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RESEARCH ARTICLE

Flavor Characteristics of Rice-grape Wine with Starch-hydrolyzing Enzymes

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Abstract A brewing process for rice-grape wine, in which rice powder and grapes are concurrently fermented, was developed. Rice powder was mixed with α-glucosidase, glucose isomerase, and yeast, and then incubated for 2 days at 25ºC. Then a mixture of 'Muscat Baily A' and 'Campbell Early' grape must was added to the fermented mixture of rice and maintained at 4ºC to allow for complete ethanol fermentation. The rice-grape wine contained 11.6% ethanol, compared to 9.6% ethanol for grape wine. The aroma profile revealed that 2-methyl-1-propanol, 2-methyl propyl acetate, and phenethyl acetate were in greater abundance in the rice-grape wine, whereas ethyl hexanoate, diethyl succinate, and ethyl decanoate were more abundant in the grape wine. The esters formed from fatty acids and ethanol increased during 2 years of storage for both wines. An electronic nose analysis revealed no significant difference in the aromas of the rice-grape and grape wine samples.

Keywords: flavor, rice-grape wine, grape wine, starch hydrolyzing enzyme, aroma

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Introduction

Due to the inherent difficulties of adapting grape vines to the soil and climate conditions of Korea, Korean wine makers are limited in their selection of wine-grape varieties and cultivars. The most desired cultivars may not be ideal choices. As a possible work-around to this problem, the traditional wine-making process has been modified by adding cooked rice to grape must or grape juice (1). 'Campbell Early' is one of the principal classic grape varieties native to Korea and is also a popular edible grape. Because this domestic grape cultivar contains high levels of malic and tartaric acid, a de-acidification process was developed by Lee and Kim (2). Recently, 'Muscat Baily A' has become increasingly popular as a cultivated variety in the southern part of the Korean peninsula. This variety can be blended with 'Campbell Early' to enhance the fruity aroma of wine.

The sugar content of grapes cultivated in Korea is typically 16-18°Bx, which results in less than 10% ethanol content in the final wine (3). Currently, the most common method for dealing with sugar-deficient fermentation is to add sucrose as a supplementary sugar. Consequently, most domestic wineries have been fortifying their musts with sucrose to enhance the final ethanol content to approximately 12%.

Traditional Korean rice wine (more accurately described as 'rice beer') has long been brewed using cooked rice and yeasts. Much research has been done to improve the quality and functionality of rice wine using microbes and various herbs containing physiologically active compounds. Recent studies have focused on standardization of the manufacturing process and safety-control of the distribution system (4-6). In addition, attempts have been made to combine traditional rice wine with grape wine by co-fermenting rice and grape must with amylolytic enzymes and yeast (1). However, the unique characteristics, acceptability, and functionality of rice-grape wine are poorly understood. Also, the effect of concurrent rice fermentation on the growth of yeasts and the resulting volatile compounds of rice-grape wine have not been determined.

Biomass and mineral content govern the fermentation rate during wine making (7,8). Similarly, sugar content is critical to yeast growth and metabolism during wine fermentation. The principal sugars in grapes are glucose and fructose, which usually occur in roughly equal proportions. Therefore, in this study, rice starch was converted into glucose and fructose by α-glucosidase and glucose isomerase, so that the yeast could ferment sugars in a similar composition as that found in grape must. Pre-fermentation of rice may also influence the wine aroma in a decisive way due to the major groups of volatile compounds formed from rice. Quantitative data on the quality and aromatic compounds of rice-grape wine would be useful information for consumers. Therefore, we investigated the effects of rice pre-fermentation on the quality of rice-grape wine during fermentation and storage and assessed the volatile compounds and acceptability of rice-grape wine compared to grape wine. We systematically evaluated both types of wine using analytical methods, including GC-MS and electronic nose and tongue systems (9,10).

Materials and Methods

Microorganisms, chemicals, and samples Sumizyme (glucoamylase) was purchased from Shin Nihon Kagaku Kogyo (Aichi, Japan), and glucose isomerase was purchased from Genencor (Palo Alto, CA, USA). *Saccharomyces cerevisiae* was obtained from La Parisienne (Casteggio Lieviti SRL, Casteggio, Italy). Commercial wine ('1865'; San Pedro, Chile) was purchased from a local market in Seoul, Korea.

