acid produced during the fermentation process of *Makgeolli* [16]. In this study, the range of the volatile acid concentrations was 24.8–38.8 ppm for the commercial *Makgeolli* samples, 51.2–60.4 ppm for rice wine prepared with wheat-bran *koji*, and 34.4–44.0 ppm for rice wine prepared with rice *koji* (Table 2). A previous study produced similar results, with volatile acid contents of less than 40 ppm for rice wine prepared with rice *koji* and less than 80 ppm for those with added plant material [8].

Amino acids play an important role in moderating the savory taste of *Makgeolli*. However, a high concentration of amino acids will impart a greasy taste and reduce the quality of the rice wine [17]. In the present study, the



**Table 3 Organic acid contents of rice wine prepared with *Aspergillus oryzae* KSS2 (AW and AR) and *Rhizopus oryzae* KJJ39 (RW and RR) and commercial *Makgeolli* (CA, CB and CC) (mean  $\pm$  SD, n = 3, mg/mL)**

Sample	Organic acid				
	Citric acid	Malic acid	Fumaric acid	Lactic acid	Acetic acid
CA	0.95 $\pm$ 0.00	0.15 $\pm$ 0.01	0.02 $\pm$ 0.00	0.29 $\pm$ 0.01	0.12 $\pm$ 0.00
CB	ND <sup>a</sup>	ND	ND	2.01 $\pm$ 0.01	0.30 $\pm$ 0.00
CC	0.67 $\pm$ 0.01	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00	0.27 $\pm$ 0.00	0.26 $\pm$ 0.01
AW	0.19 $\pm$ 0.02	0.75 $\pm$ 0.10	0.19 $\pm$ 0.01	0.77 $\pm$ 0.06	0.28 $\pm$ 0.05
RW	0.25 $\pm$ 0.01	0.43 $\pm$ 0.05	0.09 $\pm$ 0.01	1.02 $\pm$ 0.22	0.55 $\pm$ 0.01
AR	0.22 $\pm$ 0.01	0.96 $\pm$ 0.06	0.17 $\pm$ 0.00	0.51 $\pm$ 0.05	0.14 $\pm$ 0.04
RR	0.26 $\pm$ 0.02	0.13 $\pm$ 0.04	0.03 $\pm$ 0.00	0.86 $\pm$ 0.01	0.58 $\pm$ 0.06

<sup>a</sup> Not detected

amino acid content was 0.06–0.09% in commercial *Makgeolli*, 0.10–0.26% in rice wine prepared with wheat-bran *koji* and 0.08–0.25% in rice wine prepared with rice *koji* (Table 2), shown to be similar to a previous report [8].

#### Organic acid concentration

The organic acid concentrations of the rice wine samples are listed in Table 3. The citric acid concentrations varied markedly among the commercial *Makgeolli* samples, being 0.95 mg/mL in CA, 0 mg/mL in CB, and 0.67 mg/mL in CC. Rice wine prepared with wheat-bran *koji* showed citric acid concentrations in the range of 0.19–0.25 mg/mL, while they were 0.22–0.26 mg/mL in rice wine prepared with rice *koji*. Park et al. reported that the citric acid concentration differed significantly between different commercial *Makgeolli* samples, which they attributed to the type of imported rice and the quantity of rice used during the production of the *Makgeolli* [4]. The malic acid concentrations also differed greatly among the commercial *Makgeolli* samples, with values ranging from 0 to 0.15 mg/mL. For rice wine prepared with wheat-bran *koji* and rice *koji*, the malic acid concentration was 0.75 mg/mL for AW, 0.43 mg/mL for RW, 0.96 mg/mL for AR, and 0.13 mg/mL for RR. The fumaric acid concentration was 0–0.02 mg/mL in commercial *Makgeolli*, 0.19 mg/mL in AW, 0.09 mg/mL in RW, 0.17 mg/mL in AR, and 0.03 mg/mL in RR. These results showed that the concentrations of malic acid and fumaric acid were 2–5 times higher in the samples prepared with *A. oryzae* KSS2 than in those inoculated with *R. oryzae* KJJ39. The lactic acid concentrations also differed markedly among the commercial *Makgeolli* samples, at 0.29 mg/mL in both CA and CC, but 2.01 mg/mL in CB. Rice wine prepared with wheat-bran *koji* showed lactic acid concentrations of 0.77 mg/mL (AW) and 1.02 mg/mL (RW), while

the AR and RR samples prepared with rice *koji* had lactic acid concentrations of 0.51 and 0.86 mg/mL, respectively.

