

Simultaneous Determination of Volatile Organic Compounds in Commercial Alcoholic Beverages by Gas Chromatography with Flame Ionization Detection

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A simple and fast method was developed for the determination of volatile organic compounds in alcoholic beverages. Eleven volatile organic compounds (acetaldehyde, methanol, 2-propanol, *tert*-butanol, 1-propanol, ethyl acetate, 2-butanol, isobutanol, 1-butanol, 3-methyl-1butanol, and 2-methyl-1-butanol) in alcoholic beverages were analyzed with a simple direct-injection method using GC with flame ionization detection. These compounds should be monitored in the QC of production processes because they are detrimental to human health. The method was validated with four types of alcoholic beverages (beers, fruit wines, rice wines, and spirits) to confirm the versatility of the method. Linearity showed r^2 values from 0.9986 to 0.9995, with LODs ranging from 0.010 to 1.000 mg/L. Precision and accuracy showed acceptable results, proving the effectiveness of the method. The developed method was applied to 40 commercial samples representing the four types of alcoholic beverages, and principal component analysis was performed to determine profiles of the volatile organic compounds, depending on the type of alcoholic beverage.

Today, alcoholic beverages are widely consumed throughout the world and produced in large quantities. An alcoholic beverage is a drink containing ethanol, which is one of the most commonly abused substances. Representative alcoholic beverage types are beers, spirits, fruit wines, and rice wines. The types of alcoholic beverages vary, depending on the ethanol content, raw materials (grains or fruits), and production

methods (fermentation and/or distillation; 1–4). Beers are made from brewing such materials as grains, water, yeast, and hops (5, 6). Spirits are produced from the distillation of fermented liquor, which increases the ethanol content. Spirits are classified in detail by the species of ingredients (7, 8). Fruit wines and rice wines are produced by the fermentation of its base ingredients (fruits and rice, respectively). This diversity in processing leads to the presence of a wide range of byproducts called congeners. Frequently observed congeners in alcoholic beverages are volatile organic compounds (VOCs; 3).

VOCs are carbon-containing chemicals with high vapor pressure at room temperature (9). VOCs in alcoholic beverages are formed from various mechanisms such as yeast metabolism from amino acids and enzymatic reactions. These mechanisms can be affected by storage conditions (10). According to previous studies, acetone, acetaldehyde, esters, aldehydes, and fusel alcohols can be detected as VOCs in alcoholic beverages (9, 10). VOCs are generally known to have harmful effects on human health or the environment and, as new information about these effects is revealed, public interest and concern has increased. Thus, the analysis of VOCs is important for the safe manufacturing of alcoholic beverages.

For the analysis of VOCs in alcoholic beverages, liquid–liquid extraction, SPE, and headspace (HS) solid-phase microextraction (SPME) have been used for pretreatment steps, followed by GC with flame ionization detection (FID) or GC-MS equipped with a WAX or FFAP column (1, 11–16). Because these extraction methods are complex and laborious, we applied a direct-injection method in the present study. Usually GC–FID or GC-MS is used for the detection of VOCs (12, 13, 17). In the case of GC-MS, the target mass fragments need to be greater than m/z 50 because other possible interferences such as air (m/z 28) and carbon dioxide (m/z 44) can be detected when this ratio is set too low. However, most of the targets in this experiment were low-MW compounds. For example, the MW of methanol is approximately 32.04 g/mol and the MW of acetaldehyde is 44.05 g/mol; these compounds would not be appropriate for GC-MS. GC–FID showed excellent and comparable accuracy and precision to HPLC and GC-MS in the quantitative analysis.

For the separation of VOCs, a DB-624 column was used, consisting of 6% cyanopropylphenyl and 94%

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dimethylpolysiloxane. This column is specifically designed for the analysis of volatile compounds such as residual solvents and volatile pollutants. Several studies have been already done with this column for the analysis of residual solvents and organic solvents in water (18–21). Therefore, because we are planning to analyze volatile compounds, the DB-624 column is expected to work efficiently to analyze these compounds in alcoholic beverages.

Based on these facts, GC–FID with a DB-624 column was applied to the alcoholic beverages to determine the level of VOCs. The method was validated with four types of alcoholic beverages (beers, fruit wines, rice wines, and spirits) to determine the versatility of the method. Validation was performed with tests of linearity, accuracy, precision, LOD, and LOQ to determine the quality of method. The method was then applied to 40 alcoholic beverage samples commonly available in markets to quantify the volatile compounds. Principal component analysis (PCA) was applied to the results to determine the tendencies of the VOCs to occur depending on the type of alcoholic beverage. This method simplifies the analytical method for VOCs and could lead to a better understanding of the VOCs in alcoholic beverages.