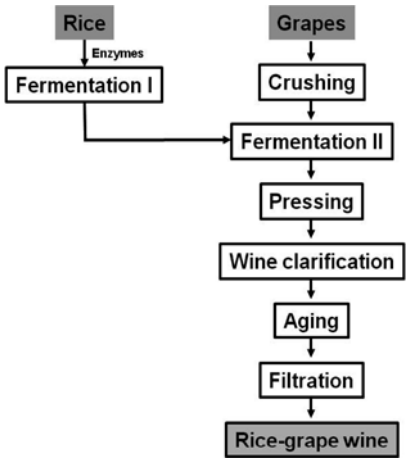

Fig. 1. Schematic diagram of the fermentation process during making of rice-grape wine.

Wine making Fully ripened *Vitis vinifera* cv. Muscat Baily A and *Vitis vinifera* cv. Campbell Early grapes were purchased from a vineyard in Gyeongsan in October, 2010. After stemming and crushing 300 kg 'Campbell Early' and 1,200 kg 'Muscat Baily A' grapes, the must was mixed with 300 g $K_2S_2O_5$ (200 ppm), followed by skin maceration. The fermentation took place in a water-jacketed 1,000-L fermenter with a vent to allow the escape of CO_2. The fermentation was carried out under a controlled temperature (25-28°C) with 300 g commercial yeast (*S. cerevisiae*). The wine-making followed the generally accepted methods for production of red wine (Fig. 1). For rice-grape wine, the process included 2 additional steps. In the 1st step, fermentation was carried out by mixing 200 kg glutinous rice in 300 L water (70% milling yield) containing 600 kU glucoamylase, 68 kU glucose isomerase, and 300 g yeast and allowing the mixture to incubate at 25°C for 2 days (fermentation I in Fig. 1). After the end of the rice fermentation, 1,000 kg must of 'Muscat Baily A' and 'Campbell Early' grapes at a ratio of 8:2 (w/w) and containing 300 g $K_2S_2O_5$ (200 ppm) was added to the fermented rice mixture. To extract maximum color from the skins and to discourage aerobic spoilage, the cap was pushed back down into the wine by pumping wine from the bottom of the fermenter over the top, where it was sprayed evenly across the surface of the cap every 8 h during the fermentation (fermentation II in Fig. 1). To reduce excess acidity, the precipitate was removed after 5 days of storage at 4°C. The evolution of fermentation was followed by daily measurement of the sugars by the refraction index, using a refractometer (Atago, Tokyo, Japan).

General analysis The pH values were measured using a pH meter (Neomet, Korea), and the titratable acidity was estimated by titration with 0.1 N NaOH to pH 8.2. The Brixes were analyzed with a refractometer (Atago).

Determination of ethanol content The ethanol content during fermentation was analyzed by HPLC (Waters 600; Waters, Milford, MA, USA) equipped with an RI detector and a Rezex ROA column (Phenomenex, Torrance, CA, USA). The flow rate was 1.0 mL/min, and the mobile phase was water.

Extraction of aromatic compounds from wines using headspace-solid phase microextraction (HS-SPME) HS-SPME was used to extract aromatic compounds from the wine. The wine (7 mL) was put into a 20-mL vial and allowed to reach equilibrium in a 50°C water bath for 30 min. SPME fiber (50/30 μm divinylbenzene/carboxen on polydimethylsiloxane) (Supelco Co., Bellefonte, PA, USA) was exposed to the headspace (1 cm) of the sample, with full extension of the fiber, and the volatiles were adsorbed onto the fiber for 30 min at 50°C. After adsorption, the aromatic compounds were desorbed in the GC injection port (200°C) for 1 min (splitless mode; 60 s valve delay). The SPME needle was left in the GC injector until the end of the GC run to eliminate any possible contamination. Prior to use, the SPME fibers were cleaned by baking in the GC injector for 30 min. The HS-SPME experiments were carried out in duplicate.

GC-MS The GC-MS system used in this study consisted of an Agilent 6890N GC/Agilent 5973N mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA, USA) with a DB-5 ms column (60 m×0.25 mm, 0.25-μm film thickness; J & W Scientific, Folsom, CA, USA). The carrier gas was helium with a constant flow rate of 1.0 mL/min. The injector and ion source temperatures were 200 and 250°C, respectively. The oven temperature was isothermal at 40°C for 5 min, was increased to 200°C at a rate of 5°C/min, and then was isothermal at 200°C for 20 min. The following MSD conditions were used: transfer line temperature, 280°C; ionization energy, 70 eV; mass range, 33-350 atomic mass units (amu).