It has been reported previously [6], that as fermentation progresses, the concentrations of lactic acid and succinic acid increase significantly to become the most abundant organic acids in *Makgeolli*. Organic acids are important ingredients that give a sour taste to the rice wine and play an important role in enhancing its taste and aroma if they are present in trace amounts. However, excessive acetic acid can alter the taste of the rice wine, reducing its quality [5]. In the present study, the commercial *Makgeolli* samples contained 0.12–0.30 mg/mL acetic acid, those prepared with wheat-bran *koji* contained 0.28 mg/mL (AW) and 0.55 mg/mL (RW), and those prepared with rice *koji* contained 0.14 mg/mL (AR) and 0.58 mg/mL (RR). Overall, the acetic acid concentrations were 2–3 times higher for the samples inoculated with *R. oryzae* KJJ39 than for those prepared with *A. oryzae* KSS2.

#### Free sugar concentration

The free sugar concentrations of the samples are listed in Table 4. Glucose is a major sugar in *Makgeolli* that plays an important role in fermentation, since the degradation of starch into glucose allows the production of alcohol. The present study did not detect fructose, and only detected maltose in AW (2.78 mg/mL) and RR (0.61 mg/mL). The glucose concentrations were high in CC (65.51 mg/mL), RW (42.39 mg/mL) and RR (44.99 mg/mL) and low in CA (0.69 mg/mL), AW (19.64 mg/mL) and AR (1.18 mg/mL). During the fermentation of *Makgeolli*, starch is broken down into monosaccharides such as glucose through the action of amylase, which is produced by molds. In general, the concentration of glucose becomes high during early stages of fermentation, and

**Table 4 Free sugar contents of rice wine prepared with *Aspergillus oryzae* KSS2 (AW and AR) and *Rhizopus oryzae* KJJ39 (RW and RR) and commercial *Makgeolli* (CA, CB and CC) (mean  $\pm$  SD, n = 3, mg/mL)**

Sample	Free sugar (mg/mL)				
	Fructose	Sucrose	Glucose	Maltose	Mannitol
CA	ND <sup>a</sup>	0.03 $\pm$ 0.06	0.69 $\pm$ 0.60	ND	1.76 $\pm$ 0.13
CB	ND	0.75 $\pm$ 0.08	ND	ND	1.46 $\pm$ 0.03
CC	ND	3.10 $\pm$ 1.06	65.51 $\pm$ 8.50	ND	2.22 $\pm$ 0.52
AW	ND	4.44 $\pm$ 0.73	19.64 $\pm$ 14.05	2.78 $\pm$ 2.89	3.45 $\pm$ 0.21
RW	ND	1.56 $\pm$ 0.41	42.39 $\pm$ 6.32	ND	5.67 $\pm$ 0.82
AR	ND	0.35 $\pm$ 0.36	1.18 $\pm$ 0.91	ND	4.10 $\pm$ 0.82
RR	ND	0.43 $\pm$ 0.37	44.99 $\pm$ 10.19	0.61 $\pm$ 0.62	4.96 $\pm$ 0.74

<sup>a</sup> Not detected

**Table 5** Amino acid analyses of rice wine prepared with *Aspergillus oryzae* KSS2 (AW and AR) and *Rhizopus oryzae* KJJ39 (RW and RR) and commercial *Makgeolli* (CA, CB and CC) (mean  $\pm$  SD, n = 3, mg/mL)