Materials and Methods

Materials

(a) Chemicals.—Methanol (Cat. No. 34860), 2-propanol (Cat. No. 650447), 1-propanol (Cat. No. 402893), 1-butanol (Cat. No. 34867), 2-butanol (Cat. No. B85919), 3-methyl-1-butanol (Cat. No. 320021), 2-methyl-1-butanol (Cat. No. 65990), 4-methyl-2-pentanol (Cat. No. 109916), *tert*-butanol (Cat. No. 308250), and acetaldehyde (Cat. No. 00071) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl acetate (Cat. No. 9282-03) was purchased from J.T. Baker (Milwaukee, WI), and isobutanol (Cat. No. 1052) from Duksan Pure Chemicals (Ansan, Korea).

(b) Alcoholic beverage products.—A total of 40 alcoholic beverages were purchased from Korean local markets. These samples represent four different types of alcoholic drinks: beers, fruit wines, rice wines, and spirits ($n = 10$ of each type).

Methods

(a) Sample preparation.—Standard solutions were prepared for use in the validation of the developed methods. All the VOC standards were dissolved and diluted in 20% ethanolic solution. 4-Methyl-2-pentanol was selected as the internal standard (IS), which did not exist in the samples and has been shown to have good separations with the target compounds (22, 23). For sample preparation, the IS was directly spiked to the samples and the standard solutions. A 10 μ L aliquot of IS was spiked to each solution to make a final volume of 5 mL. After spiking, the samples were directly transferred to crimp-capped vials to avoid loss of the target compounds and then directly injected for immediate analysis by GC–FID.

(b) Instrumental conditions.—For the GC system, a GC-2010 (Shimadzu, Kyoto, Japan) was used with FID. An Agilent DB-624 column (60 m \times 0.25 mm id \times 1.4 μ m film thickness) was used for the separation of VOCs. The column temperature program was set at 40°C for 10 min; raised at 5°C/min to 90°C,

2°C/min to 120°C, and 10°C/min to 240°C; and then held for 10 min for the separation of these compounds. Nitrogen gas was used as the carrier gas at a rate of 1 mL/min. Temperatures of the injection port and detector were set at 200 and 250°C, respectively. For stability of the signal and the detector flame, H₂ gas and air were run through the detector at a constant rate of 40 and 400 mL/min, respectively. The injection volume was 1 μ L, with a split ratio of 1:10.

(c) Method validation.—(1) *Linearity.*—The linearity of the VOC determination method was evaluated using standard solutions dissolved in 20% ethanolic solution. These solutions were serially diluted with same solvents, making ranges from 1 to 1000 mg/L. Calibration plots were made by plotting the relative analyte-to-IS peak area ratio against concentration of the target standards. Linearity was analyzed by the correlation coefficient of the calibration curve.

(2) *Accuracy and precision.*—Accuracy and precision were validated with low, medium, and high concentrations of each VOC standard. These concentrations were set as 1, 10, and 100 mg/L, respectively, according to calibration range. The 11 VOC standards were spiked to four kinds of target alcoholic beverages (beers, fruit wines, rice wines, and spirits) and analyzed in six replicates ($n = 6$). Precision was expressed as the RSD of six replicates, and accuracy was expressed as percentage recovery, calculated as the peak area of the standard in the sample divided by the peak area of the standard in ethanol solution.

(3) *LOD and LOQ.*—The LOD and LOQ were determined using the S/N approach. The LOD and LOQ were the concentrations leading to the detection or quantification of the signals, calculated by 3 or 10 times the baseline noise, respectively.

(d) Application of method to commercial alcoholic beverages.—(1) *GC–FID analysis.*—The developed GC–FID method was applied to four kinds of alcohol samples collected from Korean commercial markets. These samples were spiked with the IS and then vortex-mixed and filtered through a 0.5 μ m syringe filter before injection.

(2) *PCA.*—After the analysis, PCA was performed to find out clustering behaviors of VOCs for each alcohol type. To perform PCA, MetaboAnalyst 3.0 used the mean-centered peak areas of the 11 VOCs as input data (24). Clustering tendency was verified by a scores plot, and the VOCs responsible for the clustering were identified by a loadings plot.

Results and Discussion

Method Validation

In this study, a GC–FID method for the determination of 11 kinds of VOCs in alcoholic beverages was developed. Eleven VOC standards and the IS were well separated using the developed method (Figure 1). The developed method was validated with measures of linearity, accuracy, precision, LOD, and LOQ.

