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Quantitative analysis of volatile flavor components in Korean alcoholic beverage and Japanese sake using SPME-GC/MS

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Abstract Quantitative analysis of the volatile flavor components in Korean alcoholic beverages (*makgeolli* and *yakju*) and Japanese sake was carried out using SPME-GC/MS. Fusel oils (n-butyl alcohol, isobutyl alcohol, isoamyl alcohol, and phenethyl alcohol), ethyl esters (ethyl acetate, isoamyl acetate, ethyl caproate, ethyl caprylate, and ethyl caprate) and aldehydes (furfural and benzaldehyde) were analyzed quantitatively by an 85 μm SPME fiber (carboxen/polydimethylsiloxane) using internal standards (1-pentanol-1-^{13}C and methyl nonanoate). Phenethyl alcohol (85-216 ppm) and isoamyl alcohol (38-115 ppm) constituted the majority of fusel oils in all the samples. Acetic acid was detected in sour *makgeolli* at a high level (0.02-0.14 ppm) compared with *yakju* and sake. A very high level of total ethyl esters (ethyl acetate, isoamyl acetate, ethyl caprate, and phenethyl acetate), having fruit and flower flavor, was found in *makgeolli*. Processing the volatile flavor data by multivariate partial least squares discriminant analysis, *makgeolli*, *yakju,* and sake showed cluster separation.

Keywords: volatile flavor components, *makgeolli, yakju*, sake, SPME-GC/MS

Introduction

The traditional alcoholic beverages such as *makgeolli* (*takju*) and *yakju* are cultural products that retain Korean history and tradition. Globalization of these Korean alcoholic beverages is being promoted via emphasis on their tradition and modernization of their quality. *Makgeolli* has diverse characteristics according to its raw materials, production methods, regional characteristics, and whether it is sterilized or non-sterilized. Thus, *makgeolli* can be referred as the most distinctively Korean alcoholic beverage with more uniqueness than any other alcoholic beverages in the world (1,2). *Makgeolli* and *yakju* are traditionally produced by complex fermentation methods that use grain and yeast together. They are not distilled, and the rice wine mash is drunk. The beverage in which the fermented rice wine mash is merely filtered through a sieve leaving a visually cloudy appearance with white crystals is called *makgeolli* or *takju*, while the clear liquid collected by adding water to the fermented liquid is called *cheongju* or *yakju* (3). Balance between the color and quality of the traditional alcoholic beverage is formed by flavor components. These are a result of sugar, amino acids, and organic acids from the breakdown of raw material through the enzymatic activities of the yeast after preparation. In addition, the volatile flavor components from the alcoholic fermentation by yeast and also have an impact (4). To achieve standardization, scientification, and globalization of Korea's traditional alcoholic beverage, there must first be an advancement in studies such as excellent strain discovery and strain improvement. Ultimately, there must be studies on volatile flavor compounds that have important effects on quality, together with other flavor components. In addition, research regarding the selection of raw material, the preparation method of the raw material, the setting of optimal fermentation conditions, and sensory improvement needs to be conducted (5). Until recently, preceding studies on the volatile flavor compounds of the Korean traditional alcoholic beverage have included comparative analyses of volatile flavor compounds (5-7) by varying the raw materials and microorganisms. For studies on the volatile flavor compounds of Japanese sake, there have been researches on the unique volatile flavor pattern of sake, such as studies on the raw materials (8) and the fatty acids (9). However, there has been no research on the comparative analysis of the volatile flavor compounds between the Korean traditional alcoholic beverage and Japanese sake, under the same conditions.

Currently, analysis of volatile flavor compounds makes use of subjective sensory analysis and accurate instrumental analysis such as GC/MS. Among these analytical methods, chemically significant absolute values can be obtained through the instrumental analysis method, discovering the types of compounds related to the flavor and their concentration levels. Moreover, unlike sensory analysis, the instrumental analysis method has the benefit of not being affected by the surrounding environment (10). In the present study, the analysis was carried out by collecting the volatile flavor compounds from the headspace using solid phase microextraction (SPME). The SPME method does not use any organic solvents, so the substance to be analyzed is adsorbed onto the coated fiber on the stationary phase. Subsequently, it is inserted into the opening of the GC and desorbed at a high temperature in order to collect the volatile flavor compounds. In this way, the loss of the volatile flavor compounds during the collecting process can be minimized. The general headspace method is selective towards highly volatile substances, while the headspace method using the SPME shows good results even with compounds with a low volatility such as *makgeolli* (11-13).

