FOOD QUALITY and CULTURE
Food Quality Culture 2(1): 32~36 (2008)

Analysis and Comparison of Volatile Flavor Components in Rice Wine Fermented with *Phellinus linteus* Mycelium and Regular Commercial Rice Wine

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ABSTRACT This study identified and compared the volatile flavor components of two commercial rice wines: one fermented using the mycelium of *Phellinus linteus* and a regular commercial rice wine. The volatile flavor components were isolated from the infusions by Porapak Q (50-80 mesh) column adsorption. The concentrated aroma extracts were then analyzed and identified by GC and GC-MS. Thirty-four kinds of flavor components were identified in the mycelium-fermented rice wine, including 11 alcohols, 8 esters, 3 ketones, 6 acids, 3 hydrocarbones, and 4 others. In the regular commercial rice wine, 36 kindss of flavor compounds were identified, including 9 alcohols, 6 esters, 4 ketones, 6 acids, 9 hydrocarbones, and 2 others. Therefore, the data indicate that the primary flavor components in the rice wines were alcohols and esters.

KEYWORDS: mycelium of *Phellinus linteus*, rice wine, volatile flavor compounds, Porapak Q

INTRODUCTION

Traditional Korean rice wines are primarily made from cereals such as rice. And traditional liquors, infused with various oriental medicine materials, have been made for their health benefits as well as good flavor (Min YK and Jeong HS 1995, Min YK and Lee MK 1997, Seo SB et al 2002). Several Korean researchers have analyzed the volatile flavor components of liquors such as "takju" (Lee TS and Han EH 2001, 2000) and folk "sojues" (Ahn HY et al 1995), and have reported that alcohols, organic acids, esters, and aldehydes are their main flavor compounds. *Phellinus linteus* is known as a medicinal mushroom, whose pharmaceutical effects on tumors and inflammatory diseases have long been recognized in traditional oriental medicine (Bae JS et al 2003, Ji JH et al 2000, Song CH et al 1998). Food production using the mycelium of *Phellinus linteus* was developed by Song HN and Oh SW 2002. Fermented rice wine has been prepared with this mycelium, using a rice-based medium to increase the production yield of ethyl alcohol; the wine's manufacturing method as well as pharmacological effects on the liver have been reported by Ahn SM et al (2006).

This paper reports on the characteristic flavor components of a rice wine prepared using *Phellinus linteus* mycelium and of another commercial rice wine having the same alcohol percentage.

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Received March 27, 2008; Accepted May 8, 2008

MATERIALS AND METHODS

Materials and chemicals

Materials: A: Commercial rice wine fermented with *P. linteus* mycelium(name: Millennium promise, 14% Alc. Vol., 2004 commercial product).

B: Standard commercial rice wine (name: Bekhwa subok, 14% Alc. Vol., 2004 commercial product).

Chemicals: Authentic flavor compounds were purchased from Sigma-Aldrich Chemical Co., Wako Co. (Osaka, Japan), and Tokyo Kasei (Tokyo, Japan)., Several compounds were also acquired from a laboratory at the Department of Nutrition and Food Science, Ochanomizu University (Tokyo, Japan).

Preparation of flavor concentrate

A previously described adsorption method was used for the analysis (Ito Y et al 2002). One-hundred milliliters of

rice wine was subjected to chromatography in a column packed with Porapak Q (Porapak Q, 50-80 mesh, 2.0 g+ purified water 50 mL). Water-soluble compounds such as amino acids and sugars were removed with 50 mL of purified water, followed by elution of the adsorbed compounds with 80 mL of diethyl ether. The elutate was dried over anhydrous sodium sulfate after adding the internal standard (80 ppm of tetradecane/dichloromethane) (Jang EY et al 2006), and the solvent was evaporated at 40 at atmospheric pressure. Finally, the volatile compounds were concentrated with a nitrogen stream immediately before GC inject on and GC-MS analysis.

Gas chromatography (GC)

A Shimadzu model GC 17A (Kyoto, Japan) equipped with a flame-ionization detector was employed. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min with a split ratio of 50:1. The column was 30 m×0.25 mm i.d. and coated with HP-5 (J and W scientific). The oven temperature was held at 35°C for 5 min and then increased to 210°C at a rate of 5°C/min. The injector and detector temperatures were set at 220°C and 200°C, respectively.

Gas Chromatography-Mass spectrometry (GC-MS)

An HP 6890 series gas chromatograph equipped with an HP 5973 mass selection detector and Wiley library was used for the GC-MS analysis. The GC conditions were the same as those described above for the GC analysis. Mass spectra were scanned at 70 eV. The components were tentatively identified by matching the mass spectra with those of reference compounds in the Wiley library data system. These identifications were confirmed by the GC retention times of authentic standards.