Linearity.—The calibration plots for 11 VOCs were produced by plotting the target analyte-to-IS peak area ratio of against the concentration of the target standards, and the linearity results are summarized in Table 1. All the VOCs were linear from 10 to 1000 mg/L. Correlation coefficients of the calibration plots ranged from 0.9986 to 0.9995, which were determined to show linearity.

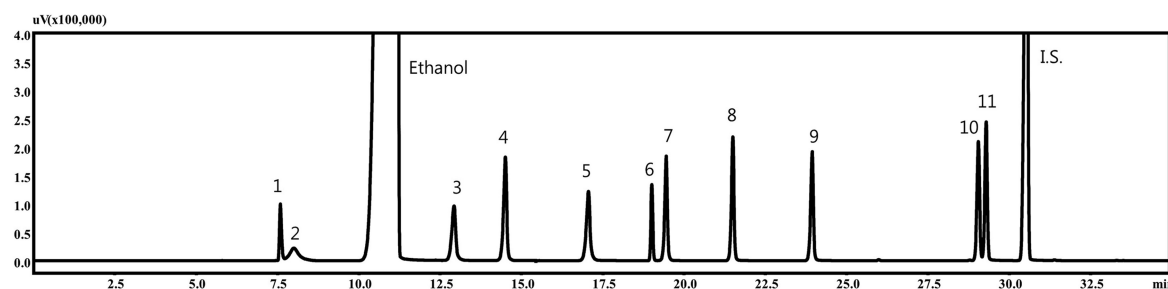


Figure 1. GC chromatogram for the VOC standards: 1, acetaldehyde; 2, methanol; 3, 2-propanol; 4, *tert*-butanol; 5, 1-propanol; 6, ethyl acetate; 7, 2-butanol; 8, isobutanol; 9, 1-butanol; 10, 3-nethyl-1-butanol; 11, 2-methyl-1-butanol; I.S., 4-methyl-2-pentanol.

Table 1. Retention times, correlation coefficients of calibration curves, LODs, and LOQs of 11 VOC standards

VOC	Retention time, min	Slope	Intercept	r^2	LOD, mg/L	LOQ, mg/L
Acetaldehyde	7.53	32.215	-0.0001	0.9995	0.010	0.030
Methanol	8.00	34.613	0.0005	0.9986	0.010	0.030
2-Propanol	12.94	65.257	-0.0051	0.9991	0.100	0.300
<i>tert</i> -Butanol	14.51	99.736	-0.0098	0.9989	0.100	0.300
1-Propanol	17.08	79.066	-0.0088	0.9989	1.000	3.000
Ethyl acetate	18.99	43.140	-0.0041	0.9991	0.033	0.100
2-Butanol	19.44	83.276	-0.0087	0.9990	0.250	0.750
Isobutanol	21.45	96.468	-0.0108	0.9989	0.250	0.750
1-Butanol	23.65	90.910	-0.0106	0.9989	0.250	0.750
3-Methyl-1-butanol	27.77	101.187	-0.0120	0.9988	0.100	0.300
2-Methyl-1-butanol	27.94	107.073	-0.0114	0.9990	0.100	0.300

Accuracy and precision.—Accuracy and precision were measured by analyzing three concentrations of VOCs with six replicates in four kinds of alcoholic beverages. Concentrations for VOCs were 1, 10, and 100 mg/L, expressed as low, medium, and high, respectively. The results of accuracy and precision are summarized in Tables 2 and 3, respectively. Accuracy was

Table 2. Accuracy of four types of alcoholic beverages^a

VOC	Beers			Fruit wines			Rice wines			Spirits		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Acetaldehyde	86.5	97.3	82	87.3	96.6	104.6	86	103.3	95.2	87.9	85.4	86.1
Methanol	89.5	83.6	87.6	105.9	110.8	108.4	89.9	114.0	112.8	90.3	94.3	102.1
2-Propanol	95.1	94.9	86.6	76.8	95.3	92.6	83.0	94.7	95.7	85.7	86.9	86.6
<i>tert</i> -Butanol	100.0	86.6	81.0	86.0	86.0	84.1	95.5	94.2	90.8	89.7	79.6	78.4
1-Propanol	111.1	96.5	85.8	102.1	98.5	92.8	101.5	101.4	98.9	116.3	91.9	86.2
Ethyl acetate	102.8	102.0	95.0	105.2	99.3	103.8	93.7	93.5	97.7	102.0	90.7	93.7
2-Butanol	88.0	89.4	82.7	92.2	90.4	86.1	94.6	98.3	95.6	100.5	86.0	83.3
Isobutanol	91.7	94.5	85.8	104.8	95.9	87.9	85.0	99.8	95.4	99.4	86.1	82.6
1-Butanol	90.4	91.8	83.7	98.6	96.2	89.2	102.1	94.6	97.9	107.4	90.1	85.7
3-Methyl-1-butanol	95.5	97.8	85.8	118.2	111.5	96.8	97.1	96.6	89.0	103.5	86.4	82.1
2-Methyl-1-butanol	89.4	95.1	87.4	94.3	85.4	79.8	82.8	96.1	97.4	101.7	85.0	83.2