Analysis by electronic nose For the analysis, 10 mL sample was placed in a 10-mL vial (PharmaFix, Chemmea, Slovakia). The vials were hermetically sealed with PTFE/silicone septa and caps (Shimadzu, Kyoto, Japan) immediately after filling. Volatile compounds from wine were analyzed using an MS electronic nose (SMart Nose 300; SMart Nose, Marin-Epagnier, Switzerland). This instrument is equipped with a quadrupole mass spectrometer (QMS422l; Balzers Instruments, Marin-Epagnier, Switzerland), a programmable autosampler (Combl PAL; SMart Nose), and a computer equipped with a statistics program (SMart Nose 151; SMart Nose). The samples were conditioned for 10 min at 70°C with agitation at 350 rpm prior to sampling. The sample headspace (2.5 mL) was taken with a syringe (syringe temperature 90°C) and injected into the electronic nose at 90°C. The ionic masses at 10-200 amu were scanned 3 consecutive times at 0.5 s/amu. Each cycle of sample measurement and scanning was programmed to run for 3 min. A purging by syringe with nitrogen (99.999% at 0.5 bar) at a rate of 230 mL/min also took 3 min. New samples were immediately available as the vials were individually conditioned by the programmable autosampler. The injector was continuously purged with nitrogen except during measurements. For data analysis the average intensity over 3 scanning cycles/injection in electronic nose was taken as the intensity of each ionic mass. The raw data from electronic nose were normalized with the ionic mass of ^{40}Ar as an internal reference. Ionic fragments lighter than 40 amu indicated air composition; therefore, major peaks were observed between 40 and 200 amu. For discriminant function analysis, there should be more than 3 groups of samples. In order to discriminate flavor pattern of 2 rice-wine samples, commercial wine, '1865' was used as a control. Discrimination function analysis (DFA) was performed using the statistics program (11,12).

Analysis by electronic tongue For the analysis, 25 mL sample was filtered through Whatman no. 6 paper (Whatman, Kent, UK). It was analyzed using an electronic tongue (The ASTREE II Liquid and Taste Analyser; Alpha M.O.S., Tulouse, France) consisting of an array of 7 sensors (SRS, GPS, STS, UMS, SPS, SWS, and BRS). GPS and SPS were used as standard sensors, SRS was used for sourness, STS was used for saltiness, UMS was used for umami, SWS was used for sweetness, and BRS was used for bitterness.

Results and Discussion

Fermentation characteristics of rice-grape wine Two fermentations were performed under wine-making conditions. The processes differed only in the presence and absence of supplementary rice powder. Sumizyme (glucoamylase) and glucose isomerase were added to slurry of rice powder; the amount of enzymes added was optimized to keep the ratio of glucose and fructose at 1:1 in the fermentation broth. Then the broth was fermented for 2 days. Ethanol was produced to 8.6% during this first step of rice fermentation. The blending of 2 varieties, 'Muscat Baily A' and 'Cambel Early' was preliminarily optimized to achieve a delicate expression of fruit flavor with balanced acidity and

Table 1. Changes in fermentation characteristics of rice-grape wine and grape wine during fermentation

Fermentation period (day)	Temp. (°C)	pH	Brix (°Bx)	Acidity	Reducing sugar (mg/mL)	Ethanol (%)	Operation
Rice-grape wine							
0	20	4.60	-	-	-	0	
1	20	4.60	10.6	0.272	23.65	1.0	
2	24	3.39	11.5	0.532	21.44	8.6	Must added
3	24	4.03	12.8	0.560	21.65	11.1	2nd fermentation
4	20	3.62	13.8	0.600	21.16	5.1	Circulation 4 times
5	-	-	-	-	-	-	Circulation 3 times
6	30→28	3.84	6.0	0.640	21.37	11.6	Circulation 5 times
7	24	3.85	6.5	0.630	5.75	11.6	Removal of grape skin and seed
Grape wine							
0	20	3.69	18.6	0.615	15.01	0	
1	20	3.64	15.8	0.675	14.78	0.7	Circulation 5 times
2	25	3.60	10.6	0.690	18.33	4.8	Circulation 4 times
3	-	-	-	-	-	-	Circulation 3 times
4	26→25	3.76	6.5	0.600	8.66	9.6	Circulation 5 times
5	-	-	-	-	-	-	
6	24	3.86	6.0	0.620	6.20	9.6	Removal of grape skin and seed

