J. Microbiol. Biotechnol. (2010), **20**(12), 1702–1710
doi: 10.4014/jmb.1011.11004
First published online 28 November 2010

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Characterization of *Yakju* Brewed from Glutinous Rice and Wild-Type Yeast Strains Isolated from *Nuruks*

Kim, Hye Ryun[1,2], Jae-Ho Kim[1], Dong-Hoon Bae[2], and Byung-Hak Ahn[1]*

[1]*Korean Alcoholic Beverage Research Center, Korea Food Research Institute, Seongnam 463-746, Korea*
[2]*Department of Food Engineering, Dankook University, Cheonan 330-714, Korea*

Received: November 3, 2010 / Revised: November 24, 2010 / Accepted: November 25, 2010

Korean traditional rice wines *yakju* and *takju* are generally brewed with *nuruk* as the source of the saccharogenic enzymes by natural fermentation. To improve the quality of Korean rice wine, the microorganisms in the *nuruk* need to be studied. The objective of this research was to improve the quality of Korean wine with the wild-type yeast strains isolated from the fermentation starter, *nuruk*. Only strain YA-6 showed high activity in 20% ethanol. Precipitation of Y89-5-3 was similar to that of very flocculent yeast (>80%) at 75.95%. Using 18S rRNA sequencing, all 10 strains were identified as *Saccharomyces cerevisiae*. Volatile compounds present in *yakju* were analyzed by gas chromatography−mass selective detector. The principal component analysis (PCA) of the volatile compounds grouped long-chain esters on the right side of the first principal component, PC1; these compounds were found in *yakju* that was made with strains YA-6, Y89-5-3, Y89-5-2, Y90-9, and Y89-1-1. On the other side of PC1 were short-chain esters; these compounds were found in wines that were brewed with strains Y183-2, Y268-3, Y54-3, Y98-4, and Y88-4. Overall, the results indicated that using different wild-type yeast strains in the fermentation process significantly affects the chemical characteristics of the glutinous rice wine.

Keywords: *Yakju*, glutinous rice, wild-type yeast strain, *nuruk*, volatile compounds

Recently, increasing efforts have been made in Korea to popularize the consumption of traditional Korean rice wine in order to enjoy and maintain the native culture and promote local agriculture. The Korean liquor industry has been heavily dependent on imported raw materials, rather than relying on domestic agriculture, and the luxury liquor market has been dominated by imported liquors. Based on store sales, the Korean liquor market grossed 8.6 trillion Korean won in 2008. S*oju*, beer, and whiskey made up 87% of the total sales, whereas traditional rice wines, including *yakju* and *takju*, made up only 3.6%.

After soaking, the rice used to make *yakju* and *takju* is fermented with the traditional fermentation starter *nuruk*. Fermentation produces a harmonious blend of tastes and colors due to the sugars, amino acids, organic acids, and volatile flavor compounds produced by this process [22]. Korean traditional rice wines are generally brewed using the parallel double fermentation process, with *nuruk* as the source of the enzymes and fermentation-causing microorganisms, including naturally occurring fungi, yeast, and bacteria [4]. Mash, a small batch of specially soaked and prepared rice used to grow the starter culture, and *nuruk* are used to ferment *yakju* and *takju,* so these wines contain various live microorganisms. Among the organisms identified in *yakju* and *takju* are live molds including *Mucor*, *Rhizopus*, and *Aspergillus*, yeast including *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, and *Torulopsis*, and bacteria including *Micrococcus*, *Bacillus*, *Lactobacillus*, *Leuconostoc*, and *Aerobacter* [4, 15, 16, 25].

To give Korean rice wines a competitive advantage over imported liquors, it is critical to study the microorganisms that are contained in traditional starters to identify and isolate the best fermentation-inducing microorganisms for use in the manufacturing of these wines. For centuries, Korean brewers have relied on *nuruk*, but its value has been diminished with the modernization of the brewing industry; however, the usefulness of *nuruk* in contemporary wine-making has not been evaluated. With little research focused on traditional rice wine brewing, this Korean cultural process is becoming lost. Moreover, owing to the recent market growth of *yakju*, investigations have focused on producing high-alcohol-content beverages (in contrast to the popular low-alcohol content in *soju*) fermented with wild yeast that are isolated from *nuruk,* and improving the

*Corresponding author
Phone: +82-31-780-9102; Fax: +82-31-709-9876;
E-mail: bhahn@kfri.re.kr

taste and quality of these beverages is very important. One benefit of these studies is that, regardless of the findings, various *makgeolli* beverages can be manufactured in the process of experimentation and sold for a profit. In fact, studies of microorganisms used in making rice wine and experiments to improve the quality of traditional rice wines manufactured with *nuruk* have already identified yeast strains that improve the quality and fermentation characteristics of *cheongju*, a beverage closely related to *yakju* [24]. Other studies have isolated, identified, and optimized production conditions for yeast strains containing abundant, biologically active glutathione [19]; identified and characterized *Saccharomyces cerevisiae* SE211, a strain that is tolerant of high alcohol concentrations [23]; defined the characteristics of *hahyangju* that is manufactured with *S. cerevisiae* strain HA3 isolated from *nuruk* [7]; characterized the best strains for *jeju* foxtail millet wine [13, 14]; and characterized *yakju* that is produced with yeast strains isolated from fruit [26, 27].

Previous reports regarding microorganisms that are related to brewing or the development of the fermentation process in traditional *nuruk* have focused mostly on *Aspergillus* molds, which are also used to make Japanese sake [11, 12, 20]. Studies that are focused on selecting, breeding, and producing suitable yeast for making traditional Korean rice wine have been sporadic.

In this study, our group searched a database listing of approximately 1,500 yeast strains isolated from 300 *nuruk* and 10 pericarp samples that were collected around Korea. The 10 best yeast strains, as identified by the database, were used as starters to brew glutinous rice wines (*yakju*) using the double-soaking method. We then analyzed these yeast strains for characteristics that are important to wine making, including ethanol and flavor production, ethanol and glucose tolerance, flocculence, and acid production. In addition, we analyzed the characteristics of the resulting wines. The study provides basic data for improving traditional Korean brewing techniques and assesses the feasibility of using wild-type yeast strains isolated from the traditional *nuruk* in manufacturing glutinous rice wine.

