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Chemical characteristics of Korean turbid rice wine prepared with partially gelatinized wheat flour brewed using different starters

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Abstract

Turbid Rice Wine (TRW) is a native traditional alcoholic beverage of Korea. The objective of this study was to evaluate the feasibility of using partially gelatinized wheat flour to produce TRW and characterized its chemical characteristics. Six different types of commercial starter namely *Geryang*, *Jinju*, *Sangju*, *Songhag*, *Urimil*, and *Sanseong* manufactured at different regions of South Korea were used in brewing. The alcohol content of TRW that used *Geryang* and *Songhag* was relatively higher than those used other starters ($p < 0.05$). The total acid content of TRW that used *Geryang* and *Songhag* was relatively lower than those used other starters ($p < 0.05$). *Geryang* and *Songhag* also produced considerably high iso-amylalcohol, the prominent volatile compound in TRW. By using *Geryang* and *Songhag*, iso-amylalcohol content of TRW were 289.9 ppm and 324.98 ppm respectively. *Geryang* was observed to produce the lowest lactic acid in TRW, followed by *Songhag*. The highest amino acidity and soluble solid content were produced in TRW that was using *Jinju* (5.12±0.04 and 9.23±0.40% respectively). The highest reducing sugar was observed in TRW that was using *Urimil* (1.39±0.07mg/mL). However, *Jinju, Urimil*, *Sangju* and *Sanseong* produced inadequate alcohol in TRW (<6%). Thus, *Geryang* and *Songhag* were suggested to be used as starter in TRW prepared with partially gelatinized wheat flour.

Introduction

Turbid rice wine (TRW) or "drunken rice" is well known as *Makgeolli*. This is a native alcoholic beverage of Korea. Practically, TRW is made from steamed non-glutinous rice, incorporated with a natural starter, or *nuruk*, and water, then, fermented for several days. TRW is unfiltered beverage, that makes its appearance to be turbid or unclear due to its ingredients such as essential amino acids, proteins, sugars, and live yeasts. Hence, it has particular nutritional characteristics (Lee and Shim, 2010; Kim *et al*., 2012).

Nowadays, the marketability of TRW increase year by year not only in Korea but also in Japan. In Korea, the liquor market share has always reached more than 5% during last 5 years. The market share increased from 7.8% in 2009 to 12% in the first quarter of 2011 (Kim *et al.*, 2012). The public demand of TRW was driven by the understanding of its functionality and superiority to contribute human health such as, antihypertensive activity (Min *et al.*, 2012), anti-inflammatory effects (Kim *et al*., 2008), and anticancer effect (Shin *et al*., 2008).

Despite TRW has been known since 918 from dinasty of Goguryeo, TRW are still studied up to now. Some studies have been done by modifying the raw materials, including; the addition of fruit in different forms of processed persimmon (Im, *et al*., 2012), the use of uncooked germinated black rice (Kim *et al*., 2012), the combination of wheat-rice as a raw material (Seo *et al*., 2012), and the use of different rice cultivars and milling degrees of rice (Lee *et al*., 2012). In addition, some studies on optimizing the fermentation and the mashing process were done by selecting the type of fermenting microbes and combining the different starters for the fermentation process, including; the use of *Pichia anomala* a non-saccharomyces yeast (Kim *et al*., 2012), utilization of starter-contained rice made by *Rhizophus oryzae* CCS01 (Seo *et al*., 2012), the use of purple sweet potato-rice starter (Cho *et al*., 2012), and the use of the various combinations of mashing types in making TRW (Park *et al*., 2012).

Generally, TRW production is initiated by heating a raw material until fully gelatinized.

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This process is not only time consuming but also requiring enormous energy. However, gelatinization simplify the degradation of starch into simpler sugar. Starch is then degraded to glucose, maltose, and maltooligosaccharides by some amylolytic enzymes essentially α-amylase, β-amylase and glucoamylase (Sarikaya *et al.*, 2000). Subsequently, the yeast Saccharomyces cerevisiae accomplishes the process by converting the simpler sugars into alcohol and CO_2.