Thus, this study aims to quantitatively analyze the volatile flavor compounds in the Korean traditional alcoholic beverage (*makgeolli* and *yakju*) and Japanese sake using the SPME-GC/MS method under the same conditions, and to conduct a multivariate statistical analysis in order to compare with Japanese sake. As such, this study aims to contribute to the promotion of the Korean traditional alcoholic beverage industry by understanding the current issues and proposing plans for improvement.

Table 1. Characteristics of *makgeolli, yakju,* and sake

No.	Sample codes	Alcohol content (% v/v)	Starch source	Additional ingredient
1	M[1)]1	6.0	Non-glutinous rice	Nuruk
2	M2	7.0	Non-glutinous rice	Nuruk
3	M3	6.0	Non-glutinous rice	Nuruk
4	M4	6.0	Non-glutinous rice	Nuruk
5	M5	6.0	Non-glutinous rice	Koji
6	Y[2)]1	13.0	Non-glutinous rice	Nuruk
7	Y2	13.0	Non-glutinous rice, wheat flour	Nuruk
8	Y3	13.0	Non-glutinous rice, glutinous rice	Nuruk, Koji
9	Y4	13.0	Non-glutinous rice	Koji
10	Y5	18.0	Non-glutinous rice, glutinous rice	Nuruk
11	S[3)]1	14.5	Non-glutinous rice,	Koji
12	S2	13.5	Non-glutinous rice,	Koji, alcohol
13	S3	10.5	Non-glutinous rice,	Koji
14	S4	16.5	Non-glutinous rice,	Koji
15	S5	14.5	Non-glutinous rice	Koji

[1)]M (*makgeolli*): M1 (Gyungju-bubju rice *makgeolli*, Gyungju-bubju, Gyungju), M2 (Organic *makgeolli*, Baesangmyun-juga, Pochun), M3 (Neulinmaeul, Baesangmyun-juga, Pochun), M4 (Daebak, Kooksoondang, Gangwondo), M5 (Jangsu, Seoul Jangsu, Jinchun)

[2)]Y (*yakju*): Y1 (Yedam, Kooksoondang, Gangwondo), Y2 (Myungpum, Chilgapsan-jujo, Chungyang), Y3 (Gyungjububju, Gyeongjubeobju, Gyeongju), Y4 (Bakhwasubok, Lotte, Gunsan), Y5 (Hansan Sogokju, Hansan-sokokju, Suchun)

[3)]S (sake): S1 (rate of polished rice: 70%, COUTEN DOCUBECH JUNMYSHU, HAKUREI BREWING CO LTD), S2 (added brewer's alcohol, NOHOHON HONJOJOSHU, KING BREWING), S3 (rate of polished rice: 60%, GINLEGOTSAN SASALACHKI SUNMIJU, GASSAN SAKE BREWING), S4 (rate of polished rice: 50%, ONNA NAKASE JUNMY DYGINJOSHU, OMURAYA SHUZOJOCO), S5 (rate of polished rice: 45%, JOJENMIZNOGOTOSI JUNMYGINJOSHU, SHIRATAKI SAKE BREWERY)

Materials and Methods

Reagents and materials Pentanol-1-^{13}C and methyl nonanoate used as internal standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). n-Alkane standards (C10-C26) were purchased from Supelco (Supelco, Bellefonte, PA, USA). Sodium chloride used as a salt was purchased from Junsei (Junsei Chemical Co. Ltd., Tokyo, Japan). Ethanol and distilled water used as solvents were obtained from J.T. Baker (J.T. Baker Chemical Co., Phillipsburg, NJ, USA).