Materials and Methods

Materials

The 10 wild-type yeast strains used in this study were isolated from preserved *nuruk* and pericarp samples that were collected from various regions of Korea. The reagents used were *chiaksan* rice (Shinlimnonghyup, Wonju, Gangwon-do, Korea), 100% rye malt powder (Buannonghyup, Sangseo, Buan, Jeollabuk-do, Korea), and *jinju-nuruk* [saccharogenic power (SP) 300, Jinju, Korea] (Table 1).

Yakju Brewing

Mash [glutinous rice:*nuruk* (SP 300):water=1:0.2:1.4; 2% of the final *yakju* volume] containing 0.01% yeast was incubated for 1 day at 20°C. For the first soak (66% of the final volume), mash was incubated with glutinous rice:*nuruk* (extracted after stirring with 5 volumes of water, SP 300):water=1:0.4:11.27 for 4 days at 15°C. Then, in the second soaking (32% of the final volume), mash was incubated with glutinous rice:*nuruk*:malt=1:0.037:0.029 for 17 days at 15°C. All brewing was performed in triplicate.

Table 1. *Nuruk* sources of the wild-type yeast strains.

Yeast strain	*Nuruk* source (region of Korea where collected)
Y54-3	Chungcheongbuk-do, Cheongju
Y88-4	Jeollabuk-do, Sunchang
Y89-1-1	
Y89-5-2	Gyeongsangnam-do, Hapcheon II
Y89-5-3	
Y90-9	Gyeongsangnam-do, Hapcheon I
Y98-4	Chungcheongnam-do, Kyeryong-paekilju
Y183-2	Jeollanam-do, Gokseong
Y268-3	Chungcheongnam-do, Yesan
YA-6	Gyeongsangnam-do, Hapcheon

Ethanol Resistance, Glucose Tolerance, and Flocculence

To determine the ethanol resistance of each yeast strain, 16, 18, 20, or 22% (v/v) of anhydrous ethanol was added to the liquid YPD culture (2% glucose, 0.5% yeast extract, 1% bacto-peptone; Sigma-Aldrich, St. Louis, MO, USA) immediately after yeast injection. The absorbance at 660 nm was determined after a 72 h incubation at 20°C. Glucose tolerance was determined by measuring the absorbance at 660 nm after incubating the yeast strains for 48 h at 20°C in liquid YPD culture containing 20, 25, 30, 35, or 40% glucose [13, 14]. To measure flocculence, yeast strains were incubated with shaking in PDB (potato dextrose broth; Difco, Detroit MI, USA) liquid culture, centrifuged, and divided into 2 samples. Sample A was resuspended in distilled water and 0.5 M EDTA. Sample B was resuspended in a $CaSO_4$ solution, centrifuged again, resuspended in $CaSO_4$, and left to settle for 6 min. Samples A and B were then diluted 10-fold, and the absorbance at 600 nm was measured [1]. Flocculence was calculated as %=(A−B)×100/A.

Microorganism Identification

Microorganisms were sent to Macrogen Inc. (Seoul, South Korea) for identification by PCR using the PTC-225 Peltier Thermal Cycler (MJ Research, Reno, NV, USA) and the primers ITS1 (5'-TCCGTA GGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The 18S rRNA gene sequencing was conducted using the same primers that were used for PCR and the ABI PRISM BigDye Terminator Cycle Sequencing Kits in the ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were identified using BLAST (http://www.ncbi.nlm.nih.gov).

Chemical Analysis of *yakju*

The ethanol content of *yakju* was analyzed by gas chromatography (GC; Hewlett-Packard 6890N; Palo Alto, CA, USA) after filtering the samples with 0.45-μm syringe filters (Xpertek, Rivonia, Republic of South Africa). The DB-ALC2 column (30 m length×0.53 mm diameter; 2 μm film thickness; J & W Scientific, Folsom, CA, USA) was used, with helium as the carrier gas and the temperatures as follows: oven, 70°C isothermal; inlet, 200°C; detector FID, 250°C. A standard curve was generated, and alcohol content was calculated as the peak area. The concentration of soluble solids was measured with a hand-held refractometer (ATAGO Pocket PAL-1; ATAGO Co. Ltd., Tokyo, Japan) and recorded in Brix units (%, sucrose). The pH was measured with the Orion Model EA 940 pH meter (Thermo Fisher Scientific, Waltham, MA, USA). Total acid was measured as succinic acid concentration and determined by adding 2−3 drops of phenolphthalein indicator into a 10-ml sample and titrating with 0.1 N NaOH until the solution turned light green; phthalic acid was used as the standard. The amino acid (glycine) concentration was determined by adding 2−3 drops of phenolphthalein indicator and 5 ml of a neutral formalin solution to 10-ml sample and titrating with 0.1 N NaOH until the solution turned pink; phthalic acid was used as the standard [18]. The *yakju* color was determined by measuring absorbance at 430 nm. The absorbance of ultraviolet light was measured at 280 nm in 25-fold diluted samples, and the concentration was calculated using the formula of absorbance/cell thickness (mm)×10×dilution factor [6]. Reducing sugar concentration absorbance was measured at 550 nm using the HP 8453 diode array UV−Vis spectrophotometer (Hewlett-Packard), the dinitrosalicylic acid method, and a glucose standard (Sigma, St. Louis, MO, USA) to calculate the concentration [17].

Analysis of Free Sugars and Organic Acids

Free sugar was analyzed by HPLC (Jasco UV-975 UV/VIS detector; Jasco, Tokyo, Japan). To analyze the sugars, a 1-ml sample was filtered through a 0.45-µm syringe filter, applied onto an Aminex HPX-87C column (300 mm×7.8 mm; flow rate, 0.6 ml/min, oven temperature, 60°C, injection volume, 10 µl; Bio-Rad Laboratories, Hercules, CA, USA), and analyzed with a refractive index detector. For organic acid analysis, a 1-ml sample was filtered through a 0.45-µm syringe filter, applied onto an Aminex HPX-87H column (300 mm×7.8 mm; flow rate, 0.6 ml/min, oven temperature, 35°C, injection volume, 10 µl), and UV absorbance was measured at 210 nm [30].