Commonly, α- and β-amylase hydrolyzed raw starch inadequately because starch granules were greatly resistant to be digested (Hyun and Zeikus., 1985). However, α- and β-amylase could hydrolyse raw starch granules in a different degradation patterns. The hydrolyzation of starch by α-amylase was more efficient than that of β-amylases. Sarikaya *et al.* (2000) have shown that rice granules were found to be the best substrate for α- and β-amylase, followed by wheat. They also reported that α-amylase from *Bacillus amyloliquefaciens* could degrade starch of rice, corn, and wheat into maltose.

Rice wine's starter or *nuruk* is extensively made in almost all provinces in Korea and this presented different microorganisms. The TRW quality depended on the microorganisms presented in the starter and resulted some important characteristics of TRW such as aroma, sourness, taste, sweetness and the others. Unfortunately, some important microorganisms were not present in the starter. Song *et al.* (2013) have shown that only 13 out of 42 starters samples contained some important microorganisms such as *Aspergillus oryzae* and *Lichtheimia corymbifera. Pichia jadinii* was predominant yeast strain instead of *Saccharomyces cerevisiae,* which was producing alcohol in TRW.

In Korea, some TRW producers have been used wheat flour to substitute rice in the range of 20% to 60% due to high market value of rice. The price of wheat flour is cheaper than rice and replacing the rice might give more economic benefit. However, there is limited study about quality characteristics of TRW prepared with partially gelatinized wheat flour using different starters. To fill in this gap, the feasibility of using wheat flour in TRW using different types of starters need to be done.

Materials and Methods

Materials

Wheat flour (13% protein content), was purchased from market in Suwon City, Korea. Saccharomyces cerevisiae was purchased from La-pharjang Inc. (French). Six types of commercial starters namely *Geryang*, *Jinju*, *Sangju*, *Songhag*, *Urimil,* and *Sanseong* were purchased from different area in Korea. *Geryang* (SP 1800) was purchased from Korea Enzyme Co., Ltd. (Hwaseong, Gyeonggi, Korea). *Jinju* (SP 300) was purchased from Jinju City, Korea. *Sangju* (SP 300) was purchased from Sangju City, Korea. *Songhag* (SP 500) was purchased from Gwangju City, Korea. *Urimil* (SP 300) was purchased from Yesan City, Korea. *Sanseong* (SP 300) was purchased from Busan City, Korea. The Saccharogenic Power (SP) represent the saccharifying activity of β-amylase. The greater SP value exhibits the greater saccharifying activity.

Turbid rice wine (TRW) brewing

Standard process of TRW at Brewing Research Center, RDA, Suwon City, Korea was used to produce TRW with partially gelatinized wheat flour. The schematic diagram for making of TRW is presented in Figure 1.

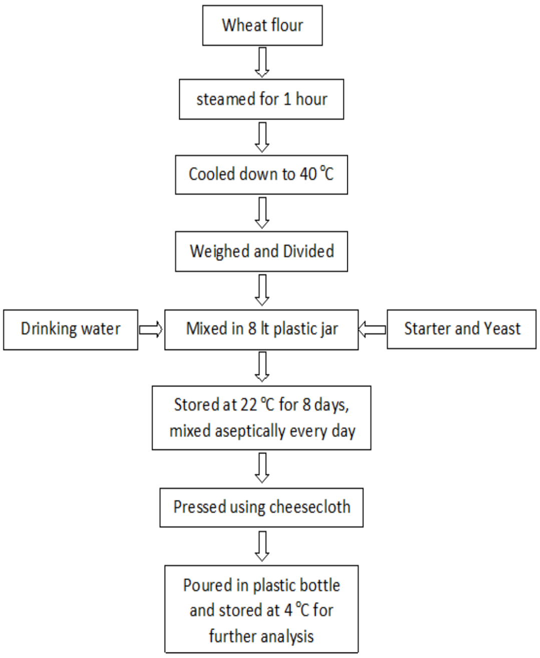

Figure 1. Schematic diagram for making Turbid Rice Wine (TRW) prepared with partially gelatinized wheat flour