Alcoholic beverage samples Each of the three major kinds of sample (*makgeolli, yakju*, and sake) was purchased from different manufacturing companies (Table 1).

Sample preparation A 5 mL aliquot of sample was placed into a 20 mL glass vial (Restek Co., Bellefonte, PA, USA) containing 1 g sodium chloride, spiked with 10 μL internal standards (methyl nonanoate at 100 mg/L and pentanol-1-^{13}C at 2,000 mg/L), and the vial was sealed with a PTFE-lined cap (Supelco).

HS-SPME extraction Five commercially available SPME fibers from Supelco were examined for this study. These were divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB; 65 μm), polyacrylate (PA; 85 μm), carboxen/polydimethylsiloxane (CAR/PDMS; 85 μm), and polydimethylsiloxane (PDMS; 100 μm), which showed different affinities to diverse compounds. The 85 μm CAR/PDMS Stable fiber was selected because this fiber showed a higher affinity toward a wide range of volatile compounds in fermented alcoholic beverages (data not shown). Before use, the fiber was conditioned as recommended by the manufacturer. SPME was performed under the following parameters: incubation/extraction temperature: 50°C; vial penetration length: 22 mm; incubation time: 20 min; extraction time: 40 min; and agitation rate: 250 rpm. Desorption was performed in the injector at 250°C for 1 min under analysis conditions. After desorption of the volatiles, the SPME fiber was cleaned in the injector at 250°C for 5 min.

Volatile compound analysis An Agilent 7890A GC (Agilent, Palo Alto, CA, USA) and a Stabilwax-DA column (30 m length x 0.25 mm diameter x 0.25 μm film thickness; Restek Co.) were used for the analysis. The GC oven temperature was programed at 60°C for 5 min, set to rise to 210°C at a rate of 4°C/min, and then held at 210°C for 20 min. The injector temperature was 250°C in splitless mode; helium was used as the carrier gas and the flow rate was 2 mL/min. The direct capillary interface of the mass spectrometric detection (MSD) (5975C; Agilent) was set at a temperature of 250°C; the ion source temperature was 230°C; ionization voltage was 70 eV; mass range was 30-450 amu; and scan rate was 2.2 scans/s. The volatile compounds were identified by comparing retention indices (RIs), and mass library (NIST 05a). RI values of the compounds were determined via injection with a homologous series of alkanes (C10-C26). For quantification of the volatile compounds, samples were run in duplicate, and integrated areas based on the total ion chromatogram (TIC) were normalized to IS average values.

Multivariative statistical analysis Deconvolution of GC/MS signals was performed using the MassHunter Workstation Data Acquisition software v. B. 05.00 (Agilent). The data were aligned and normalized using Mass Profiler Professional (Agilent), and partial least square-discriminant analysis (PLS-DA) was performed using SIMCA-P+ 12.0.1 (Umetrics, Umea, Sweden).

Results and Discussion

Volatile compound composition of the 15 samples is presented in Table 2. The 19 identified volatile compounds comprised 5 alcohols, 11 esters, 2 aldehydes, and 1 acid. These 19 compounds were selected as the representative factors of fermented alcoholic beverages (5).

Alcohols The fusel alcohols in the traditional alcoholic beverage are mainly formed by the fermentation of the amino acids in the yeast during the fermentation process. The amount produced differs according to the type of yeast, fermentation temperature, oxygen concentration, degree of agitation, and amino acid content in the raw material rice. According to previous studies, traditional Korean alcoholic beverage contains n-propyl alcohol and n-butyl alcohol that are formed through α-ketobutyric acid by the Enrlich pathway. Isobutyl alcohol that is formed by valine, and isoamyl alcohol that is formed by leucine and carries the sweet aroma of banana, are also present. Another component of this beverage is phenethyl alcohol, which has the scent of rose and honey and is found in natural oils such as rose petals and orange blossoms. Phenethyl alcohol is formed by phenylalanine and is one of the most important aromatic alcohol compounds in beer (14,15).