Volatile Compound Analysis

After equilibrating a 20-ml sample for 20 min at 60°C, the sample was applied onto a 100-µm polydimethylsiloxane (PDMS) fiber for 40 min and analyzed by GC for 1 min using solid-phase microextraction (SPME). A Hewlett-Packard 7890A GC, Hewlett-Packard 5975C mass selective detector (MSD) and a Stabilwax-DA column (30 m length×0.25 mm diameter×0.25 µm film thickness; Restek Corp., Bellefonte, PA, USA) were used for the analysis. The oven temperature of the GC was held at 50°C for 5 min and then set to rise to 200°C at the rate of 3°C/min. The injector temperature was 250°C, helium was used as the carrier gas, and the flow rate was 2 ml/min. The direct capillary interface of the MSD was set at a temperature of 250°C; ion source temperature, 230°C; ionization voltage, 70 eV; mass range, 45−550 amu; and a scan rate of 2.2 scans/s. The identities of the volatile compounds were confirmed by comparing retention indices (RI), mass spectra (NIST 05 library), and aromatic properties.

Statistical Analysis

Principal Component Analysis (PCA) of the volatile components was performed using SAS for Windows ver. 7.2 (Statistical Analysis Systems Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Characteristics of the Wild-Type Yeast Strains

The results of the ethanol resistance and glucose resistance analyses of the wild-type yeast strains isolated from *nuruk* are shown in Table 2. The activity of Y183-2 was best in 16% ethanol, whereas Y88-4 and Y89-1-1 were slightly active in 18% ethanol. Only YA-6 was highly active in 20% ethanol, whereas the other strains showed little activity at that concentration. However, YA-6 had less activity in 20% ethanol than was previously reported [8, 14]. Most of the yeast strains, except the 2 strains previously examined [8], were highly active in 29% glucose, so the maximum glucose concentration was elevated to 40%. Y89-5-3 was the most active strain in 20% glucose, and the activities of all yeast strains decreased as glucose concentration increased. The activity levels of Y89-5-3 and YA-6 were highly similar to previously reported results [14]. Y89-5-3 exhibited 75.95% flocculence, closest to that

Table 2. Ethanol resistance and glucose tolerance of the wild-type yeast strains.

Yeast strains	Ethanol resistance[a] (OD_{660})				Glucose tolerance[b] (OD_{660})				
	16	18	20	22	20	25	30	35	40
Y54-3	0.74±0.40	0.11±0.02	0.04±0.01	0.04±0.02	1.97±0.4	1.19±0.04	0.88±0.11	0.88±0.02	0.81±0.05
Y88-4	0.16±0.03	0.04±0.01	0.04±0.01	0.03±0.00	1.49±0.64	1.01±0.46	1.01±0.01	0.71±0.03	0.47±0.01
Y89-1-1	0.53±0.10	0.04±0.01	0.02±0.01	0.02±0.01	1.66±0.41	0.66±0.08	0.73±0.04	0.83±0.11	0.72±0.27
Y89-5-2	1.15±0.15	0.66±0.29	0.04±0.01	0.04±0.00	1.48±0.29	1.21±0.25	1.38±0.06	0.74±0.16	0.76±0.00
Y89-5-3	1.11±0.14	0.71±0.26	0.05±0.02	0.04±0.00	2.11±0.04	2.14±0.11	1.53±0.19	1.06±0.14	0.73±0.12
Y90-9	1.38±0.08	1.09±0.26	0.03±0.01	0.02±0.00	1.57±0.04	1.38±0.10	1.36±0.08	0.80±0.05	0.74±0.15
Y98-4	0.85±0.58	0.96±0.11	0.02±0.01	0.02±0.01	1.57±0.12	1.14±0.47	1.06±0.44	0.88±0.38	0.85±0.03
Y183-2	1.64±0.10	1.06±0.08	0.03±0.01	0.02±0.01	1.78±0.30	0.69±0.12	0.35±0.09	0.90±0.06	0.75±0.06
Y268-3	1.14±0.18	0.22±0.27	0.03±0.00	0.03±0.05	1.75±0.25	1.27±0.50	0.56±0.01	0.90±0.13	0.57±0.15
YA-6	1.17±0.27	0.99±0.22	0.47±0.18	0.04±0.01	1.83±0.76	1.48±0.20	1.52±0.12	1.09±0.01	0.41±0.02

[a]Yeast strains were cultured at 20°C for 72 h in YPD broth containing 16%, 18%, 20%, or 22% ethanol. [b]Yeast strains were cultured at 20°C for 48 h in YPD broth containing 20%, 25%, 30%, 35%, or 40% glucose. Results shown are mean ± SD from 3 independent experiments.

Table 3. Flocculence, 18S rRNA sequencing, and aroma description of wild-type yeast strains.

Yeast strain	Flocculence (%)	18S rRNA sequencing	18S rRNA sequencing (%)	Aroma description [a]
Y54-3	8.23	*Saccharomyces cerevisiae* strain M01614 internal transcribed spacer 1, partial sequence	90	Refreshing, sweet, sour
Y88-4	41.59	*Saccharomyces cerevisiae* strain M01614 internal transcribed spacer 1, partial sequence	91	Fruity, sweet, sour
Y89-1-1	23.40	*Saccharomyces cerevisiae* strain M01614 internal transcribed spacer 1, partial sequence	93	Refreshing, fruity, sweet, sour
Y89-5-2	21.33	*Saccharomyces cerevisiae* isolate ST 3352/1-03 18S rRNA gene, partial sequence	97	Pleasant
Y89-5-3	75.95	*Saccharomyces cerevisiae* isolate ST 3352/1-03 18S rRNA gene, partial sequence	96	Fruity, sweet, flowery, fermented
Y90-9	27.99	*Saccharomyces cerevisiae* strain M01614 internal transcribed spacer 1, partial sequence	98	Refreshing, sweet, sour, fermented
Y98-4	9.99	*Saccharomyces cerevisiae* partial 18S rRNA gene, type strain CBS4054	92	Fruity, sweet, sour
Y183-2	20.14	*Saccharomyces cerevisiae* isolate NN691 18S rRNA gene, partial sequence	92	Sweet, fermented yogurt
Y268-3	65.92	*Saccharomyces cerevisiae* partial 18S rRNA gene, type strain CBS4054	84	Banana-like, sweet
YA-6	55.65	*Saccharomyces cerevisiae* genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence, strain: JP	97	Sweet, fermented yogurt