Total weight of TRW produced in this study was 5600 g, which comprise of 2000 g of wheat flour and 3600 g of drinking water. Starter was adjusted to 10% of wheat flour weight, instead of *Geryang* was only 2%. *Geryang* has the highest SP value compare to those of other starters. Yeast *(Saccharomyces cerevisiae)* was adjusted to 1% of total weight. For making of TRW, the wheat flour was steamed for 1 hour, then allowed to cold down at 40°C. Partially gelatinized wheat flour was then divided into 2000 g each. Afterward, starter, yeast, and water were mixed thoroughly, followed by fermentation at 22°C for eight days. TRW brewing was done in triplicate (3 jar/starter). Samples were taken from each treatment after the brewing accomplished at day 8.

Sample preparation

Approximately 200 ml TRW sample was taken from each treatment. To clarify the samples, centrifugation at 10.000 g for 10 minutes was done using large-capacity High-Speed Refrigerated Centrifuge Hitachi CR22GIII (Hitachi, Japan). The supernatant was taken from tubes and poured in a sample bottle. Samples were stored at freezing temperature until required for chemical analysis.

Chemical analysis

The pH was measured with Orion 3 Star pH Benchtop (Thermo Scientific). Soluble solids was measured with a portable refractometer (ATAGO PR32-α; ATAGO Co. Ltd., Tokyo, Japan) and recorded in brix units. Firstly, standard measurement was done using distilled water as zero brix units. After cleaning the loading side of refractometer, approximately 4 to 5 drops of sample was loaded into loading side. Then, soluble solid was measured by pressing read knob, and brix unit was recorded on the refractometer display. Amino acidity was determined by titration method using Burette Digital III (Brand). Three drops of phenolphthalein indicator were added to a 10 ml sample and then titrating with 0.1 N NaOH until the solution turned pale pink. Five ml of formalin was added to solution, then mixed thoroughly until pale pink color disappeared and titrating again with 0.1 N NaOH until the solution turned pale pink. Amino acidity was calculated as a volume needed for titration after formalin addition. Total acid was determined by titrating 10 ml of sample with 0.1 N NaOH until the pH reached 8.2-8.3. Total acid was calculated as lactic acid (%) by using lactic acid molecular weight number as a conversion value. Reducing sugar was determined by dinitrosalicylic acid method. Samples absorbance were recorded using spectrophotometer at 550 nm (Shimadzu UV-2450, UV-Vis Spectrophotometer). Reducing sugar content was calculated by using glucose as a standard (Sigma-Aldrich, St. Louis, MO, USA) and was presented in mg ml-1. Alcohol content was determined by distillation method, whereby 100 ml distilled water was added to 100 ml sample in erlenmeyer flask, followed by adding 3-4 drops anti-foaming agent. Then, sample was distilled by using parallel distillation machine. The distillate sample was collected in 100 ml graduated cylinder to gain of 80 ml distillate. After that, distilled water was added to reach 100 ml volume. Furthermore, the distillate sample was cooled down at refrigerator to reach 15^{o}C, then, alcohol content was measured by using alcohol meter. Alcohol content was calculated as percent volume (v v-1).