Isoamyl alcohol is a high quality alcohol that has been evaluated to be an important volatile flavor compound in *makgeolli* (6,16). The analysis showed that the *makgeolli* (M) group had an average of 84±21 ppm, while M2 had average of 116 ppm, which was the highest value. The *yakju* (Y) group had an average of 83±29 ppm, which was almost identical to the result of M group. However, Y1 had 116 ppm, while Y4 had 38 ppm, showing a large difference between the samples. The sake (S) group had an average of 78±13 ppm, which showed a lower detected amount compared with the results of the other groups, but all the samples showed similar values, with S1 having the highest detected result of 96 ppm. In the case of isobutyl alcohol, the Y group had the highest detected amount, with an average of 18±12 ppm. Y1 had 36 ppm and Y5 had 24 ppm, showing the highest amount among all the samples. Unlike the Y group, the detected amount in the M group (8±3 ppm) and S group (10±2 ppm) was mostly within 10 ppm (Table 3). In the case of phenethyl alcohol, the M group had an average of 160±47 ppm, having a higher detected amount than the other groups. Among the M group, M4 had the highest detected amount of 216 ppm. In the case of the S group, the samples showed overall similar results having an average of 142±30 ppm, with the exception of S1, which was

Table 2. Characteristics of the target volatile flavor compounds

No.	RI[1)]	Compound	Molecular formula	Mass	Selected ions (*m/z*)
1	1000	Ethyl acetate	$C_4H_8O_2$	88.05	43, 61, 70, 88
2	1043	Ethyl butanoate	$C_6H_{12}O_2$	116.08	43, 71, 88
3	1051	n-Propyl alcohol	C_3H_8O	60.06	31, 59, 42
4	1110	Isobutyl alcohol	$C_4H_{10}O$	74.07	43, 33, 74
5	1123	Isoamyl acetate	$C_7H_{14}O_2$	130.10	43, 70, 55, 87
6	1153	n-Butyl alcohol	$C_4H_{10}O$	74.07	56, 31, 41
7	1215	Isoamyl alcohol	$C_5H_{12}O$	88.09	55, 42, 70
8	1237	Ethyl caproate	$C_8H_{16}O_2$	144.12	88, 43, 99
9	1444	Ethyl caprylate	$C_{10}H_{20}O_2$	172.15	88, 101, 127, 57
10	1458	Acetic acid	$C_2H_4O_2$	60.02	43, 45, 60
11	1477	Furfural	$C_5H_4O_2$	96.02	96, 39, 29
12	1535	Benzaldehyde	C_7H_6O	106.04	106, 77, 51
13	1541	Ethyl pelargonate	$C_{11}H_{22}O_2$	186.16	88, 101, 141, 186
14	1644	Ethyl caprate	$C_{12}H_{24}O_2$	200.18	88, 101, 155, 200
15	1822	Phenylethyl acetate	$C_{10}H_{12}O_2$	164.08	104, 43, 91
16	1847	Ethyl laurate	$C_{14}H_{28}O_2$	228.21	88, 101, 43, 228
17	1917	Phenethyl alcohol	$C_8H_{10}O$	122.07	91, 122, 65
18	2052	Ethyl myristate	$C_{16}H_{32}O_2$	256.24	88, 101, 43, 256
19	2258	Ethyl palmitate	$C_{18}H_{36}O_2$	284.27	88, 101, 43, 284

[1)]Linear retention index calculated on the Stabilwax-DA column using C_{10}-C_{26} as external reference.

distinctively higher at 190 ppm. The Y group had an average of 121±28 ppm, with the least amount of inter-group deviation. Phenethyl alcohol, which has superior aromatic properties, was detected in a higher amount than that of isoamyl alcohol in all the samples. This is consistent with previous study results that reported the volatile flavor compounds of commercial *makgeolli* (17). The amount of phenethyl alcohol could be higher in the M group compared with the Y or S group due to the presence of phenylalanine compounds, which are the precursors of phenethyl alcohol in aspartame that is added to increase sweet taste.