^a Data presented as average percentage recoveries ($n = 6$) using low (1 mg/L), medium (10 mg/L), or high (100 mg/L) concentrations of the VOC standards in each of the four matrices.

calculated as percentage recoveries (peak area of the standard in sample divided by peak area of the standard in ethanol solution). Precision was calculated as RSD. Most of the accuracy results were in the range of $\pm 20\%$. There were some exceptions, such as at the low concentration of 2-propanol in fruit wines. Most of the precision results were below 20%, except for the lowest concentrations of methanol in rice wines and spirits.

LOD and LOQ.—The LOD and LOQ were calculated based on the S/N of 3 and 10 times the baseline noise, respectively, and these results are shown in Table 1. The LODs ranged from 0.010 to 1.000 mg/L, and the LOQs from 0.030 to 3.000 mg/L, depending on the compound (Table 1).

Application of Method to Commercial Alcoholic Beverages

The developed method was applied to real samples of commercial alcoholic beverages. The method can detect 11 volatile organic compounds, nine of which were detected in the 40 alcoholic beverage samples. *tert*-Butanol and 2-butanol did not exist in our samples. VOCs occurred in different patterns according to the type of alcoholic beverage, as shown in Figure 2, and VOC content is summarized in Table 4.

After quantification, we performed PCA to determine clustering behaviors of VOCs in alcoholic beverages and to verify which VOCs are responsible for the clustering (Figure 3). In the scores plot, the four types of alcoholic

Table 3. Precision of four types of alcoholic beverages^a

VOC	Beers			Fruit wines			Rice wines			Spirits		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Acetaldehyde	13.5	3.2	14	6.6	7.9	11.2	6.8	1.5	11.4	5.2	12.7	5.3
Methanol	11.2	3.7	8.2	14.0	3.8	13.6	27.1	0.4	3.5	23.2	4.5	1.2
2-Propanol	3.9	2.4	10.9	4.8	1.0	16.5	8.6	0.9	3.3	9.2	2.8	0.8
<i>tert</i> -Butanol	15.0	11.2	17.3	3.0	3.2	18.1	11.3	8.3	9.7	12.8	6.5	5.4
1-Propanol	6.0	1.4	6.9	2.2	2.7	17.0	3.3	2.5	6.1	10.2	4.1	2.1
Ethyl acetate	9.7	1.5	10.0	4.1	4.5	19.8	0.6	0.1	5.5	12.0	2.2	1.8
2-Butanol	8.3	5.1	12.9	3.5	2.2	14.5	8.4	7.9	8.6	13.1	2.2	6.2
Isobutanol	3.6	3.1	8.1	2.6	2.4	15.5	2.8	0.5	1.6	12.1	2.9	1.8
1-Butanol	4.2	2.3	5.8	2.5	6.1	17.9	8.3	8.2	7.6	15.3	6.6	5.6
3-Methyl-1-butanol	2.4	4.6	8.1	2.7	7.4	13.4	2.7	0.4	0.8	12.5	7.8	7.3
2-Methyl-1-butanol	6.9	12.1	9.0	3.5	1.9	13.8	2.3	1.5	4.8	14.4	8.2	8.2

^a Data presented as the average RSDs ($n = 6$) using low (1 mg/L), medium (10 mg/L), or high (100 mg/L) concentrations of the VOC standards in each of the four matrices.