[a]Aroma describes the *yakju* brewed with different wild-type yeast strains.

of very flocculent yeast (>80%). Y54-3 and Y98-4 were characterized as non-flocculent yeast (<20%), and the rest of the strains were moderately flocculent (20~60%). Based on analysis of the 18S rRNA, all 10 strains were identified as *Saccharomyces cerevisiae*.

The results of aroma description of the *yakju* samples brewed with different yeast strains are presented in Table 3. *Yakju* made with Y54-3 and Y98-4 had a sweet and sour aroma. A sweet aroma and a sour, refreshing, fruity aroma were detected in *yakju* made with Y88-4 and Y89-1-1, and a pleasant aroma was detected in *yakju* made with Y89-5-2. *Yakju* made with Y183-2 and YA-6 had the aroma of fermented yogurt with sweet notes, and *yakju* made with Y89-5-3 and Y90-9 possessed a fermented odor with a refreshing, flowery aroma. *Yakju* made with Y268-3 had a banana-like aroma.

Chemical Properties of *Yakju* Made with Different Yeast Strains

The chemical properties of *yakju* made with different yeast strains are shown in Tables 4 and 5. *Yakju* samples were analyzed after 17 days of fermentation. Y89-5-3 produced *yakju* with the highest ethanol content (18.4±0.23%). All yeast strains except Y183-2 resulted in 16.8% or higher ethanol content. *Yakju* made with YA-6 (isolated from apple residue) produced a higher ethanol content than the yeast strain S-1 (isolated from pericarp), which was previously reported to produce a maximum of 11.17% ethanol in *yakju* [27]. This result can be attributed to the use of both malt and glutinous rice to produce *yakju*, as *yakju* made with these two components has the highest ethanol content.

The highest pH (4.07±0.06) was found in *yakju* made with Y268-3, whereas the pH of *yakju* made with the other strains was 3.75–3.98±0.01. The latter values are relatively low for *yakju* [10, 21]. Total acid concentration was highest in *yakju* made with Y183-2 (0.44±0.02%), which also had the lowest pH, followed by *yakju* made with Y98-4 (0.36±0.05%). *Yakju* samples brewed with the remaining strains all had similar acid concentrations of 0.28–0.32±0.05%. The highest glycine concentration (0.41±0.01%) was found in *yakju* brewed with Y89-5-2, Y268-3, and YA-6, and the lowest glycine concentration (0.28±0.02%) was found in *yakju* made with Y88-4. The concentration of soluble solids was highest in *yakju* made with Y183-2, at 13.2±0.05%, whereas the other yeast strains had 10.7–11.8±0.10% of soluble solids. This value is higher than previously determined by sensory analysis in wines made with non-glutinous rice [5] and is similar to the highest values seen in *yakju* made with glutinous rice [9].

The color degrees of the *yakju* samples made with each yeast strain were similar at 0.15–0.17±0.01%. The absorption of ultraviolet light serves as an index of aromatic amino

Table 4. Chemical content of *yakju* brewed with different wild-type yeast strains (mean±SD, n=3).

Yeast strain	Ethanol (%)	pH	Total acid (%, succinic acid)	Amino acid (%, glycine)	Soluble solid (%, sucrose)	Color degree (OD_{430})	Ultraviolet light (OD_{280})	Reducing sugar (mg/ml)
Y54-3	18.2±0.30	3.85±0.05	0.31±0.03	0.34±0.10	11.8±0.10	0.15±0.01	2.04±0.18	14.75±0.11
Y88-4	17.2±0.22	3.83±0.02	0.32±0.05	0.28±0.02	11.7±0.20	0.15±0.02	1.26±0.20	16.40±0.04
Y89-1-1	17.2±0.28	3.86±0.12	0.31±0.10	0.35±0.01	11.6±0.10	0.16±0.01	2.46±0.08	15.94±0.05
Y89-5-2	17.7±0.29	3.88±0.09	0.28±0.04	0.41±0.01	11.7±0.25	0.15±0.01	2.34±0.16	15.09±0.28
Y89-5-3	18.4±0.23	3.98±0.01	0.28±0.12	0.39±0.02	11.2±0.26	0.15±0.02	2.24±0.05	13.47±0.28
Y90-9	17.3±0.31	3.95±0.03	0.30±0.05	0.38±0.06	11.4±0.08	0.15±0.01	2.34±0.02	14.43±0.26
Y98-4	16.8±0.25	3.75±0.04	0.36±0.08	0.35±0.01	10.7±0.12	0.16±0.01	2.10±0.07	12.26±0.07
Y183-2	13.5±0.21	3.64±0.10	0.44±0.02	0.33±0.05	13.2±0.05	0.17±0.01	2.17±0.44	26.98±0.32
Y268-3	17.0±0.17	4.07±0.06	0.22±0.01	0.41±0.03	11.1±0.13	0.15±0.02	1.92±0.24	12.89±0.18
YA-6	17.6±0.09	3.97±0.10	0.28±0.02	0.41±0.01	11.8±0.17	0.15±0.01	2.56±0.04	15.43±0.08

acid content. UV absorption was lowest in *yakju* brewed with Y88-4 (1.26±0.20%), which also produced the lowest concentration of amino acid. The highest amino acid concentration (2.56±0.04%) was observed when YA-6, a strain isolated from apple residue, was used. Reducing sugar concentration was highest (26.98±0.32 mg/ml) when Y183-2 was used. This same strain produced *yakju* with the lowest ethanol content and the highest concentration of soluble solids. Reducing sugar concentration was lowest (12.26±0.07 mg/ml) in *yakju* made with Y98-4, which also produced the lowest concentration of soluble solids. This correlation between reducing sugar and soluble solid content is similar to the results of previous studies [10, 28].