Taste	Amino acid	CA	CB	CC	AW	RW	AR	RR
Sweet	Glycine	0.05 $\pm$ 0.01	0.04 $\pm$ 0.02	0.05 $\pm$ 0.01	0.25 $\pm$ 0.11	0.05 $\pm$ 0.01	0.18 $\pm$ 0.01	0.01 $\pm$ 0.00
	Alanine	0.14 $\pm$ 0.03	0.18 $\pm$ 0.04	0.15 $\pm$ 0.03	0.94 $\pm$ 0.11	0.23 $\pm$ 0.06	0.71 $\pm$ 0.06	0.12 $\pm$ 0.02
	Serine	0.01 $\pm$ 0.00	0.03 $\pm$ 0.01	0.05 $\pm$ 0.02	0.40 $\pm$ 0.02	0.04 $\pm$ 0.01	0.21 $\pm$ 0.02	0.01 $\pm$ 0.00
	Threonine <sup>a</sup>	0.00 $\pm$ 0.00	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.24 $\pm$ 0.02	0.03 $\pm$ 0.00	0.14 $\pm$ 0.01	0.01 $\pm$ 0.00
	Methionine <sup>a</sup>	0.00 $\pm$ 0.00	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.13 $\pm$ 0.03	0.03 $\pm$ 0.01	0.10 $\pm$ 0.01	0.01 $\pm$ 0.00
	Proline	0.12 $\pm$ 0.02	0.10 $\pm$ 0.01	0.12 $\pm$ 0.04	0.73 $\pm$ 0.07	0.20 $\pm$ 0.02	0.50 $\pm$ 0.04	0.06 $\pm$ 0.01
Umami	Aspartic acid	0.01 $\pm$ 0.00	0.05 $\pm$ 0.03	0.03 $\pm$ 0.02	0.48 $\pm$ 0.03	0.08 $\pm$ 0.02	0.28 $\pm$ 0.02	0.04 $\pm$ 0.02
	Glutamic acid	0.05 $\pm$ 0.02	0.10 $\pm$ 0.01	0.25 $\pm$ 0.06	1.07 $\pm$ 0.07	0.22 $\pm$ 0.07	0.67 $\pm$ 0.08	0.07 $\pm$ 0.02
	Asparagine	0.01 $\pm$ 0.00	0.03 $\pm$ 0.02	0.06 $\pm$ 0.01	0.35 $\pm$ 0.03	0.07 $\pm$ 0.02	0.22 $\pm$ 0.02	0.02 $\pm$ 0.00
	Glutamine	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.15 $\pm$ 0.05	0.46 $\pm$ 0.11	0.05 $\pm$ 0.02	0.35 $\pm$ 0.06	0.02 $\pm$ 0.01
Bitter	GABA	0.01 $\pm$ 0.01	0.05 $\pm$ 0.05	0.03 $\pm$ 0.01	0.14 $\pm$ 0.01	0.04 $\pm$ 0.01	0.07 $\pm$ 0.00	0.02 $\pm$ 0.01
	Histidine	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.18 $\pm$ 0.04	0.01 $\pm$ 0.01	0.12 $\pm$ 0.03	0.01 $\pm$ 0.00
	Arginine	0.21 $\pm$ 0.04	0.03 $\pm$ 0.05	0.32 $\pm$ 0.07	1.18 $\pm$ 0.05	0.07 $\pm$ 0.01	1.08 $\pm$ 0.06	0.04 $\pm$ 0.01
	Valine <sup>a</sup>	0.01 $\pm$ 0.01	0.05 $\pm$ 0.03	0.12 $\pm$ 0.03	0.44 $\pm$ 0.02	0.10 $\pm$ 0.04	0.28 $\pm$ 0.04	0.03 $\pm$ 0.00
	Isoleucine <sup>a</sup>	0.00 $\pm$ 0.00	0.03 $\pm$ 0.02	0.07 $\pm$ 0.02	0.32 $\pm$ 0.04	0.06 $\pm$ 0.02	0.18 $\pm$ 0.03	0.02 $\pm$ 0.00
	Leucine <sup>a</sup>	0.01 $\pm$ 0.00	0.08 $\pm$ 0.04	0.21 $\pm$ 0.06	0.71 $\pm$ 0.08	0.20 $\pm$ 0.06	0.39 $\pm$ 0.07	0.06 $\pm$ 0.03
	Phenylalanine <sup>a</sup>	0.01 $\pm$ 0.00	0.06 $\pm$ 0.03	0.17 $\pm$ 0.05	0.51 $\pm$ 0.03	0.16 $\pm$ 0.05	0.29 $\pm$ 0.05	0.05 $\pm$ 0.03
	Lysine <sup>a</sup>	0.01 $\pm$ 0.00	0.04 $\pm$ 0.02	0.11 $\pm$ 0.04	0.47 $\pm$ 0.08	0.02 $\pm$ 0.01	0.35 $\pm$ 0.05	0.02 $\pm$ 0.00
Acerbity	Tyrosine	0.02 $\pm$ 0.02	0.04 $\pm$ 0.03	0.14 $\pm$ 0.04	0.54 $\pm$ 0.02	0.12 $\pm$ 0.03	0.36 $\pm$ 0.02	0.04 $\pm$ 0.01
	Tryptophan <sup>a</sup>	0.01 $\pm$ 0.00	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.09 $\pm$ 0.00	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	0.02 $\pm$ 0.00
$\Sigma^b$		0.74 $\pm$ 0.01	1.03 $\pm$ 0.02	2.23 $\pm$ 0.03	9.62 $\pm$ 0.05	1.82 $\pm$ 0.02	6.50 $\pm$ 0.03	0.66 $\pm$ 0.02