RESULTS AND DISCUSSION

As indicated in the Materials and Methods, the adsorption method of Ito Y et al (2002) was used in the analysis. If we use the distillation method that is ordinarily used in flavor compound analysis of wine, the detection of other various flavor compounds is difficult relatively, because ethyl alcohol content is high The flavor of the rice wine fermented using *phelliuns linteus* mycelium (sample A) consisted of variable odors such as a honey, beer, rice wine, and wine. The flavor of the other commercial product (sample B) had sweet, pine, and peach-like odors. The aroma concentrates of both samples had floral and honey-like odors in common. The identified compounds are summarized in Table 1. Thirty four compounds, including 11 alcohols, 8 esters, 6 acids, 3 hydrocarbons, 3 ketones, 2 phenols, and 2 others,

Table 1. Volatile aroma compounds identified in rice wine fermented using *Phellinus linteus* mycelium (A) and regular commercial rice wine (B)

	t_R (min)	Compound	Sample[1)]		
			A	B	Evidence[2)]
Alcohols					
	2.164	1-Propanol	29.38	62.68	MS,GC
	2.719	2-methyl propanol	194.22	176.50	MS,GC
	5.143	3-methyl butanol	2885.59	1597.43	MS,GC
	5.185	2-methyl butanol	398.54	326.96	MS,GC
	6.813	2,3-butanediol	264.55	28.98	MS,GC
	8.872	3-ethoxy-1-propanol	3.33	-	MS,GC
	10.033	hexanol	6.35	-	MS,GC
	14.111	heptanol	3.75	4.70	MS,GC
	14.404	methionol	19.42	-	MS,GC
	16.302	2-ethyl hexanol	12.90	39.22	MS,GC
	17.826	unknown	49.82	215.66	MS
	19.982	phenylethyl alcohol	9887.33	4909.56	MS,GC
Esters					
	15.227	ethyl hexanoate	-	38.23	MS,GC
	22.096	ethyl octanoate	10.02	50.97	MS,GC
	22.929	diethyl butanedioate	62.18	3.74	MS,GC
	24.011	2-phenylethyl acetate	21.45	62.70	MS
	24.225	ethyl decanoate	4.09	-	MS
	32.610	α-hydroxy-benzenepropanoate	5.90	-	MS
	32.748	ethyl decanoate	26.02	16.16	MS,GC
	33.156	hexyl salicylate	3.58	-	MS
	42.105	ethyl hexadecanoate	5.90	9.52	MS,GC

Table 1. Continued

	t_R (min)	Compound	Sample[1)] A	Sample[1)] B	Evidence[2)]
Ketones					
	11.087	dihydro-2(3H)-furanone	3.50	6.79	MS
	24.250	3-ethyl acetophenone	-	18.00	MS
	24.832	4-ethyl acetophenone	-	7.43	MS,GC
	27.196	dihydro-5-pentyl-2(3H)-furanone	50.30	3.70	MS
	30.954	δ-decalactone	4.74	-	MS
Acids					
	3.242	acetic acid	6.10	3.64	MS,GC
	10.839	3-methylbutanoic acid	-	6.54	MS,GC
	17.335	hexanoic acid	14.87	37.98	MS,GC
	18.041	heptanoic acid	51.57	-	MS,GC
	22.672	octanoic acid	-	32.23	MS,GC
	25.212	phenylacetic acid	4.09	7.86	MS
	27.355	decanoic acid	6.24	6.68	MS,GC
	37.267	tetradecanoic acid	3.16	-	MS,GC
Hydrocarbons					
	2.415	hexane	-	112.58	MS,GC
	9.739	2-methyl octane	-	2.61	MS
	10.019	3-methyl octane	-	2.65	MS
	11.199	nonane	-	17.91	MS,GC
	13.647	propyl benzene	11.85	14.50	MS,GC
	15.157	decane	-	21.45	MS,GC
	16.162	L-limonene	3.39	18.04	MS,GC
	18.788	undecane	-	13.18	MS,GC
	21.866	dodecane	33.50	13.03	MS,GC
Others					
	13.842	benzaldehyde	14.39	19.90	MS,GC
	14.871	2-pentylfuran	7.42	-	MS
	21.018	ethylbenzaldehyde	-	5.63	MS
	25.883	4-vinyl-2-methoxy phenol	590.50	-	MS
	29.703	4-methyl methoxy phenol	20.63	-	MS

[1)]Peak area of each compound/peak area of internal standard (I.S.)× 100.
[2)]Compounds identified on the basis of the following criteria: tentative identification based on mass spectral characteristics (MS), GC retention times were confirmed with those of standard samples (GC).

were identified or tentatively identified in sample A. In sample B, 36 compounds were identified, including 9 alcohols, 6 esters, 9 hydrocarbons, 6 acids, 4 ketones, and 2 others. The following are the results of the component classifications by functional group for both samples.