Citric acid concentration was similar in all of the *yakju* samples, at 0.02−0.05±0.01 mg/ml. Malic acid concentration was highest (0.45±0.12 mg/ml) in *yakju* brewed with Y89-5-2 and was not detected in *yakju* made with Y268-3. Succinic acid content was highest (1.92±0.24 mg/ml) in *yakju* made with Y90-9, followed by YA-6 (1.65±0.21 mg/ml). Lactic acid concentration was highest at 6.55±0.32 mg/ml in *yakju* made with Y183-2, which also had the lowest pH and highest total acid content. Therefore, *yakju* made with Y183-2 is expected to be quite sour. Acetic acid was highest at 0.45±0.21 mg/ml in *yakju* made with Y88-4 and lowest when Y90-9 and YA-6 were used. Pyroglutamic acid concentrations were 0.01−0.02 mg/ml in all samples. Maltose and glucose levels were highest at 3.29±0.24 and 19.71±1.16, respectively, when Y183-2 was used; this strain also produced the highest levels of reducing sugars. Fructose, which gives a refreshing sweet taste, was detected only in *yakju* made with Y89-1-1 or Y90-9.

Volatile Compounds in *Yakju* Brewed with Wild-Type Yeast Strains

The volatile, flavor-adding compounds were collected from *yakju* samples using the SPME (solid phase microextraction) method and were analyzed with GC−MSD (gas chromatography−mass selective detector). The relative peak area values of these compounds are presented in Table 6. In total, 50 compounds were detected, including 6 alcohols, 36 esters, 3 acids, 2 aldehydes, and 3 other compounds.

Besides ethanol, a main component in all samples, the second-highest relative peak area (12.809−22.723%) was produced by hexadecanoic acid ethyl ester, which gives

Table 5. Organic acid and free sugar contents of *yakju* prepared with different wild-type yeast strains (mean±SD, n=3, mg/ml)

Yeast strain	Citric acid	Malic acid	Succinic acid	Lactic acid	Acetic acid	Pyroglutamic acid	Maltose	Glucose	Fructose
Y54-3	0.04±0.01	0.18±0.06	0.50±0.01	4.14±0.29	0.14±0.01	0.01±0.00	2.18±0.12	8.98±0.35	-[a]
Y88-4	0.05±0.02	0.15±0.08	0.87±0.15	4.27±0.23	0.45±0.21	-	2.89±0.15	11.48±1.12	-
Y89-1-1	0.02±0.00	0.06±0.01	0.74±0.12	3.89±0.16	0.14±0.02	0.01±0.00	2.14±0.10	10.11±0.52	0.48±0.02
Y89-5-2	0.02±0.00	0.45±0.12	1.26±0.17	4.04±0.20	0.26±0.10	0.02±0.00	2.01±0.10	9.76±0.45	-
Y89-5-3	0.03±0.01	0.06±0.01	1.31±0.21	3.27±0.14	0.09±0.08	0.01±0.00	1.53±0.12	6.97±0.20	-
Y90-9	0.03±0.02	0.07±0.02	1.92±0.24	3.21±0.12	0.03±0.01	0.01±0.00	1.74±0.13	8.40±0.26	0.25±0.01
Y98-4	0.04±0.02	0.14±0.01	0.83±0.11	5.55±0.25	0.38±0.17	0.01±0.00	1.31±0.09	8.04±0.18	-
Y183-2	0.03±0.00	0.23±0.10	0.62±0.08	6.55±0.32	0.40±0.20	0.02±0.00	3.29±0.24	19.71±1.16	-
Y268-3	0.03±0.00	-	1.27±0.15	2.94±0.17	0.20±0.09	0.01±0.00	1.69±0.05	6.81±0.35	-
YA-6	0.05±0.01	0.06±0.01	1.65±0.21	3.15±0.34	0.02±0.01	0.01±0.00	1.93±0.08	8.59±0.32	-

[a] Not detected.

yakju a barely detectable, slightly waxy, creamy, and sweet taste [2]. Ethyl oleate was the next most abundant compound, at 2.279−9.200%; this compound produces a rare aroma, and a soft, oily, and sweet taste [2]. The relative peak areas of long-chain esters were also high.

The majority of esters are formed by esterification during fermentation. High levels of ethyl acetate, a compound that is unpalatable at higher concentrations [2], were not detected in *yakju* produced with Y90-9. Ethyl acetate concentration was lowest (0.29%) in *yakju* made with YA-6, and highest (1.276%) when Y88-4 was used.

Isoamyl alcohol is an important component in high-quality alcoholic beverages [29], strongly impacting the aroma and taste by adding a sweet, banana-like aroma.

Table 6. Volatile compound content of *yakju* prepared with different wild-type yeast strains.