Organic acid analysis

Sample was filtered by using 0.2 μm membrane filter (Millipore Co., Cork, Ireland) before injection. Separation and detection were performed subsequently by using HPLC post column method (LC-20A, Shimadzu Co., Kyoto, Japan). HPLC equipped with a Shodex RSpak KC-G (6 mm x 50 mm) as guard column and Shodex RSpak KC-811 (8 mm x 300 mm) separation column. For separation, the column oven temperature was 63^{o}C and mobile phase was 3 mM perchloric acid and adjusted to 0.8 ml min^{-1} flow rate. After the reaction solution (0.2 mM bromothymol blue, 15 mM $NaHPO_4$, and 2 mM NaOH) reacted with the separated liquid, the absorbance was detected at 440 nm. At that time the cell temperature was 25^{o}C, and the flow rate was 1.0 ml min^{-1}.

Volatile compound analysis

The sample was prepared by adding 5 ml sample with 0.5 ml 2.5% acetonitrile and placed into 10 ml volumetric flask and filled up with 3 degree distilled water. After that, sample was filtered by using 0.2 μm membrane filter, then finally, 200 μL sample was mixed with 1800 μL 3th distilled water and was put into 20 mL bial. GC (Shimazu, Shimadzu Co., Kyoto, Japan) and a HP-INNOWAX column (60 m x 0.25 mm x 0.25 μm, Agilent, Santa Clara, USA) were used for the analysis. The GC column oven temperature was held at 45^{o}C for 5 min and set to 100^{o}C for 5 min at a rate of 5^{o}C min^{-1}, then raised to 200^{o}C at a rate of 10^{o}C min^{-1} and held at this temperature for 10 min. The injection temperature was 250^{o}C, Nitrogen was used as the carrier gas and the flow rate was 22 cm sec^{-1} (Linear velocity). The direct capillary interface of the FID (flame ionization detector) was set at a temperature of 250^{o}C.

Statistical analysis

Total acid content, pH, amino acidity, reducing sugar, total solid, and alcohol were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test at the significance level of $p<0.05$. The statistical analysis was carried out using SPSS for Windows ver. 19 (IBM corp., USA). These data were also analyzed by using Principal Component Analysis (PCA) (XLSTAT ver. 2014, Addinsoft).

Results and Discussion

Comparison of chemical characteristics of TRW

After terminating TRW brewing at day 8, each data of chemical parameters was evaluated using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). The comparison of chemical parameters are presented in Table 1.

Table 1. Comparison of chemical characteristics of Turbid Rice Wine (TRW)

Starter	pH	Total Acid (%)	Amino Acidity (%)	Reducing Sugar (mg/mL)	Soluble Solid (brix)	Alcohol (% v/v)
Geryang	3.67 ± 0.07^{a}	0.87 ± 0.12^{d}	2.44 ± 0.30^{c}	1.05 ± 0.04^{b}	8.90 ± 0.04^{ab}	9.20 ± 0.20^{a}
Jinju	3.38 ± 0.04^{bc}	1.65 ± 0.16^{ab}	5.12 ± 0.04^{a}	1.06 ± 0.15^{b}	9.23 ± 0.40^{a}	4.07 ± 0.46^{c}
Sangju	3.40 ± 0.06^{b}	1.46 ± 0.11^{b}	4.36 ± 0.90^{b}	0.66 ± 0.04^{c}	8.30 ± 0.53^{bc}	3.40 ± 0.69^{c}
Songhag	3.34 ± 0.07^{bc}	1.09 ± 0.23^{cd}	1.32 ± 0.44^{d}	0.94 ± 0.08^{b}	7.73 ± 0.61^{c}	7.33 ± 1.22^{b}
Urimil	3.30 ± 0.02^{c}	1.16 ± 0.02^{c}	2.51 ± 0.09^{c}	1.39 ± 0.07^{a}	6.57 ± 0.50^{d}	3.87 ± 0.42^{c}
Sanseong	3.18 ± 0.01^{d}	1.88 ± 0.03^{a}	3.63 ± 0.07^{b}	0.54 ± 0.03^{c}	7.97 ± 0.06^{c}	2.93 ± 0.12^{c}

*Values indicate the mean ± SD of three replication (*n*=3).
*Means with superscript letters within a column (a-d) indicate statistically differences ($p<0.05$), as determined by Duncan's multiple range test.