<sup>a</sup> Essential amino acid<sup>b</sup> Sum of amino acid

then reduces as the growth of yeast and lactic acid bacteria progresses [8].

### Amino acids

The concentrations of total amino acids and essential amino acids are presented in Table 5. In the commercial *Makgeolli* samples, the total amino acid concentrations were 0.74–2.23 mg/mL and those of essential amino acids ranged from 0.04 to 0.80 mg/mL. Rice wine prepared with wheat-bran *koji* had total amino acid concentrations in the range of 1.82–9.62 mg/mL and essential amino acid concentrations of 0.64–2.90 mg/mL; the corresponding concentrations in rice wine prepared with rice *koji* were 0.66–6.50 and 0.22–1.77 mg/mL, respectively.

The essential amino acids (threonine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, and lysine) represented 27.3–35.8% of the total amino acids in all of the analyzed samples except for CA. Essential amino acids play important roles in different biological functions, such as lysine being essential for the synthesis of tissue, and methionine playing an important role in preventing fatty liver and by promoting phospholipids in the liver. Among the essential amino acids, lysine was measured much higher in AW (0.47 mg/mL

and AR (0.35 mg/mL) than the commercial products CC (0.11 mg/mL) and CA (0.01 mg/mL). Methionine was also included about hundred times higher in AW (0.13 mg/mL) and AR (0.10 mg/mL) than CA. This result suggests that *A. oryzae* KSS2 may better function in production of lysine and methionine rather than *R. oryzae* KJJ39.

Amino acids are not only important as nutrients in *Makgeolli*, but they are also precursors for aroma: threonine, glycine, alanine, and serine produce a sweet taste; glutamic acid gives a umami taste; aspartic acid gives a sour taste; and leucine, isoleucine, lysine, and tyrosine give a bitter taste to the rice wine. Threonine, glycine, alanine, and serine involved in sweetness and were measured by higher level in AW (0.24–0.29 mg/mL) rather than other samples. Proline is the only amino acid that is soluble in alcohol, and it produces a pleasant aroma when heated with sugars [33]. AW (0.73 mg/mL) and AR (0.50 mg/mL) contained large amounts of proline. The amino acid concentration was 5–10 times higher in rice wine inoculated with *A. oryzae* KSS2 and *R. oryzae* KJJ39 than in the commercial *Makgeolli* samples. The rice wine samples inoculated with *A. oryzae* KSS2 showed concentrations of amino acids that were 5 times higher than those in samples inoculated with

*R. oryzae* KJJ39. Rice wine prepared with wheat-bran *koji* had a twofold higher concentration of amino acids compared with that prepared with rice *koji*. The results of this work will allow production of *Makgeolli* with higher nutritional and sensory qualities.