No.	RT	RI	Compounds	Y54-3	Y88-4	Y89-1-1	Y89-5-2	Y89-5-3	Y90-9	Y98-4	Y183-2	Y268-3	YA-6
1	3.139	<1000	Ethyl acetate	0.755	1.276	0.645	0.603	0.462	-	1.235	1.677	0.863	0.290
2	3.785	<1000	Ethyl alcohol	44.042	42.228	39.873	37.861	37.476	43.501	44.713	62.709	54.411	48.796
3	5.644	1030	Butanoic acid ethyl ester	0.215	0.054	0.047	0.113	0.143	0.215	0.043	0.100	0.047	0.073
4	7.293	1098	Propanoic acid	0.157	-	0.112	0.123	0.156	0.027	0.065	0.090	0.175	0.162
5	7.679	1112	Isoamyl acetate	0.054	0.060	0.029	0.046	0.024	0.009	0.056	0.226	0.035	0.002
6	10.623	1210	Isoamyl alcohol	5.209	2.377	3.589	3.738	3.795	3.654	2.870	3.788	4.726	5.755
7	11.139	1226	Hexanoic acid ethyl ester	0.674	0.606	0.459	0.478	0.446	0.584	0.384	0.583	0.271	0.305
8	14.469	1332	Heptanoic acid,ethyl ester	0.031	0.022	0.019	0.017	0.015	-	0.015	0.022	-	-
9	15.014	1348	Lactic acid ethyl ester	0.127	0.114	0.096	0.090	0.089	0.065	0.124	0.236	0.072	0.087
10	15.985	1376	3-Ethoxy-1-propanol	0.066	-	0.051	-	-	-	0.049	-	0.022	-
11	17.800	1432	Octanoic acid ethyl ester	2.181	2.472	1.604	1.790	1.861	2.258	0.793	0.885	0.378	1.281
12	18.566	1456	Isoamyl caproate	0.059	0.154	0.066	0.055	0.039	0.010	0.156	0.137	0.076	0.024
13	20.794	1527	Benzaldehyde	0.071	0.020	0.054	0.042	0.052	0.035	0.042	-	0.157	0.102
14	20.989	1534	Nonanoic acid ethyl ester	0.062	0.049	0.051	0.046	0.040	0.045	0.035	0.021	0.017	0.041
15	21.100	1537	2,3-Butanediol	-	0.013	-	-	0.010	-	0.010	-	0.015	0.015
16	24.074	1637	Decanoic acid ethyl ester	2.226	2.857	1.876	2.852	2.891	3.325	1.152	1.002	0.819	1.992
17	24.688	1658	Isoamyl caprylate	0.047	0.034	0.035	0.047	0.057	0.082	0.020	0.020	-	0.071
18	25.033	1670	Benzoic acid ethyl ester	0.030	0.028	0.023	0.020	0.018	0.021	0.022	0.029	0.024	0.020
19	25.279	1679	Succinic acid diethyl ester	0.069	0.058	0.032	0.043	0.035	0.073	0.041	0.053	0.040	0.060
20	25.700	1693	4-Decenoic acid ethyl ester	-	0.012	-	-	-	-	-	-	-	0.014
21	27.020	1741	Undecanoic acid ethyl ester	-	-	-	0.016	0.016	-	0.016	-	-	0.008
22	27.207	1748	Caprylic ether	0.031	0.033	0.028	0.020	0.013	0.029	0.023	0.047	0.029	0.017
23	27.390	1754	*n*-Capric acid isobutyl ester	-	-	-	0.009	0.018	0.010	-	-	-	0.011
24	29.821	1845	Dodecanoic acid ethyl ester	0.903	1.449	1.351	2.019	3.137	1.536	1.232	0.947	1.025	0.936
25	30.293	1862	Isopentyl decanoate	0.031	0.026	0.034	0.054	0.089	0.066	0.017	-	0.016	0.052
26	31.624	1914	Phenylethyl alcohol	1.595	1.296	1.245	1.212	0.979	1.975	1.478	1.954	1.493	2.000
27	31.894	1924	Benzyl oxy tridecanoic acid	0.022	0.028	0.015	0.009	0.014	0.023	0.007	0.026	0.020	0.031
28	32.463	1947	Ethyl tridecanoate	0.030	0.048	0.074	0.104	-	0.108	0.062	0.100	0.076	0.085
29	34.709	2038	Isopropyl tetradecanoate	0.041	0.035	0.012	0.014	-	0.029	0.014	-	0.031	0.010
30	35.037	2051	Tetradecanoic acid ethyl ester	3.684	3.482	4.903	5.841	8.272	4.184	5.240	2.186	4.086	5.653
31	35.420	2067	Isoamyl laurate	-	-	0.022	0.033	0.055	0.027	0.022	-	-	0.020
32	35.590	2074	Z-7-Tetradecanoic acid	-	-	-	0.080	0.049	0.019	0.054	0.030	0.043	0.079
33	37.071	2137	Pentadecanal	0.025	-	0.019	0.011	0.007	0.013	0.018	0.022	0.019	0.016
34	37.445	2153	Pentadecanoic acid ethyl ester	0.186	0.255	0.361	0.533	0.318	0.291	0.643	0.379	0.639	0.970
35	37.700	2164	E-11-Hexadecanoic acid ethyl ester	-	-	0.034	0.054	0.073	0.035	0.037	0.016	0.035	0.025
36	37.893	2173	6-Undecylamine	0.099	0.079	0.111	0.106	0.079	0.079	0.117	0.133	0.139	0.119
37	39.480	2243	Hexadecanoic acid isopropyl ester	0.024	0.020	0.048	0.008	-	0.019	0.013	0.027	0.037	-

Table 6. Continued.

No.	RT	RI	Compounds	Y54-3	Y88-4	Y89-1-1	Y89-5-2	Y89-5-3	Y90-9	Y98-4	Y183-2	Y268-3	YA-6
38	39.898	2262	Hexadecanoic acid ethyl ester	19.105	20.436	21.123	20.904	19.054	17.966	20.423	12.809	17.632	22.723
39	40.240	2277	Ethyl 9-hexadecanoate	0.626	0.837	0.916	1.401	0.919	1.054	1.143	0.603	1.112	0.958
40	41.722	2339	Hexadecanoic acid propyl ester	0.025	0.015	0.028	0.051	0.021	0.066	0.077	0.062	0.142	0.042
41	42.170	2357	Heptadecanoic acid ethyl ester	0.054	0.087	0.071	0.067	0.041	0.090	0.126	0.076	0.087	0.343
42	42.452	2368	Hexadecanoic acid 2-methyl propyl ester	0.029	0.024	0.038	0.045	0.047	0.038	0.029	-	0.029	0.048
43	42.680	2377	1-Hexadecanol	0.115	0.157	0.072	0.103	0.027	0.073	0.090	0.095	0.151	0.064
44	42.964	2388	4-Octadecylmorpholine	0.172	0.133	0.191	0.125	0.114	0.138	0.195	0.202	0.196	0.177
45	45.182	2460	Octadecanoic acid ethyl ester	1.463	2.173	1.632	1.586	1.038	1.675	1.766	1.373	1.238	2.431
46	45.892	2482	Ethyl oleate	7.357	9.200	9.115	8.630	8.402	8.076	7.946	4.482	5.069	2.279
47	46.122	2490	E-9-Octadecanoic acid ethyl ester	0.262	0.429	0.391	0.362	0.356	0.325	0.355	0.227	0.184	0.506
48	47.666	>2500	Ethyl linoleate	6.931	7.050	8.313	8.129	8.960	7.783	6.217	2.156	4.156	1.270
49	50.233	>2500	Ethyl 18-nonadecanoate	0.987	0.188	1.036	0.363	0.141	0.257	0.710	0.470	0.124	0.033
50	50.480	>2500	Ethyl linolenate	0.126	0.089	0.157	0.151	0.153	0.112	0.102	-	0.044	-
			Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Data are presented as % total peak area. RT, Retention time; RI, Retention indices were determined using C10~C25 as external reference.