pH of TRW

The pH value could be used as an indicator for other substances produced during fermentation such as organic acids and carbon dioxide (So *et al.,* 1999; Kim *et al*., 2012). The highest pH value was observed in TRW brewed using *Geryang* (3.67±0.07), and the lowest pH was shown by *Sanseong* (3.18±0.01). These results were quite different with those reported by Kim *et al.* (2012), which used Improved starter (similar to *Geryang*) and *Jinju* starter. They have shown that, pH values of TRW using *Jinju* and Improved starter were 4.15 ± 0.03 and 3.79 ± 0.05 respectively. These starters were employed together with *S. cerevisiae*. They also have shown that, pH values of TRW using *Jinju* and Improved starter were 3.28 ± 0.02 and 3.25 ± 0.03 respectively. These starters were employed together with *Pichia anomala.* Previous studies have been reported that pH of rice wine were around 3.00 to 4.00 (Im *et al*., 2012; Hong *et al*., 1997; Woo *et al*., 2010). The pH value of TRW made from partially gelatinized wheat flour was in agreement with those in previous studies. Some studies have been reported that pH of TRW depended on raw material, fermentation agent (starter) and yeast (Kim *et al*., 2012; Cho *et al*., 2012; Park *et al*., 2012). Sahlin (1999) reported that, the different raw materials had different buffering capacity which might affect to the pH dynamic of system. Thus, the changing of pH value during brewing might be initiated by raw material or starter which were used in brewing.

Total acid

Total acid content of TRW using different starters were relatively high in the range of 0.87±0.12% to 1.88±0.03%. The lowest total acid content was shown by TRW brewed with *Geryang*. There was significantly different ($p < 0.05$) between total acid of TRW using *Geryang* and other starters. Total acid content of TRW tend to be high when wheat flour was used as a raw material. Previous studies have been reported that total acid content in TRW ranged from 0.2% to 1.3% which was influenced by raw materials (Kim *et al*., 2012; Seo *et al*., 2012; Lee *et al*., 2012; Cho *et al*., Park *et al*., 2012). The similar results have been reported by Seo *et al.* (2012) that total acid content of TRW increased, (0.68±0.02% to 1.04±0.02%) when the proportion of wheat increased. Lactic acid bacteria might grow faster in wheat flour media. They also reported that the viable cell numbers of lactic acid bacteria increased when the wheat proportion increased to substitute rice for making TRW. In addition, the titratable acidity increased as a result of LAB growth in initial fermentation stage and subsequently altered the general bacteria, LAB, and yeast (Kim *et al*., 2011). Total acid content in TRW is contributed by lactic acid which was produced during fermentation. In this present study, lactic acid production in TRW using geryang was significantly lower than those of other starters. There were various species of lactic acid bacteria presented in starters such as *Enterococcus faecium* and *Pediococcus pentosaceus* (Song *et al*., 2013), *Aerobacter*, and *Bacillus* sp. (Hong *et al*., 1997). *Rhizopus oryzae,* the representative saccharifying fungi which existed in starters, was able to produce lactic acid by saccharifying and fermenting starch to produce lactic acid (Anuradha *et al*., 1999; Hang, 1990; Yu and Hang, 1989). It might contributed to lactic acid content in TRW. In lactic acid fermentation, carbon sources could be derived by raw materials such as molasses, cheese whey, and starch (Anuradha *et al*., 1999).

Amino acidity

Amino acidity is an important factors in TRW. Amino acidity is required to make better taste (savoury or umami taste) in TRW. The highest amino acidity content was observed in TRW using *Jinju* (5.12±0.04%). There was significantly different between amino acidity content of TRW using *Jinju* than those of other starters ($p < 0.05$).