moderate tannin. During the following fermentation of grape must for 5 days at 24-28°C, ethanol was maximally produced to 11.6%. The alcohol content of the grape wine was 9.6% lower than that of the rice-grape wine, suggesting that the rice starch contributed to a higher content of ethanol (Table 1). The titratable acidity slightly increased up to 0.69% and decreased to 0.62% during the fermentation of grape wine. The titratable acidity of the rice-grape wine was 0.630%; it increased from 0.272 to 0.532% during the 1st fermentation period, but by the end of the fermentation it was essentially the same for both rice-grape wine and grape wine. The acidity of both grape wine and rice-grape wine was 0.620% less than the 0.740-0.760% acidity reported for wines made from 'Campbell Early' grapes (2). The initial concentration of reducing sugars was 23.65 mg/mL in the rice-grape wine and 15.01 mg/mL in the grape-wine, while the final concentration was reduced to 6.20 and 5.75 mg/mL, respectively (Table 1). Most of the data from these general analyses were in good agreement with those for rice-grape wine made in a laboratory by mixing rice and grape wines at a ratio of 1:5 (w/w) (1).

Aroma characteristics of grape wine and rice-grape wine Total ion chromatograms (TIC) of the grape and rice-grape wines are shown in Fig. 2. These chromatograms allow a comparison of the aroma profiles of these 2 types of wine after fermentation and storage for 2 years. There was no significant difference in the quantity of these 2 types of wine. For grape wine, most of the aromatic compounds increased after 2 years of storage compared to the grape wine after fermentation. Similarly, compared to rice-grape wine after fermentation, rice-grape wine after storage for 2 years showed higher amounts of most of the aromatic compounds.

The aromatic compounds identified in the grape and rice-grape wines and their respective peak areas are shown in Table 2. The effect of rice on the flavor of the wine was insignificant in this study. That is, 39 aromatic compounds were identified from the 2 types of wine, and the aroma pattern was essentially the same for both types of wine except for some differences in the amount of specific compounds. The peak areas of most of the aromatic compounds were similar in both grape wine and rice-grape wine. However, the peak areas of 2-methyl-1-propanol (no. 3 in Fig. 2), 2-methyl propyl acetate (no. 6), styrene (no. 13), and phenethyl acetate (no. 31) were greater in rice-grape wine, whereas ethyl hexanoate (no. 16), diethyl succinate (no. 26), and ethyl decanoate (no. 35) were present in greater amounts in grape wine.

A comparison of the aroma profiles of grape wine after fermentation versus after storage for 2 years showed that the peak areas of ethyl hexanoate (no. 16), 1-octanol (no. 21), diethyl succinate (no. 26), ethyl decanoate (no. 35), ethyl dodecanoate (no. 36), ethyl tetradecanoate (no. 37), isopropyl myristate (no. 38), and ethyl hexadecanoate (no. 39) increased significantly in grape wine after 2 years of storage. These compounds are esters formed from fatty acids and alcohols by esterification during storage except for 1-octanol and diethyl succinate. Rice-grape wine showed the same trend: esters, such as ethyl hexanoate, diethyl succinate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, isopropyl myristate, and ethyl hexadecanoate, increased significantly in quantity after 2 years of storage. These medium and long-chain fatty acids are known to be

Table 2. Aromatic compounds identified from grape wine and rice-grape wine after fermentation and 2 years of storage