Isoamyl alcohol was highest in *yakju* made with YA-6 (5.755%), followed by Y54-3 (5.209%). The relative peak area of hexanoic acid ethyl ester, which lends a fruity aroma [2], was highest (0.674%) in samples brewed with Y54-3, and lowest (0.271%) in *yakju* made with Y268-3. The relative peak areas of phenylethyl alcohol, which is found in natural oils such as rose and orange oils and produces a flowery, honey-like aroma [3], were highest at 2.0% and 1.975% when YA-6 and Y90-9 were used, respectively.

Tetradecanoic acid ethyl ester gives wine soft, oily taste characteristics that are similar to orris root or violets [3]; its concentration was highest (8.272%) in *yakju* made with Y89-5-3 and lowest (2.186%) when Y183-2 was used. The

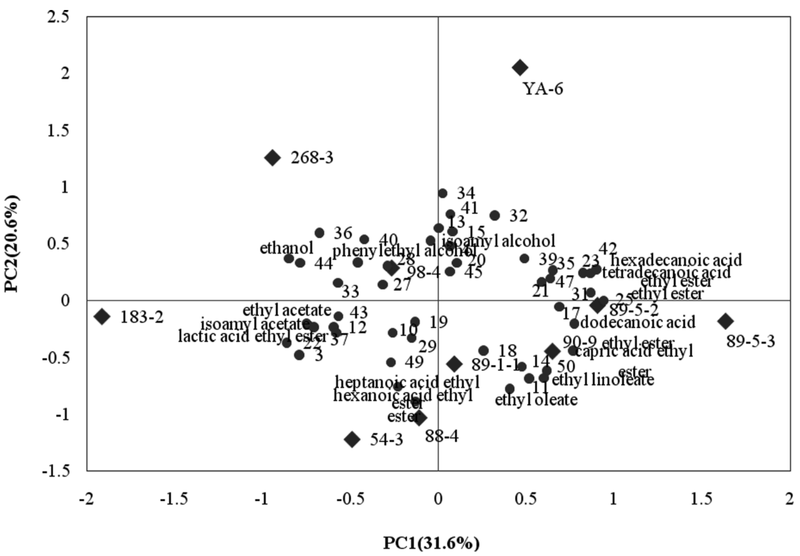

Fig. 1. Principal component analysis (PCA) of the 50 volatile compounds of *yakju* brewed with 10 different wild-type yeast strains.
PC1 and PC2 account for 31.6% and 20.6% of the variance, respectively. Letters correspond to the volatile compound designations as presented in Table 6.
◆, *Yakju* brewed with different wild-type yeast strains; ●, Volatile compounds.

relative peak area of ethyl linoleate, which has little odor, but has soft, sweet, oily taste characteristics [2], was also highest when Y89-5-3 was used, at 8.96%, and lowest, at 1.27%, in samples produced with YA-6.

The relative peak area percentages of the compounds that were identified in samples brewed with Y89-5-3 were as follows: alcohols, 42.287%; esters, 57.229%; acids, 0.218%; aldehydes, 0.059%; and other compounds, 0.206%. The relative peak area of alcohols was lower than that of esters. On the other hand, the relative peak area percentages in *yakju* brewed with Y183-2 were alcohols, 68.546%; esters, 30.904%; acids, 0.146%; aldehydes, 0.022%; and other compounds, 0.382%. Thus, in this sample, the relative peak areas of the alcohols were higher than those of esters.

The biplot of PC1 versus PC2 from the principal components analysis of the matrix of volatile compounds from all samples is presented in Fig. 1. The first dimension, accounting for 31.6% of the variance, shows a contrast between *yakju* samples brewed with YA-6, Y89-5-3, Y89-5-2, Y90-9, and Y89-1-1, and *yakju* made with Y183-2, Y268-3, Y54-3, Y98-4, and Y88-4. The second principal component, which accounts for 20.6% of the variance, shows a contrast between samples made with YA-6, Y268-3, and Y98-4 and the other samples. Long-chain esters, such as decanoic acid ethyl ester, which has a sweet and nutty odor; dodecanoic acid ethyl ester, which has a fruity, flowery odor and oily characteristics [2]; hexadecanoic acid ethyl ester, which produces extremely soft, sweet, and creamy characteristics but almost no aroma; ethyl oleate, which has little odor and soft, sweet, oily characteristics; and ethyl linoleate are all plotted along the far right of PC1. On the other side of the PC1 are short-chain esters; alcohols besides ethanol; ethyl acetate, which gives a fruity, brandy-like aroma but is unpalatable at high concentrations; isoamyl acetate, with a sweet, refreshing, fruity aroma [2]; and phenylethyl alcohol, which produces the aroma of roses and honey.

Acknowledgment

This research was supported by a grant from the Traditional Food Globalization Research and Development Projects (flavor improvement of Korean traditional rice wine for worldwide trade) of the Korea Food Research Institute.