Reducing sugar

Reducing sugar content was observed to be the highest in TRW using *Urimil* (1.39±0.07% mg/mL), and to be the lowest in TRW using *Sanseong* (0.54±0.03% mg/mL). There was significantly different between reducing sugar in TRW using *Urimil* than those of other starters ($p < 0.05$). The higher reducing sugar resulted stronger sweet taste that indicated incomplete fermentation (Kim *et al.*, 2012).

Soluble solid

Sugar are the major soluble solids in TRW, whereas other soluble materials are an organics and amino acids. As in the fruits and vegetables, soluble solids level often to be used as an indicator of the presence of sugars (Bumgarner and Kleinhenz, 2012). In this study, the highest soluble solid content was observed in TRW brewed with *Jinju* (9.23±0.40obrix)>*Geryang* (8.90±0.04obrix)>*Sangju* (8.30±0.53 obrix)>*Sanseong* (7.97±0.06 obrix)>*Songhag* (7.73±0.61 obrix)>*Urimil* (6.57±0.50 obrix). In previous studies, soluble solid content could be attributed to alcohol production in TRW. In addition, soluble solid content inversely proportional to alcohol content. The greater level of soluble solid indicated incomplete fermentation and resulted lower alcohol content (Kim *et al.*, 2012; Kim *et al.*, 2011). This was on the contrary with those of other studies (Cho *et al.*, 2012; Park *et al.*, 2012; Seo *et al.*, 2012). In our study, there was no relation between soluble solid content and alcohol content. However soluble solid content was concomitant with the alcohol content when *Geryang* and *Songhag* used as the starter.

Alcohol content

Alcoholic fermentation, a complicated biochemical process, is carried out by yeast. Cereal crops such as wheat, maize, barley, rye malt are commonly used for alcoholic fermentation (Nikolova *et al.*, 2008). Alcohol is produced from simple sugar by *S. cerevisiae* via Embden-Meyerhof pathway of glycolysis (Maris *et al.*, 2006). Raw starch is degraded into dextrin, oligosaccharide, and maltose by α- and β-amylase. This is followed by producing simple sugar by glucoamylase and converted into alcohol by *S. cerevisiae* (Sarikaya *et al.*, 2000). Ethanol, the most important ingredient in TRW, influences the overall acceptance and quality of TRW (Kim *et al.*, 2007). In this present study, the alcohol content of TRW ranged from 2.93±0.12% to 9.20±0.20%. The alcohol content of TRW brewed using *Geryang* and *Songhag* was significantly different than those using other starters ($p < 0.05$). The highest alcohol content of the TRW was produced using *Geryang* (9.20±0.20%), and the lowest alcohol content was produced using *Sanseong* (2.93±0.12%). Previous studies have been reported that, alcohol content of TRW were diverse in the range of 6 to 13% (Kim *et al.*, 2012; Im *et al.*, 2012; Seo *et al.*, 2012; Cho *et al.*, 2012; Park *et al.*, 2012). TRW making is quite different with other alcoholic beverages such as beer. Beer making does not require microbial saccharification. On the contrary, saccharification is required by TRW to produce a good quality of TRW. The saccharification of raw materials in alcohol fermentation using mixed starters was accomplished by *Mucoraceae* and *Tricho-comaceae*, including the genera *Mucor, Rhizopus* and *Aspergillus* (Nout, 2009). Such as in the Sake manufacturing, *A. oryzae,* which is prominent in Koji, produces α-amylase and amylglucosidase, which hydrolyse starches to dextrin, maltotriose, maltose, glucose, and acids. Afterward, the primary alcohol producing yeast *(S. cerevisiae)* convert those simple sugar into alcohol (Jung *et al.*, 2012). In addition, *S. fibuligera* has been found to be the prominent amylase producer in traditional Thai and Vietnamese alcohol fermentation (Limtong *et al.*, 2002).