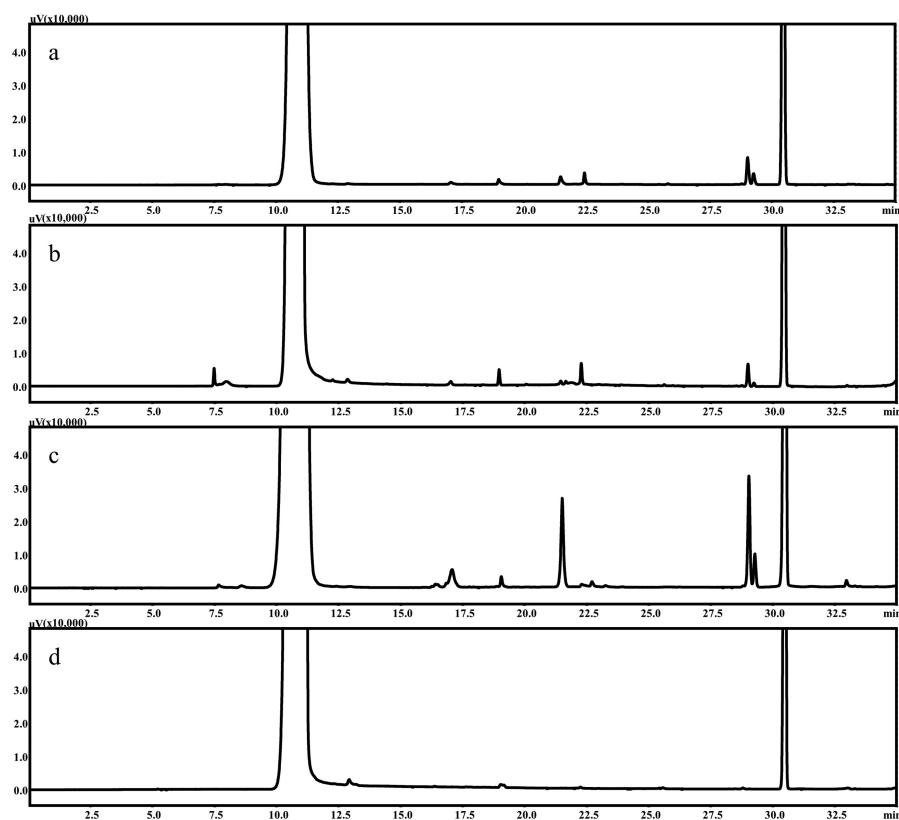


Figure 2. GC chromatogram for the four types of alcoholic beverages: (a) beers, (b) fruit wines, (c) rice wines, and (d) spirits.

beverages are clearly distinguished, confirming that VOCs are generated differently in each type. A loadings plot shows which compounds are important for separation. The plots in Figure 3 demonstrate that methanol amounts were higher in fruit wines, 2-propanol amounts were highest in spirits, and fusel alcohols were the major VOCs in rice wines. These distinct profiles of VOCs result from the diverse production processes of the alcoholic beverages.

Although all four types of alcoholic beverages had small amounts of acetaldehyde, the highest level (218.55 mg/L) was

found in rice wines. This level, however, is lower than the Korean Ministry of Food and Drug Safety (MFDS) regulation of 700 mg/L. In previous studies, acetaldehyde was consistently detected in alcoholic beverages (2, 11, 25). This VOC exists in all types of alcoholic beverages because it is an intermediate metabolite of ethanol. It is classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC). Acetaldehyde has shown carcinogenic effects by binding to DNA and forming carcinogenic adducts (26–30). IARC working groups have reported that acetaldehyde