Alcohols

Both samples contained high levels of alcohols known as fusel oil components, which were in the order of 3-methyl butanol, 2-methyl butanol, and 1-propanol. According to one report, if 2-methyl butanol content is higher than 3-methyl butanol in beer, the organoleptic quality of the beer is reduced (Yada J 1976), In both of the rice wines, 3-methyl butanol content was higher than 2-methyl butanol content. 3-Methyl butanol has been determined as the main component of many wines, including white wine. Sample A contained a substantial amount of 3-methyl butanol, which is characterized as a sweet banana-like odor and is derived from leucine by yeast fermentation (Lee TS and Choi JY 1998). Sample A also contained a remarkable amount of phenylethyl alcohol, which is an important component of other beverages such as beer, wine, and Takju. Phenylethyl alcohol has a mild rose floral aroma and originates from phenylalanine (Yada J 1976, Lee DS et al 1994). A larger amount of 2,3-butanediol was found in A sample than in sample B. 2,3-Butanediol is recognized as the desirable

flavor of fermented foods, including liquors, breads, and cheeses (Lee TS and Han EH 2000). Phenylethyl alcohol and 2,3-butanediol may be important contributors to the flavor of sample A.

Esters

Ordinarily, wines contain smaller amounts of esters than alcohols, but esters contribute more to the flavor of wine than alcohols (Yada J 1976, Nishiya T 1977). Samples A and B both contained more alcohol compounds than ester. Ethyl acetate was found in both samples, which has a fruity aroma and is one of the main components of beer (Ahn HY et al 1995). The two samples also contained: isoamyl acetate, having a banana-like odor; 2-phenylehtyl acetate with a honey-like odor; and ethyl decanoate, having a grape-like odor. Phenylethyl acetate has been reported as an aroma component in beer and in clear strained rice wine, and is produced by the esterification of phenylethyl alcohol and acetic acid (Yada J 1976). Sample A contained more phenylethyl alcohol than sample B, however sample B contained more phenylethyl acetate than sample A. Furthermore, α-hydroxy benzene propanoate and hexyl salicylate were only found in sample A; but it did not contain ethyl butyrate or ethyl caproate, which have pineapple-like and apple-like odors, respectively. Overall, the presence and content differences of esters may greatly influence what is considered desirable flavor in these samples.

Acids

Small amounts of acetic acid were found in both samples, which is reported as an odor with a stimulating sensation in foods (Lee TS and Han EH 2001). Sample B contained octanoic acid, which has a rancid odor and is found in butter, cheese, and palm oil. In addition, sample B contained 3-methyl butanoic acid, which also has a rancid odor and is contained in butter, cheese, and safflower (Choi SH et al 2004).

Others

Both samples contained limonene, a terpene hydrocarbon that is the main aroma component of citrus fruit. Furanic compounds, including furanones, seem to originate from the thermal degradation of carbohydrates during the rice wine manufacturing. Both samples contained furanones, including dihydro-2(3H)-furanone and dihydro-5-pentyl-2(3H)-furanone, which were important factors for the sweet odors of the wines. Specifically, both samples contained high levels of dihydro-2(3H)-furanone, and sample A contained a much greater amount of dihydro-5-pentyl-2(3H)-furanone than sample B. Sample A also contained δ-decalactone, which is a pleasant odor that is produced when heat is applied to milk fat. Both samples contained benzaldehyde, possessing an almond-like odor. 4-Vinyl-2-methoxy phenol was only found in sample A, which appeared to be an important contributor to its odor. The GC patterns of the aroma components in the two samples were slightly different. And the characteristic odors of each rice wine seemed to be attributed to the content differences of their odor compounds.

CONCLUSION

This study identified and compared the characteristic flavor components of two commercial rice wines: one fermented with *Phellinus linteus* mycelium and a regular commercial rice wine. The former wine was co-cultured with *Aspergillus oryzae,* and both rice wines possessed the same alcohol percentage. For analysis, the Porapak Q column adsorption method was used. The volatile flavor components of the two samples consisted of alcohols, esters, acids, and other compounds. Large amounts of alcohol compounds, including phenylethyl alcohol, 2,3-butanediol, 3-methylbutanol, and 2-methylbutanol, were identified in both rice wines. Both wines also contained large amounts of esters. These compounds seemed to be important contributors to the flavors of the rice wines. The GC patterns of the flavor components in the two samples were slightly different. The wine fermented with *Phellinus linteus* mycelium contained a greater amount of phenylethyl alcohol than the other commercial sample. On the other hand, the mycelium-fermented wine only contained a small amount of phenylethyl acetate. Finally, the characteristic odors of each rice wine seemed to be attributed to content differences in their odor compounds.

ACKNOWLEDGMENTS

This study was supported by the Technology Development Program of the Ministry of Agriculture and Forestry, Republic of Korea.

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