Principal component analysis of TRW

Principal Component Analysis (PCA) was done to asses on how the chemical parameter could characterize the TRW brewed with various starter. PCA could separated the TRW brewed with various starter clearly and could explained 82.33% of total variation by two principal components (P1 vs P2). A bi-plot showing either scores and loadings separated data into six definite groups based on the type of starter used in TRW (Figure 2).

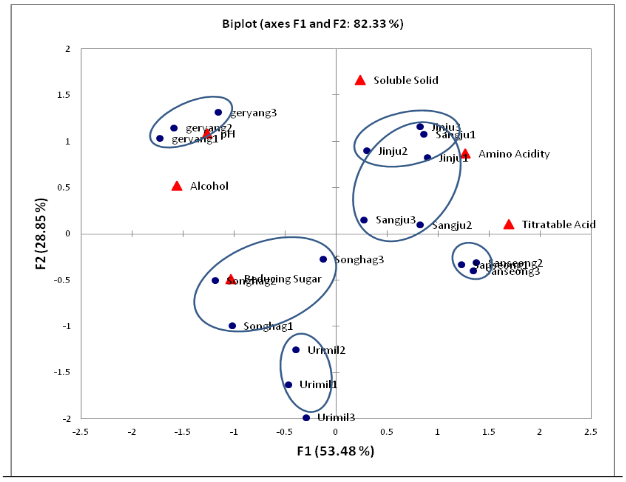

Figure 2. PCA biplot showing the effect of starter in the chemical characteristics of Turbid Rice Wine (TRW). The number following starter's name indicate replication

TRW brewed with *Geryang* was characterized by greater pH and alcohol content. TRW brewed with *Jinju* was characterized by greater soluble solid and amino acidity content. TRW brewed with *Sanseong* and *Urimil* were characterized by having greater titratable acidity and reducing sugar content respectively.

Organic acid content of TRW

In this study, six kinds of organic acid namely oxalic, citric, succinic, lactic, acetic, and pyroglutamic acid were detected in the TRW brewed using different starters (Table 2).

Table 2. Organic acid content of Turbid Rice Wine (TRW)

Starter	Organic acid (mg/ml)					
	oxalic	citric	succinic	lactic	acetic	Pyroglutamic
Geryang	0.14±0.02	0.46±0.06	1.08±0.06	4.46±0.98	1.71±0.21	ND
Jinju	0.19±0.01	1.05±0.04	0.92±0.05	11.32±0.57	2.39±0.08	0.18±0.02
Sangju	0.19±0.01	1.05±0.08	0.72±0.02	9.87±1.1	2.68±0.22	0.14±0.01
Songhag	0.12±0.02	0.96±0.09	0.90±0.10	6.54±1.66	1.58±0.22	ND
Urimil	0.09±0.03	0.87±0.23	0.22±0.04	5.87±1.66	0.98±0.32	ND
Sanseong	0.18±0.00	0.95±0.04	0.56±0.00	13.41±0.23	1.84±0.03	0.15±0.01

The oxalic, citric, succinic, lactic, acetic, and pyroglutamic acid content of TRW ranged from 0.09±0.03 to 0.19±0.01 mg ml^{-1}, 0.46±0.06 to 1.05±0.04 mg ml^{-1}, 0.22±0.04 to 1.08±0.06 mg ml^{-1}, 4.46±0.98 to 13.41±0.23 mg ml^{-1}, 0.98±0.32 to 2.68±0.22 mg ml^{-1}, and 0.14±0.01 to 0.18±0.02 mg ml^{-1}, respectively. Five kinds of organic acid contained in TRW brewed using each starter except pyroglutamic acid. This was found only in TRW brewed using *Jinju, Sangju*, or *Sanseong*. In this present study, lactic acid was the most abundant organic acid and considered to be the most important organic acid in TRW. It is attributed to round sour taste in the TRW (Kim *et al*., 2012). In this present study, *Geryang* was observed to produce the lowest lactic acid content in TRW (4.46±0.98 mg ml^{-1}) (Table 2). Oxalic and pyroglutamic acid were less abundant in TRW (Table 2.). In other study, oxalic acid was attributed to bitter taste in TRW (Kim *et al*. 2012), while, pyroglutamic acid was attributed to delicious taste similar to glutamic acid (Win, 2008). This present study observed that, acetic acid and citric acid in TRW ranged from 0.98±0.32 to 2.68±0.22 and 0.46±0.06 to 1.05±0.08, respectively. Both of them provided pungent sour and freshly sour taste, respectively (Kim *et al*., 2012). Acetic acid is produced by metabolism of glucose by lactic acid bacteria, catabolism of amino acids, and metabolism of citric acid and lactic acid (McSweeney and Sousa, 2000; Kaminarides *et al*. 2005).