Table 4. Concentration of VOCs in the alcoholic beverage samples^a

Sample	Acetaldehyde	Methanol	2-Propanol	<i>tert</i> -Butanol	1-Propanol	Ethyl acetate	2-Butanol	Isobutanol	1-Butanol	3-Methyl-1-butanol	2-Methyl-1-butanol
Beers											
Beer 1	5.54 ± 0.26	23.92 ± 1.87	ND ^b	ND	29.73 ± 1.22	42.02 ± 0.29	ND	34.44 ± 44	19.39 ± 0.07	63.81 ± 1.79	32.59 ± 1.03
Beer 2	5.96 ± 1.50	6.33 ± 1.75	ND	ND	26.68 ± 1.15	33.00 ± 1.50	ND	30.19 ± 0.80	19.91 ± 0.06	63.21 ± 3.52	37.06 ± 1.39
Beer 3	4.10 ± 0.37	ND	ND	ND	ND	27.55 ± 0.49	ND	ND	ND	50.04 ± 0.71	ND
Beer 4	7.70 ± 0.98	2.94 ± 0.98	ND	ND	ND	31.85 ± 1.47	ND	25.87 ± 0.35	ND	44.06 ± 0.57	ND
Beer 5	2.72 ± 0.33	1.13 ± 0.40	ND	ND	ND	26.90 ± 0.29	ND	ND	ND	44.43 ± 1.41	ND
Beer 6	11.63 ± 1.53	4.73 ± 0.94	ND	ND	ND	33.79 ± 1.53	ND	27.96 ± 0.35	ND	46.49 ± 0.94	ND
Beer 7	3.63 ± 0.63	1.51 ± 0.30	ND	ND	ND	32.10 ± 3.28	ND	22.79 ± 0.23	ND	48.73 ± 1.72	ND
Beer 8	4.42 ± 0.97	1.32 ± 0.53	ND	ND	ND	33.42 ± 3.53	ND	27.73 ± 0.08	ND	50.08 ± 0.30	27.33 ± 0.10
Beer 9	3.72 ± 0.12	2.17 ± 0.52	ND	ND	24.07 ± 0.75	33.72 ± 0.78	ND	31.92 ± 0.66	ND	58.19 ± 1.78	32.19 ± 0.71
Beer 10	4.01 ± 0.05	0.36 ± 0.04	ND	ND	ND	37.40 ± 1.37	ND	34.36 ± 0.10	ND	62.14 ± 0.24	32.04 ± 0.13
Fruit wines											
Fruit wine 1	16.88 ± 0.58	7.91 ± 1.72	19.5 ± 1.47	ND	ND	36.25 ± 2.07	ND	ND	ND	41.53 ± 0.89	ND
Fruit wine 2	24.04 ± 0.72	33.02 ± 1.34	19.65 ± 0.63	ND	ND	37.91 ± 0.42	ND	ND	ND	43.86 ± 1.06	ND
Fruit wine 3	10.67 ± 0.61	78.41 ± 4.49	16.86 ± 0.77	ND	69.45 ± 1.28	38.86 ± 0.78	ND	190.17 ± 4.68	ND	204.81 ± 4.36	69.45 ± 0.59
Fruit wine 4	46.77 ± 3.52	52.59 ± 0.81	20.65 ± 0.85	ND	24.88 ± 0.49	48.24 ± 1.68	ND	ND	ND	43.91 ± 1.35	ND
Fruit wine 5	17.87 ± 2.82	116.06 ± 12.36	ND	ND	26.88 ± 1.20	36.06 ± 0.48	ND	40.19 ± 5.89	ND	52.26 ± 1.93	26.36 ± 0.50
Fruit wine 6	7.46 ± 1.30	23.74 ± 14.31	23.90 ± 1.59	ND	24.10 ± 0.51	34.04 ± 1.57	ND	28.21 ± 1.75	ND	57.83 ± 2.19	25.22 ± 0.17
Fruit wine 7	16.90 ± 2.56	29.12 ± 2.98	15.96 ± 0.66	ND	ND	35.69 ± 0.08	ND	ND	ND	43.22 ± 0.98	ND
Fruit wine 8	26.10 ± 2.81	30.83 ± 1.88	21.47 ± 0.13	ND	ND	53.53 ± 1.80	ND	24.86 ± 1.27	19.40 ± 0.13	49.90 ± 1.13	ND
Fruit wine 9	12.75 ± 1.07	86.67 ± 5.37	20.81 ± 0.43	ND	27.68 ± 0.34	25.83 ± 0.22	ND	35.24 ± 0.70	ND	65.66 ± 1.77	28.34 ± 0.51
Fruit wine 10	13.01 ± 10.85	19.34 ± 18.96	18.46 ± 0.62	ND	ND	34.64 ± 0.08	ND	24.31 ± 0.72	22.50 ± 0.03	ND	ND
Rice wines											
Rice wine 1	25.54 ± 2.06	19.29 ± 4.07	ND	ND	51.41 ± 0.97	30.11 ± 0.45	ND	ND	19.87 ± 0.07	83.63 ± 2.76	34.35 ± 0.85
Rice wine 2	10.61 ± 0.26	9.00 ± 0.80	ND	ND	64.23 ± 2.97	35.31 ± 1.74	ND	120.44 ± 3.75	ND	120.28 ± 3.72	45.09 ± 1.16
Rice wine 3	11.48 ± 1.56	ND	ND	ND	69.91 ± 2.57	41.70 ± 2.97	ND	73.13 ± 1.20	ND	102.26 ± 1.46	41.76 ± 0.32
Rice wine 4	23.98 ± 0.54	19.48 ± 2.23	ND	ND	65.81 ± 4.49	38.88 ± 1.35	ND	156.23 ± 6.89	ND	162.65 ± 5.97	52.88 ± 1.76
Rice wine 5	8.44 ± 0.52	14.99 ± 1.24	ND	ND	89.54 ± 1.10	43.81 ± 1.49	ND	58.86 ± 1.64	ND	88.43 ± 2.49	37.80 ± 0.78
Rice wine 6	8.31 ± 0.74	3.22 ± 1.58	ND	ND	56.45 ± 0.35	36.54 ± 0.91	ND	59.20 ± 0.35	ND	85.51 ± 0.45	37.43 ± 0.20
Rice wine 7	218.55 ± 17.20	ND	13.84 ± 0.16	ND	88.98 ± 3.62	42.49 ± 1.82	ND	92.63 ± 4.01	19.76 ± 0.05	105.74 ± 4.69	34.75 ± 1.03
Rice wine 8	10.16 ± 1.53	ND	13.74 ± 0.10	ND	88.06 ± 6.07	26.10 ± 0.94	ND	69.17 ± 4.63	22.55 ± 0.31	103.24 ± 7.54	45.89 ± 2.51
Rice wine 9	11.73 ± 1.95	ND	14.28 ± 0.25	ND	61.34 ± 4.57	34.64 ± 1.60	ND	85.46 ± 4.79	19.92 ± 0.08	117.87 ± 8.78	44.88 ± 2.06
Rice wine 10	16.11 ± 0.63	ND	ND	ND	53.73 ± 2.65	31.08 ± 1.44	ND	69.57 ± 2.53	19.95 ± 0.07	104.33 ± 3.88	36.16 ± 0.83
Spirits											
Spirit 1	ND	ND	30.64 ± 1.77	ND	19.20 ± 0.26	40.30 ± 5.05	ND	ND	ND	ND	ND
Spirit 2	ND	ND	26.17 ± 4.61	ND	ND	28.51 ± 11.63	ND	ND	ND	ND	ND