No.	RI[1)]	Aromatic compound	Peak area			
			Grape wine		Rice-grape wine	
			After fermentation	2 years storage	After fermentation	2 years storage
1	503	Ethanol	4.2×10^{8} [2)]	7.5×10^{8}	5.5×10^{8}	1.3×10^{9}
2	613	Ethyl acetate	1.0×10^{8}	6.9×10^{7}	1.3×10^{8}	1.5×10^{8}
3	624	2-Methyl-1-propanol	2.3×10^{7}	1.8×10^{7}	1.1×10^{8}	1.4×10^{8}
4	737	3-Methyl-1-butanol	2.3×10^{8}	3.2×10^{8}	2.6×10^{8}	3.4×10^{8}
5	744	2-Methyl-1-butanol	1.1×10^{8}	1.2×10^{8}	1.1×10^{8}	1.4×10^{8}
6	758	2-Methyl propyl acetate	1.7×10^{6}	3.0×10^{6}	1.7×10^{7}	1.5×10^{7}
7	800	Ethyl butanoate	3.7×10^{6}	6.2×10^{6}	2.6×10^{6}	5.5×10^{6}
8	782	2,3-Butanediol	8.1×10^{6}	2.0×10^{7}	1.0×10^{7}	4.7×10^{6}
9	782	1,3-Butanediol	1.8×10^{6}	7.1×10^{6}	2.8×10^{6}	2.1×10^{6}
10	868	1-Hexanol	8.1×10^{6}	9.3×10^{6}	7.8×10^{6}	8.6×10^{6}
11	876	Isoamyl acetate	1.3×10^{8}	1.2×10^{8}	1.5×10^{8}	1.1×10^{8}
12	878	2-Methyl butyl acetate	2.7×10^{7}	1.6×10^{7}	4.4×10^{7}	2.5×10^{7}
13	893	Styrene	3.2×10^{6}	2.4×10^{7}	1.6×10^{7}	6.1×10^{7}
14	970	Hexanoic acid	5.8×10^{6}	4.6×10^{6}	1.1×10^{6}	3.8×10^{6}
15	980	Methionol	3.3×10^{6}	5.7×10^{6}	3.2×10^{6}	3.9×10^{6}
16	996	Ethyl hexanoate	8.1×10^{7}	1.2×10^{8}	3.0×10^{7}	6.0×10^{7}
17	1009	Hexyl acetate	8.4×10^{6}	3.3×10^{6}	5.8×10^{6}	2.9×10^{6}
18	1027	2-Ethyl hexanol	2.7×10^{6}	2.6×10^{6}	1.0×10^{6}	2.0×10^{6}
19	1037	1,8-Cineole	1.3×10^{6}	2.7×10^{6}	2.6×10^{6}	4.2×10^{6}
20	1051	Ethyl 4-hydroxy butanoate	1.8×10^{6}	2.1×10^{6}	3.2×10^{6}	4.5×10^{6}
21	1068	1-Octanol	5.2×10^{6}	7.3×10^{7}	2.6×10^{6}	3.3×10^{6}
22	1094	Ethyl heptanoate	9.3×10^{5}	2.0×10^{6}	1.1×10^{6}	1.9×10^{6}
23	1103	Nonanal	1.0×10^{6}	2.1×10^{6}	1.8×10^{6}	1.5×10^{6}
24	1117	Phenethyl alcohol	3.1×10^{8}	4.7×10^{8}	2.9×10^{8}	3.6×10^{8}
25	1120	Methyl octanoate	3.9×10^{6}	2.6×10^{7}	1.4×10^{6}	2.2×10^{7}
26	1173	Diethyl succinate	3.2×10^{7}	2.1×10^{8}	2.2×10^{6}	1.1×10^{8}
27	1193	Ethyl octanoate	3.6×10^{8}	9.2×10^{8}	1.6×10^{8}	3.8×10^{8}
28	1204	Decanal	1.8×10^{6}	5.1×10^{6}	4.0×10^{6}	2.0×10^{6}
29	1224	β-Citronellol	5.3×10^{6}	4.3×10^{6}	2.2×10^{6}	2.7×10^{6}
30	1250	Isoamyl hexanoate	4.5×10^{6}	2.2×10^{6}	1.2×10^{6}	1.2×10^{6}
31	1256	Phenethyl acetate	5.3×10^{7}	3.0×10^{7}	1.0×10^{8}	3.5×10^{7}
32	1269	1-Decanol	4.0×10^{6}	7.2×10^{6}	1.1×10^{6}	3.3×10^{6}
33	1343	2-Methyl propyl octanoate	1.6×10^{6}	3.3×10^{6}	1.7×10^{6}	2.7×10^{6}
34	1353	Decanoic acid	1.9×10^{6}	1.4×10^{7}	3.9×10^{5}	4.4×10^{6}
35	1389	Ethyl decanoate	8.4×10^{7}	5.0×10^{8}	2.1×10^{7}	1.6×10^{8}
36	1588	Ethyl dodecanoate	1.1×10^{7}	9.7×10^{7}	5.6×10^{6}	2.3×10^{7}
37	1687	Ethyl tetradecanoate	6.5×10^{6}	2.4×10^{7}	5.5×10^{6}	1.3×10^{7}
38	1798	Isopropyl myristate	6.4×10^{5}	2.4×10^{7}	1.1×10^{6}	5.7×10^{6}
39	1915	Ethyl hexadecanoate	1.2×10^{7}	2.9×10^{7}	1.1×10^{7}	4.2×10^{7}