Aside from the above, n-propyl alcohol was detected in small quantities. The Y group had 3.92±2.39 ppm and the M group had 1.78±0.66 ppm. In the case of the S group, there was barely any detection except for 2.98 ppm detected in S2. In the case of n-butyl alcohol, it was either not detected or detected below 1 ppm in all the samples, with the Y group having 0.79±1.36 ppm, the M group having 0.73±0.62 ppm, and the S group having 0.57±0.64 ppm.

Esters Esters are derived from alcohols and aldehydes as the alcohol ferments. Because even a small amount of esters has an aroma that determines the quality of the alcoholic beverage, they are considered to be important flavor compounds. Their flavor contribution is greater than that of alcohols or aldehydes (17,18). Such types of esters are in the form of fatty acid ethyl esters (FAEE) and are perceived to be the important flavor compounds in beer or sake (18,19). In the present study, several esters were analyzed. Ethyl acetate (20) is formed through the esterification of low chain fatty acids contained in the rice wine mash by yeast or bacteria during the fermentation process. It has unpleasant characteristics in high concentrations, despite having a strong fruity and brandy-like scent. Isoamyl acetate is considered to be an important flavor compound in sake and is also used as a flavoring agent in carbonated water or syrups. It is the major ester that forms the flavor of pear, banana, and apple, while having a light fruity scent with maturity and roundness in the top note. Phenylethyl acetate (14) has the characteristics of a honey flavor, and ethyl butanoate (21) has fruity characteristics such as pineapple. Ethyl caproate (22,23) is known to be a flavor compound in sake with a strong diffusion force. It possesses scents of fresh fruits such as apple and grape that are important flavor compounds for the quality of sake. It is formed during fermentation by esterase and alcohol acyltransferase in the fatty acid synthesis process of the yeast, with ethanol and caproic acid as the substrate. Ethyl caprylate has fruity sweet flavor characteristics of banana and pineapple, and ethyl caprate has a sweet flavor, is oily, and has a brandy residue-like scent. Ethyl laurate, ethyl myristate, and ethyl palmitate have at least 12 carbons that contribute to the slight trace of waxy odor, smoothness, and sweetness. This allows the smooth characteristics in the mouth; however, these three esters are unable to be actually tasted (21). Ethyl caproate, which is an important flavor compound for the quality of sake, was detected in descending

Table 3. Contents (ppm[1)], w/w) of volatile flavor compounds in Korean alcoholic beverages and Japanese sakes

RI[2)]	Volatile compound	*Makgeolli*					*Yakju*					*Sake*				
		M1	M2	M3	M4	M5	Y1	Y2	Y3	Y4	Y5	S1	S2	S3	S4	S5
Alcohols																
1051	n-Propanol	1.2	2.9	1.9	1.5	1.5	6.9	3.9	5.2	1.8	1.7	-[3)]	3.0	-	-	-
1110	Isobutanol	7.8	8.5	2.9	9.3	10.0	36.0	11.4	12.5	6.3	23.5	11.2	11.8	7.9	8.7	9.4
1153	n-Butanol	0.9	0.2	0.2	0.8	1.7	3.2	0.3	-	-	1.5	1.3	1.2	0.3	-	-
1215	Isoamyl alcohol	60.6	116. 1	77.0	89.8	74.7	115.9	100.4	72.9	38.2	87.7	96.1	84.8	63.0	68.1	79.4
1917	Phenethyl alcohol	117.0	204.2	120.0	216.4	143.5	157.3	136.4	122.7	85.6	102.7	190.6	114.4	119.4	137.5	148.1
Esters																
1000	Ethyl acetate	0.116	0.194	0.448	0.066	0.155	0.041	0.149	0.056	0.034	0.230	0.047	0.036	0.038	0.078	0.039
1043	Ethyl butanoate	0.006	0.009	0.009	0.003	0.007	-	-	0.004	0.002	0.002	0.008	0.002	0.005	0.018	0.008
1123	Isoamyl acetate	0.151	0.209	0.500	0.146	0.259	0.038	0.025	0.077	0.031	0.009	0.044	0.010	0.033	0.113	0.025
1237	Ethyl caproate	0.074	0.048	0.156	0.052	0.034	0.011	0.012	0.051	0.136	0.061	0.364	0.001	0.090	0.203	0.412
1444	Ethyl caprylate	0.492	0.494	1.910	0.236	0.377	0.023	0.087	0.030	0.084	0.075	0.152	0.001	0.116	0.466	0.087
1541	Ethyl pelargonate	0.006	0.014	0.036	0.011	0.060	-	0.001	-	-	0.036	-	-	0.001	0.002	0.002
1644	Ethyl caprate	0.425	0.596	1.403	0.193	0.283	0.003	0.027	0.002	0.002	0.018	0.009	-	0.008	0.082	0.002
1822	Phenylethyl acetate	0.160	0.455	0.154	0.178	0.123	0.028	0.022	0.057	0.053	0.008	0.036	0.005	0.049	0.137	0.016
1847	Ethyl laurate	0.064	0.149	0.336	0.045	0.037	0.001	0.004	0.003	0.014	0.011	0.044	0.002	0.010	0.017	0.061
2052	Ethyl myristate	0.038	0.116	0.334	0.040	0.025	-	-	-	-	0.001	-	-	-	-	--
2258	Ethyl palmitate	0.118	0.383	0.930	0.150	0.144	0.003	0.003	0.002	0.002	0.007	0.004	0.001	0.001	0.002	0.001
Acids & Aldehyde																
1458	Acetic acid	0.025	0.035	0.144	0.024	0.041	0.007	0.033	0.006	0.005	0.03	0.002	0.003	0.004	0.004	0.002
1477	Furfural	-	0.006	-	-	-	0.004	0.026	0.01	0.002	0.002	0.004	0.008	0.007	0.002	0.004
1535	Benzaldehyde	0.008	0.023	0.028	0.004	-	0.009	0.014	0.085	0.045	0.008	0.033	0.015	0.036	0.038	0.01