Table 4. (continued)

Sample	Acetaldehyde	Methanol	2-Propanol	<i>tert</i> -Butanol	1-Propanol	Ethyl acetate	2-Butanol	Isobutanol	1-Butanol	3-Methyl-1-butanol	2-Methyl-1-butanol
Spirit 3	ND	ND	27.96 ± 2.50	ND	ND	35.31 ± 1.85	ND	ND	ND	ND	ND
Spirit 4	ND	ND	37.16 ± 3.45	ND	ND	26.31 ± 9.49	ND	ND	ND	ND	ND
Spirit 5	ND	ND	29.69 ± 2.26	ND	ND	ND	ND	ND	ND	ND	ND
Spirit 6	ND	ND	29.68 ± 2.28	ND	ND	18.43 ± 5.33	ND	ND	ND	ND	ND
Spirit 7	ND	ND	ND	ND	ND	20.52 ± 8.96	ND	ND	ND	ND	ND
Spirit 8	ND	ND	ND	ND	19.67 ± 0.12	ND	ND	ND	ND	19.79 ± 0.08	17.47 ± 0.07
Spirit 9	ND	ND	23.58 ± 1.92	ND	ND	21.79 ± 5.65	ND	ND	ND	19.87 ± 0.15	17.50 ± 0.06
Spirit 10	ND	ND	28.67 ± 2.48	ND	ND	ND	ND	ND	ND	ND	ND

^a Concentrations reported in milligrams per liter.

^b ND = Not detected.