[1)]Retention indices were determined using C7-C22 as an external reference; Samples were analyzed in 2010 (after fermentation) and in 2012 (2 years of storage).
[2)]Mean (n=2); All coefficients of variation were less than 20%.

formed via fatty acid synthesis pathway from acetyl-CoA during yeast fermentation (13).

Ester compounds, which play an important role in wine aroma, both quantitatively and organoleptically, are largely responsible for the fruity aroma of wine. Ethyl esters of organic acids and fatty acids are the most abundant compounds contributing to wine aroma. Simpson and Miller (10) reported that ethyl hexanoate was an important contributor to the aroma of Chardonnay wines. Diethyl succinate, which is an ester of succinic acid produced during fermentation, has been identified in fermented foods such as *doenjang* (14) and *gochujang* (15).

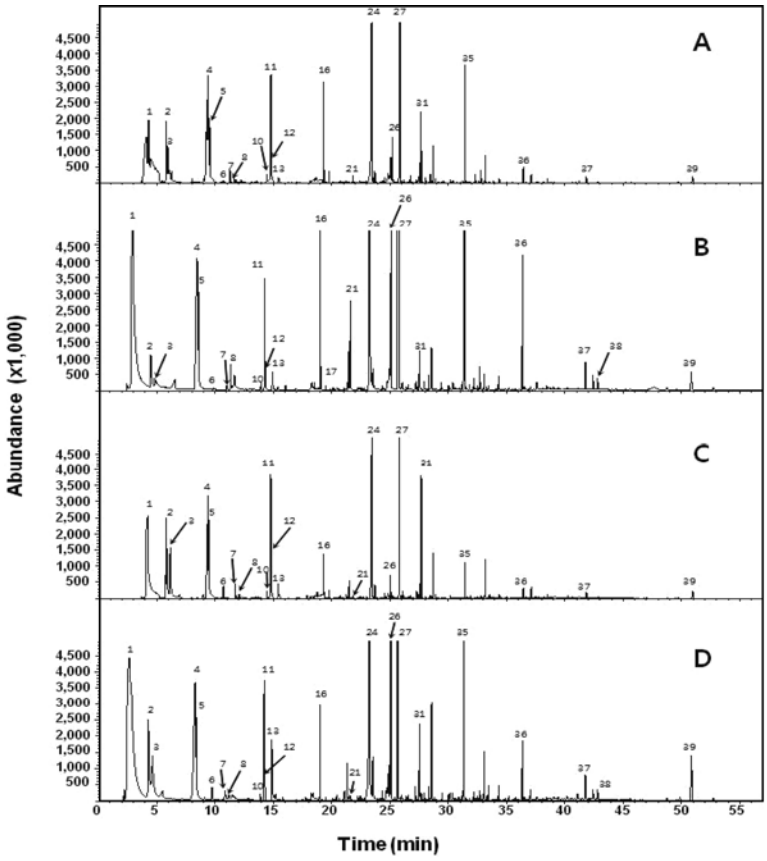

Fig. 2. Total ion chromatograms of grape wine and rice-grape wine after fermentation and 2 years of storage. Grape wine after fermentation (A) and 2 years of storage (B); Rice-grape wine after fermentation (C) and 2 years of storage (D). Peak numbers are the same as in Table 2.