[1)]Average of the ppm (n=2), ppm=(area pf each compound×amount of internal standard)/(area of internal standard×amount of sample/$10^{6)}$

[2)]Linear retention index calculated on the Stabilwax-DA column using C_{10}-C_{26} as external reference

[3)]Not detected

order in the S, M, and Y groups, with the respective quantities being 0.21±0.17, 0.07±0.05, and 0.05±0.05 ppm. S5 and S1 had high detected amounts, which were 0.41 and 0.36 ppm, respectively. In the case of ethyl caprylate, which has sweet odor characteristics of fruits among the flavor compounds of *makgeolli*, the M group had an average of 0.70±0.68 ppm, which was observed to be higher than the quantities detected in both the S and Y groups, having an average of 0.16±0.17 and 0.06±0.03 ppm, respectively. In particular, it was evident that M3 having 1.91 ppm, was more than 10 times higher than the amount in the other samples. In the case of ethyl caprate, which has smooth and sweet odor characteristics, the M group having 0.58±0.48 ppm had a higher detected amount compared with that of both the S and Y groups, which had an average of 0.02±0.03 and 0.01±0.01 ppm, respectively. M3 had the highest detected quantity, which was 1.40 ppm. In the case of phenylethyl acetate, which has flowery and fruity odor characteristics, the M group having 0.21±0.13 ppm had a higher detected amount compared with that of both the S and Y groups, which had 0.05±0.05 and 0.03±0.02 ppm, respectively. M2 had the highest detected quantity, which was 0.46 ppm, and S4 had the next highest detected amount of 0.14 ppm. In the case of isoamyl acetate, which has a fruity odor, it was observed that the M group having 0.25±0.14 ppm was once again higher than that of both the S and Y groups, which had 0.04±0.04 and 0.03±0.02 ppm, respectively. In the case of ethyl acetate, which is a necessary flavor compound of *makgeolli* (*takju*) despite being rather unpleasant at high concentrations, the M group having 0.19±0.14 ppm had a higher detected amount compared to that of both the Y and S groups, which had 0.10±0.08 and 0.04±0.01 ppm, respectively. In the case of ethyl palmitate, which is smooth and sweet and yet virtually tasteless, 0.35±0.34 ppm was detected in the M group having a relatively superior flavor than that of the Y or S group, which contained less than 0.01 ppm.