References

1. American Society of Brewing Chemists. 2004. *ASBC Methods of Analysis*, 9th Ed. Microbiology Method Yeast-11.
2. Arctander, S. 1969. *Perfume and Flavor Chemicals (Aroma Chemicals) I*. Montclair, NJ, USA.
3. Arctander, S. 1969. *Perfume and Flavor Chemicals (Aroma Chemicals) II*. Montclair, NJ, USA.
4. Chang, K. J. and T. J. Yu. 1981. Studies on the components of *sokokju*, and commercial *yakju*. *Korean J. Food Sci. Technol*. **13:** 307−313.
5. Han, E. H., T. S. Lee, B. S. Noh, and D. S. Lee. 1997. Quality characteristics in mash of *takju* prepared by using different *nuruk* during fermentation. *Korean J. Food Sci. Technol*. **29:** 555−562.
6. Japan Sake Brewers Association. 1993. *A Book with Notes National Tax Service Methods of Analysis*, pp. 27−30, 4th Ed. Tokyo, Japan.
7. Jung, H. K., C. D. Park, H. H. Park, G. D. Lee, and I. S. Lee. 2006. Manufacturing and characteristics of Korean traditional liquor, *Hahyangju*, prepared by *Saccharomyces cerevisiae* HA3 isolated from traditional *nuruk*. *J. Food Sci. Technol.* **38:** 659−667.
8. Kim, H. R., S. H. Baek, M. J. Seo, and B. H. Ahn. 2006. Feasibility of *cheonghju* brewing with wild type yeast strains from *nuruks*. *Korean J. Appl. Microbiol. Biotechnol*. **34:** 244−249.
9. Kim, H. R., S. J. Jo, S. J. Lee, and B. H. Ahn. 2008. Physicochemical and sensory characterization of a Korean traditional rice wine prepared from different ingredients. *J. Food Sci. Technol*. **40:** 551−557.
10. Kim, H. R., Y. H. Kwon, S. J. Jo, J. H. Kim, and B. H. Ahn. 2009. Characterization and volatile flavor components in glutinous rice wines prepared with different yeasts of *nuruks*. *J. Food Sci. Technol*. **41:** 296−301.
11. Kim, H. S., J. S. Hyun, J. Kim, H. P. Ha, and T. S. Yu. 1998. Enzymological characteristics and identification of useful fungi isolated from traditional Korean *Nuruk*. *J. Microbiol. Biotechnol*. **26:** 456−464.
12. Kim, H. S. and T. S. Yu. 2000. Volatile flavor components of traditional Korean *Nuruk* produced by *nuruk* fungi. *Korean J. Appl. Microbiol. Biotechnol*. **28:** 303−308.
13. Kim, J. Y. and J. S. Koh. 2004. Fermentation characteristics of *Jeju* foxtail millet-wine by isolated alcoholic yeast and saccharifying mold. *J. Korean Soc. Appl. Biol. Chem*. **47:** 85−91.
14. Kim, J. Y. and J. S. Koh. 2004. Screening of brewing yeasts and saccharifying molds for foxtail millet-wine making. *J. Korean Soc. Appl. Biol. Chem*. **47:** 78−84.
15. Lee, S. R. 1986. *Hankuk eui Balhyo Sikpum (Fermented Foods of Korea)*, pp. 222−294. Ewha Women's University, Seoul, Korea.
16. Lee, Z. S. and T. W. Lee. 1970. Studies on the microflora of *takju* brewing. *Korean J. Microbiol*. **8:** 116−133.
17. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. **31:** 426−428.
18. NTSTS Institute. 1997. *Textbook of Alcoholic Beverage-Making*, pp. 368−370. National Tax Service Technical Service Institute, Seoul, Korea.
19. Park, J. C., M. OK, J. Y. Cha, and Y. S. Cho. 2003. Isolation and identification of the high-glutathione producing *Saccharomyces cerevisiae* FF-8 from Korean traditional rice wine and optimal producing conditions. *J. Korean Soc. Agric. Chem. Biotech*. **46:** 348−352.
20. Park, J. W., K. H. Lee, and C. Y. Lee. 1995. Identification of filamentous molds isolated from Korean traditional *nuruk* and their amylolytic activies. *Korean J. Appl. Microbiol. Biotechnol.* **23:** 737−746.

21. Park, Y. M., S. J. Kim, I. S. Hwang, K. H. Cho, and S. T. Jung. 2005. Physicochemical and sensory properties of *jinyang-ju* prepared with glutinous rice and nonglutinous rice. *Korean J. Food Culture* **20:** 346−351.
22. Rhee, S. J., C. Y. Lee, K. K. Kim, and C. H. Lee. 2003. Comparison of the traditional (*samhaeju*) and industrial (*chongju*) rice wine brewing in Korea. *Food Sci. Biotechnol*. **12:** 242−247.
23. Seo, M. J. and S. R. Ryu. 2002. Screening and characteristics of ethanol tolerant strain *Saccharomyces cerevisiae* SE211. *Korean J. Appl. Microbiol. Biotechnol*. **30:** 216−222.
24. Shin, C. S., S. K. Lee, and Y. J. Park. 1996. Characteristics of the yeast strains isolated for improvement of *Choungju* quality. *Agric. Chem. Biotechnol*. **39:** 9−15.
25. Shin, Y. D. and D. H. Cho. 1970. A study on the microflora changes during *takju* brewing. *Korean J. Microbiol*. **8:** 53−64.
26. Shin, K. R., B. C. Kim, J. Y. Yang, and Y. D. Kim. 1999. Characterization of *yakju* prepared with yeasts from fruits 1. Volatile components in *yakju* during fermentation. *J. Kor. Soc. Food Sci. Nutr*. **28:** 794−800.
27. Shin, K. R., B. C. Kim, J. Y. Yang, and Y. D. Kim. 1999. Characterization of *yakju* prepared with yeasts from fruits 2. Quality characteristics of *yakju* during fermentation. *J. Kor. Soc. Food Sci. Nutr*. **28:** 801−804.
28. So, M. H., Y. S. Lee, and W. S. Noh. 1999. Changes in microorganisms and main components during *takju* brewing by a modified *nuruk*. *Korean J. Food Nutr.* **12:** 226−232.
29. Yuda, J. 1976. Volatile compounds from beer fermentation. *J. Soc. Brew. Japan* **71:** 818−830.
30. Zoecklein, B. W., K. C. Fugelsang, B. H. Gump, and F. S. Nury. 1995. *Wine Analysis and Production*. Chapman & Hall, New York, USA.