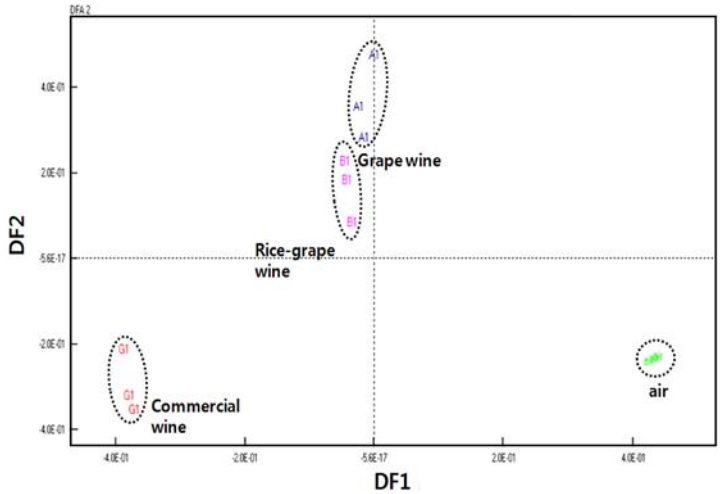

Fig. 3. Discriminant function analysis of electronic nose data for different types of wine (DF1: r^2=0.9994, *F*=4545.40, DF2: r^2=0.9575, *F*=60.15).

Wine discrimination by electronic nose and tongue

As shown in Fig. 3, the coefficient of determination, r^2, and the *F*-value of DF1 were 0.9994 and 4,545.40, respectively, and those of DF2 were 0.9575 and 60.15, respectively. The DF1 score showed a high correlation compared to the DF2 score. The obtained DFA results from the electronic nose showed that commercial wine was different from the grape wine and rice-grape wine evaluated in this study. The obtained data were mainly discriminated by DF1 score. The commercial wine was located at the negative extreme of DF1 (Fig. 3), whereas the 2 wine samples of this study exhibited DF1 scores intermediate between the commercial

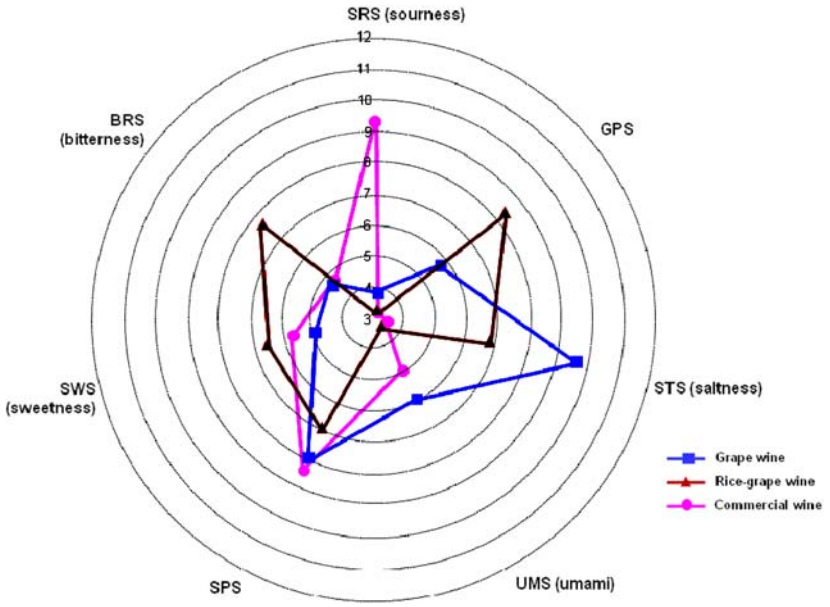

Fig. 4. Analysis profile of tastes of grape wine and rice-grape wine, as determined by an electronic tongue.

wine and an air sample. This means that the commercial wine had substantially more volatile compounds than the wines produced in this study. Much less difference was observed between the rice-grape and grape wines, as indicated by very similar DF1 scores. However, it was possible to discriminate between grape wine and rice-grape wine due to differences in the DF2 scores. Thus, the 2 wine samples could be separated based on electronic nose and discriminant function analyses.

The effect of the rice on the acceptability of rice-grape wine was analyzed using an electronic tongue. As shown in Fig. 4, the grape wine and rice-grape wine differed in saltiness, bitterness, and sweetness. Sweetness and bitterness were significantly enhanced in the rice-grape wine, whereas saltiness was diminished, relative to grape wine. The flavor pattern of grape wine, as determined by the electronic nose, was similar to that of rice-grape wine, while some differences were observed in the taste patterns of the 2 types of wine, as determined by the electronic tongue. This difference in taste was presumably due to the rice. The commercial wine was the most sour among the 3 samples.

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