From Table 3, it can be understood that most types of esters were detected in *makgeolli* in relatively larger quantities, followed by in sake and *yakju*. In the case of M3, particularly superior aromatic compounds such as the aforementioned ethyl caprylate, ethyl caproate, ethyl caprate, and phenylethyl acetate were detected in distinctively high levels. In the case of sake, quantities of aromatic compounds were detected in descending order: ethyl caproate, which has become the standard sake odor; ethyl caprylate, which has a sweet fruity flavor; and phenylethyl acetate, which has a honey and flowery scent. Other esters were also detected in small quantities. In the case of *yakju*, the esters were detected in the order of ethyl acetate and isoamyl acetate, with most other esters detected in small quantities.

Acids and aldehydes With respect to acids, acetic acid, which is formed by the heterolactic fermentation of glucose, was selected as the target substance. A small amount of acid is needed for the flavor production of *makgeolli* (*takju*), since acetic acid forms various types of acetate and ethyl esters by bonding with alcohols that are produced during the fermentation process. At the same time, it is an acidic tasting compound (6) that has a stimulating odor. 0.05±0.05, 0.01±0.01, and 0.003±0.001 ppm were detected in the M, Y, and S group, respectively (Table 3).

Aldehydes are compounds that are produced during the fermentation of alcohol. It has been reported that the activity of bacteria from hetero-fermentation and the quality and condition of the yeast greatly affect the production of aldehydes (24). Beverages with high alcohol content such as whiskey, brandy, and pot distilled soju contain a high amount of acetaldehyde, whereas beverages with lower alcohol content such as blended *soju* and beer contain a lower amount of acetaldehyde. According to the Korean Food Standards Codex (25), the level of aldehyde among the alcoholic beverages is stated to be 700 mg/L or below to be 700 mg/L or below in the case of distilled alcohol such as *soju*, whiskey, brandy, and other regular distilled alcoholic beverages. However, there is no standard for other kinds of alcoholic beverages. In the present study, among the aromatic aldehydes, benzaldehyde, which has aromatic properties such as almond while being easily oxidized in air, and furfural, which is prone to discoloration due to the automatic oxidation by air and light, were investigated. In the case of furfural, it was detected in all the samples with an average amount of 0.009±0.01 ppm for the Y group and 0.005±0.002 ppm for the S group. On the other hand, in the case of the M group, 0.006 ppm was detected only in M2. Moreover, among the Y group, Y2 had the highest detected amount of all the samples, with the quantity being 0.026 ppm. In the case of benzaldehyde, the M group had the least amount at 0.013± 0.012 ppm, followed by the S group having 0.026±0.013 ppm, and the Y group having the highest amount at 0.032±0.033 ppm. In the Y group, Y3 had the highest amount at 0.085 ppm (Table 3).

Multivariate statistical analysis of volatile compounds The SPME-GC/MS analysis data of the volatile flavor compounds in *makgeolli*, *yakju,* and sake were subjected to PLS-DA using the SIMCA-P statistical analysis program, through deconvolution, alignment, and normalization of the obtained data. As shown in Fig. 1, the different alcoholic beverages (M, Y, and S groups) were clearly distinguishable in the score plot generated by combining PC1 (21.35% of the total variance) with PC2 (12.15% of the total variance). M1, which had a below average detection of n-propanol, n-butanol, isoamyl alcohol, isoamyl acetate, ethyl myristate, ethyl palmitate, and acetic acid appeared distant from the others in the group at the upper left side. In the case of Y4 of the Y group, a leaning towards the S group was observed. This was a result of the amounts of isobutanol, ethyl acetate, ethyl caproate, ethyl caprylate, phenylethyl acetate, acetic acid, furfural, and benzaldehyde showing tendencies that were more similar to that of the S group than that of the other samples in the Y group. The contents of volatile compounds as seen in the S group were detected in similar amounts, thus it was observed that the data of the S group were plotted in proximity within the same quadrant.