Volatile compounds of TRW

In this present study, four volatile compounds, ethylacetate, EtOH, iso-butanol, and iso-amylalcohol, were identified in the TRW using each type of starters. Six volatile compounds, acetaldehyde, MeOH, 1-Propanol, n-Butanol, n-Hexanol, and acetic acid, were only detected in TRW using particular starters as shown in Table 3.

Table 3. Volatile compounds of Turbid Rice Wine (TRW)

Volatile Compound (ppm)	Starter					
	Geryang	*Jinju*	*Sangju*	*Songhag*	*Urimil*	*Sanseong*
Acetaldehyde	ND	171.85	ND	ND	277.87	ND
Ethylacetate	30.36	143.21	176.01	28.07	60.66	99.48
EtOH (%v/v)	10.40	4.54	3.98	12.27	4.80	2.98
MeOH	ND	ND	ND	45.62	ND	ND
1-Propanol	48.89	24.63	ND	59.70	13.65	15.76
Iso-butanol	7.17	3.36	2.31	8.14	2.62	1.97
n-Butanol	39.09	26.11	ND	64.64	91.43	19.12
n-Hexanol	ND	ND	20.29	ND	ND	ND
Iso-amylalcohol	289.79	173.34	140.12	324.98	295.45	106.51
Acetic acid	166.74	150.56	591.26	308.53	ND	250.35

all of volatile compounds are presented in ppm (part per million) except for EtOH is presented in %v/v.
*ND, not detected

Acetaldehyde was detected in TRW using *Jinju* and *Urimil*. MeOH and n-Hexanol were identified in TRW using *Songhag* and *Sangju*. Isoamyl alcohol and acetic acid were predominant in TRW that ranged from 106.51 ppm to 324.98 ppm and 150.56 to 591.26, respectively. The highest isoamyl alcohol content was observed in TRW brewed with *Songhag*. On the contrary, the lowest isoamyl alcohol content was observed in TRW using *sanseong*. Other study revealed that, isoamyl alcohol, which was produced from the amino acid leucine during *makgeolli* brewing, has a banana flavor, so it was considered as an important alcohol composite that significantly affects the flavor and taste of *makgeolli* (Kim *et al*., 2012; Lee *et al*., 2007). This present study detected the presence of iso-Butanol in the range of 1.97 ppm to 8.14 ppm. According to Sumby *et al.* (2010) and Jung *et al.* (2012) the flavor quality of alcoholic beverages depended on ester compounds that were formed from reactions of alcohol and carboxylic acid. This reaction was catalyzed by enzymes such as esterases, lipases and alcohol acetyltransferases produced by LAB.

Conclusions

Geryang and *Songhag* produced sufficient alcohol content and moderate total acid content to provide palatable taste in TRW. In addition, those produced high iso-amylalcohol compound to provide banana flavor in TRW. The amino acidity content in TRW brewed using *Geryang* and *Songhag* was relatively lower than those of other starters. *Urimil* was also identified to produce total acid and iso-amylalcohol at a favourable level, however, it produced insufficient alcohol content in TRW. In conclusion, *Geryang* and *Songhag* were suggested to be used as starter in TRW prepared with partially gelatinized wheat flour.

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