#### Acknowledgements

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Agricultural Microbiome R&D Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA-918010-4). The authors thank S. Y. Park for English correction and editing.

#### Authors' contributions

MK organized the experiment, collect data and summarize a main finding for the manuscript. JAS corrected and wrote some part of the first draft manuscript and manage a final full manuscript as a corresponding author. All authors read and approved the final manuscript.

#### Funding

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Agricultural Microbiome R&D Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA-918010-4).

#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Competing interests

The authors declare that they have no competing interests.

Received: 27 August 2020 Accepted: 15 December 2020

Published online: 18 February 2021

#### References

- Kim HR, Jo SJ, Lee SJ, Ahn BH (2008) Physicochemical and sensory characterization of a Korean traditional rice wine prepared from different ingredients. *Korean J Food Sci Technol* 40:551–557
- Lee TS, Han EH (2001) Volatile flavor components in mash of *Takju* prepared by using *Aspergillus oryzae* *Nuruks*. *Korean J Food Sci Technol* 33:366–372
- Lee SJ, Kim JH, Jung YW, Park S, Shin WC, Park CS, Hong S, Kim GW (2011) Composition of organic acids and physiological functionality of commercial *Makgeolli*. *Korean J Food Sci Technol* 43:206–212
- Park CW, Jang SY, Park EJ, Yeo SH, Kim OM, Jeong YJ (2011) Comparison of quality characteristics of commercial *Makgeolli* type in south Korea. *Korean J Food Preserv* 18:884–890
- Woo SM, Shin JS, Seong JH, Yeo SH, Choi JH, Kim TY, Jeong YJ (2010) Quality characteristics of brown rice *Takju* by different *Nuruks*. *J Korean Soc Food Sci Nutr* 39:301–307
- Park CS, Lee TS (2002) Quality characteristic of *Takju* prepared by wheat flour *Nuruks*. *Korean J Food Sci Technol* 34:296–302
- Song JC, Park HJ (2003) *Takju* brewing using the uncooked germed brown rice at second stage mash. *J Korean Soc Food Sci Nutr* 32:847–854
- Ha GJ, Kim NK, Je HJ, Choi SY, Seol HK, Hong GP, Lee SD (2015) Quality characteristics of *Makgeolli* produced in Gyeongnam province. *J Agric Life Sci* 49:247–257
- Huh CK, Lee JW, Kim YD (2012) Quality characteristics of rice wine according to the rice wine seed mash with lactic acid concentration. *Korean J Food Preserv* 19:933–938
- Lee WK, Kim JR, Lee MW (1987) Studies on the changes in free amino acids and organic acids of *Takju* prepared with different *Koji* strains. *J Korean Agric Chem Soc* 30:323–327
- Carroll E, Trinh TN, Son H, Lee YW, Seo JA (2017) Comprehensive analysis of fungal diversity and enzyme activity in *nuruk*, a Korean fermenting starter, for acquiring useful fungi. *J Microbiol* 55:357–365
- Kim HS, Yu TS (2000) Volatile flavor components of traditional Korean *Nuruk* Fungi. *Korean J Appl Microbiol Biotechnol* 28:303–308
- Yang S, Choi SJ, Kwak J, Kim K, Seo M, Moon TW, Lee YW (2013) *Aspergillus oryzae* strains isolated from traditional Korean *Nuruk*: Fermentation properties and influence on rice wine quality. *Food Sci Biotechnol* 22:425–432
- Han EH, Lee TS, Noh BS, Lee DS (1997) Quality characteristics in mash of *Takju* prepared by using different *Nuruk* during fermentation. *Korean J Food Sci Technol* 29:555–562