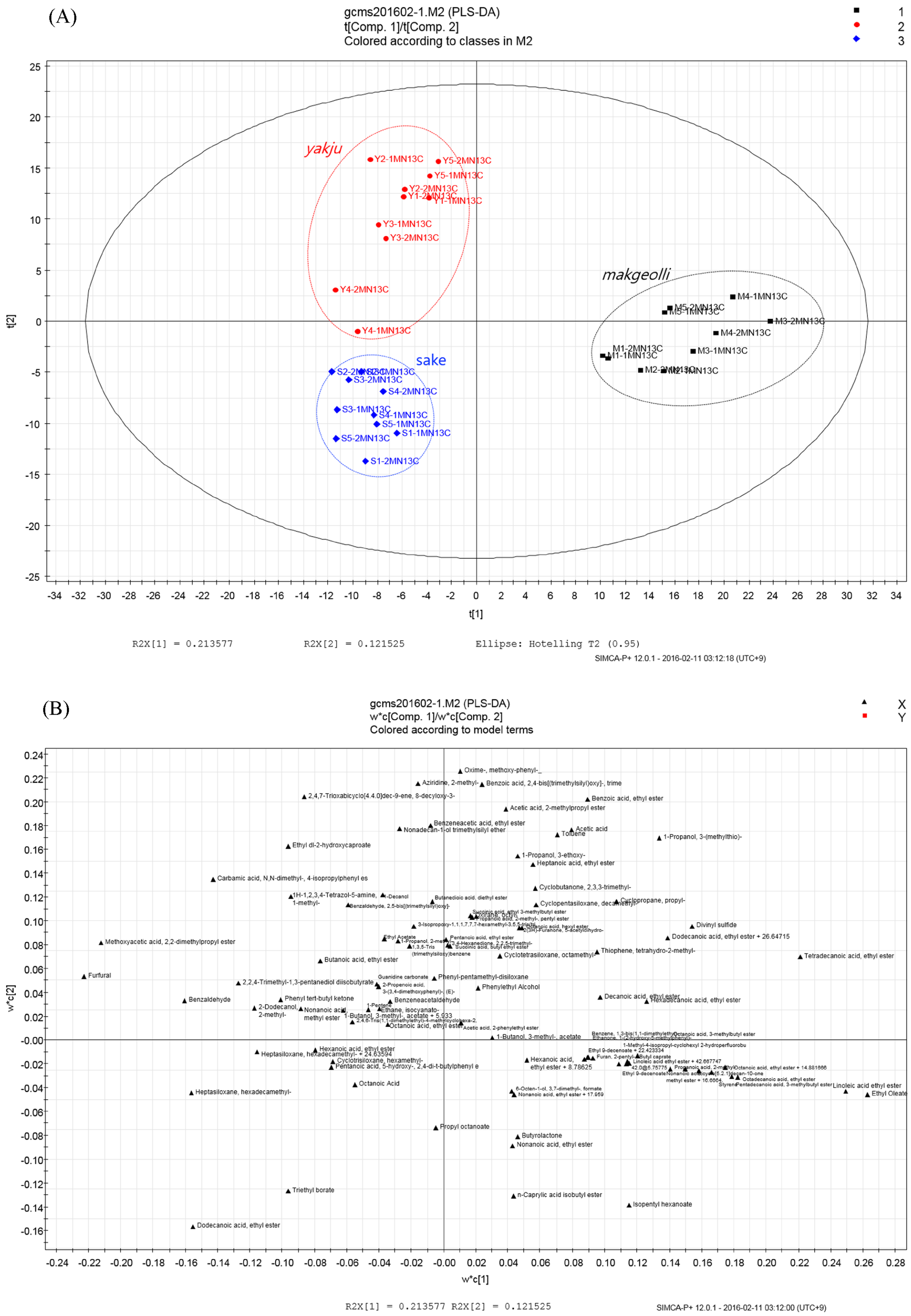

Fig. 1. PLS-DA score (A) and loading (B) plots derived from the GC/MS profiles of volatile flavor compounds in Korean alcoholic beverages and Japanese sake. PC1 and PC2 account for 21.35% and 12.15% of the variance, respectively.
M (■): *makgeolli,* Y (○): *yakju,* S (◆): sake

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