- Han EH, Lee TS, Noh BS, Lee DS (1997) Volatile flavor components in mash of *Takju* prepared by using different *Nuruks*. *Korean J Food Sci Technol* 29:563–570
- Jeong ST, Kwak HJ, Kim SM (2013) Quality characteristics and biogenic amine production of *Makgeolli* brewed with commercial *Nuruks*. *Korean J Food Sci Technol* 45:727–734
- Joung EJ, Park NS, Kim YM (2004) Studies Korean *Takju* using the by-product of rice milling. *Korean J Food Nutri* 17:199–205
- Kim IH, Park WS, Koo YJ (1996) Comparison of fermentation characteristics of Korean traditional alcoholic beverage with different input step and treatment of rice and *Nuruk*. *Korean J Diet Cult* 11:339–348
- Kim SH, Mun JY, Kim SY, Yeo SH (2018) Quality characteristics of glutinous rice-*Makgeolli* fermented with Korean yeast (SC Y204 and Y283) isolated from *Nuruk*. *Korean J Food Preserv* 25:874–884
- Kwon YH, Lee AR, Kim JH, Kim HR, Ahn BH (2012) Changes of physico-chemical properties and microbial during storage of commercial *Makgeolli*. *Korean J Mycol* 40:210–214
- Lee Y, Yi H, Hwang KT, Kim DH, Kim HJ, Jung CM, Choi YH (2012) The qualities of makgeolli (Korean rice wine) made with different rice cultivars, milling degrees of rice, and *Nuruks*. *J Korean Soc Food Sci Nutr* 41:1785–1791
- Lee JE, Lee AR, Kim HR, Lee EJ, Kim TW, Shin WC, Kim JH (2017) Restoration of traditional Korean *Nuruk* and analysis of the brewing characteristics. *J Microbiol Biotechnol* 27:896–908
- So MH, Lee YS, Han SH, Noh WS (1999) Analysis of major flavor compounds in *Takju* mash brewed with a modified *Nuruk*. *Korean J Food Nutr* 12:421–426
- So MH, Lee YS, Noh WS (1999) Improvement in the quality of *Takju* by a modified *Nuruk*. *Korean J Food Nutr* 12:427–432
- Seo WT, Cho HK, Lee JY, Kim B, Cho KM (2012) Quality characteristics of wheat-rice *Makgeolli* by making of rice *Nuruk* prepared by *Rhizopus oryzae* CCS01. *Korean J Microbiol* 48:147–155
- Son EY, Lee SM, Kim M, Seo JA, Kim YS (2018) Comparison of volatile and non-volatile metabolites in rice wine fermented by *Koji* inoculated with *Saccharomyces fibuliger* and *Aspergillus oryzae*. *Food Res Int* 109:596–605
- Korea National Tax Service Liquor Analysis of liquor regulatory (2010) National Tax Service Technical Service Institute, Korea: 35–53
- Luchsinger WW, Cornesky RA (1962) Reducing power by the dinitrosalicylic acid method. *Anal Biochem* 4:346–347
- Kim JY, Sung KW, Bae HW, Yi YH (2007) pH, acidity, color, reducing sugar, total sugar, alcohol and organoleptic characteristics of puffed rice powder added *Takju* during fermentation. *Korean J Food Sci Technol* 39:266–271
- Lee SM, Lee TS (2000) Effect of roasted rice and defatted soybean on the quality characteristics of *Takju* during fermentation. *J Nat Sci* 12:71–79
- Lee JS, Lee TS, Noh BS, Park SO (1996) Quality characteristics of mash of *Takju* prepared by different raw materials. *Korean J Food Sci Technol* 28:330–336
- Lee WY, Rhee CH, Woo CJ (2004) Changes of quality characteristics in brewing of *chungju* (*sambaekju*) supplemented with dried persimmon and *Cordyceps sinensis*. *Korean J Food Preserv* 11:240–245
- Lee MO, Youn JB, Park SA, Lee YS, Bin JH, Lee SH (2002) Investigation on the quality characteristics of *Sansung Takju* compared with commercial *Takju*. *Rep Busan Inst Health Environ* 12:48–62

#### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.