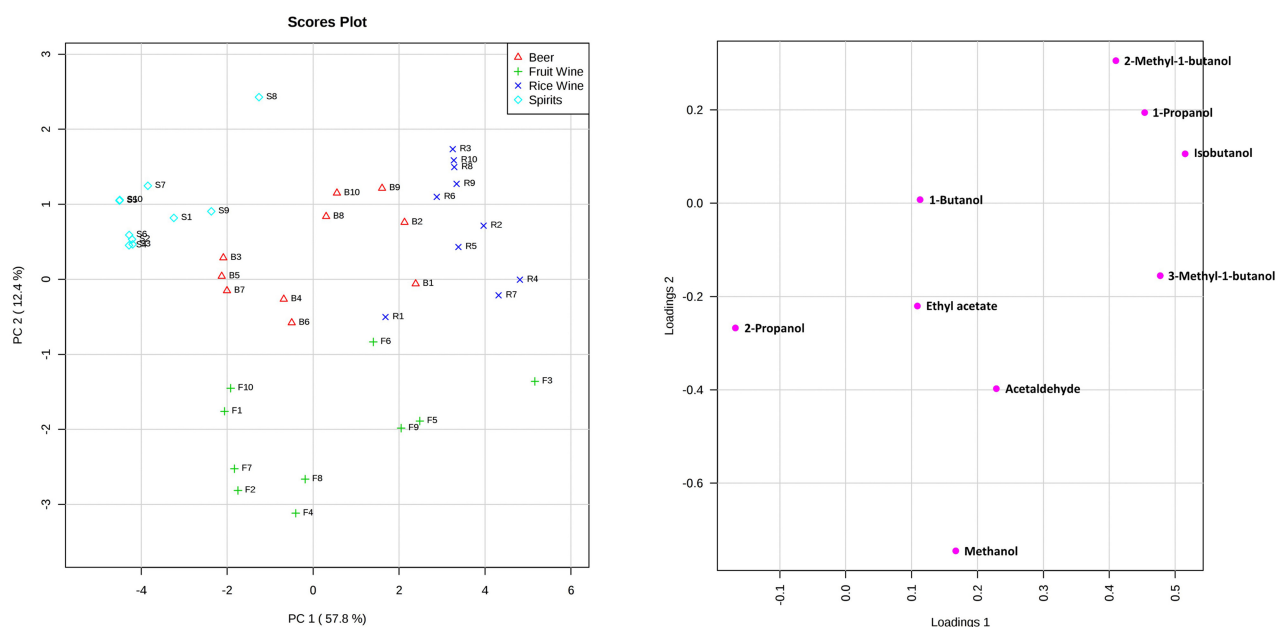


Figure 3. PCA scores plot (left) and loadings plot (right) of VOC profiling of four types of alcoholic beverages.

in alcoholic beverages contributes to the cause of malignant esophageal tumors (31, 32). Other studies have reported that acetaldehyde can increase the risk for digestive tract cancers (33, 34). Because this compound has a risk of causing cancer, it is recommended that it be continuously monitored in the production process to keep the concentration lower than regulation limits.

In the case of methanol, the highest concentration was 116.06 mg/L. It was lower than 500 mg/L, which is the MFDS limit. However, fruit wines had higher amounts of methanol than the other three types of alcohols due to the characteristic of the methanol-forming process, which is associated with pectinolytic enzymes (3, 11). Previous studies have also shown large amounts of methanol in fruit wines (25). The loadings plot in Figure 3 shows that methanol is responsible for the separation of fruit wines from the other samples. Therefore, methanol is the predominant VOC in fruit wines according to former and current results.

In the spirit samples, fusel alcohols were produced in small amount compared to other types of beverages. Beers and fruit wines had higher amounts of fusel alcohols compared to spirits, whereas rice wines had plenty of fusel alcohols, including 1-propanol, 2-propanol, 1-butanol, isobutanol, 3-methyl-1-butanol, and 2-methyl-1-butanol. In a previous study, aromatic compounds of Chinese rice wines were analyzed and found to include propanol, butanol, ethyl acetate, and 3-methylbutanol (35), similar to our results with rice wines from Korea. For fusel alcohol regulation, there are no data or set limits from the MFDS. Also, some fusel alcohols, such as 1-propanol, 1-butanol, isobutanol, isoamyl alcohol, and amyl alcohol, are considered safe by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (1999) and U.S. Food and Drug Administration (2011). Fusel alcohol is necessary for the special flavoring of alcoholic beverages, but excessive concentrations can cause side effects and produce astringent and bitter flavors (36). Even in the European Union,

there is a minimum limit of volatile substance content due to flavoring with higher-order alcohols (European Council, 1989). However, higher amounts of these compounds can have toxic effects such as nervous hyperemia, dizziness, and headaches. Therefore, limiting the maximum amount of fusel alcohol seems wise. There have been reports recommending maximum limits of fusel alcohols (10); additional study is recommended to determine their regulation levels.

For validation of the current method, alcoholic beverage samples were analyzed by the previous HS-SPME method (15). First, linearity was compared using standard solutions. The HS-SPME method showed poor linearity compared to the direct-injection method. When applied to real samples, there were differences in the sensitivity of compounds due to their distinct volatility. Therefore, some compounds were detected better by the direct-injection method and some were detected better by the HS-SPME method (data not shown). Further comparative studies should be performed to better understand various pretreatment procedures.

Conclusions

VOCs, which are often detected in alcoholic beverages, have harmful effects on human health, such as causing dizziness and being a cancer risk. Thus, the determination of VOCs in alcoholic beverages is important for production processes and research purposes. Analysis of several kinds of alcoholic beverages is needed because various production processes lead to a diversity of VOCs.

In this study, we developed and validated a simple, fast method for the determination of VOCs in four kinds of alcoholic beverages. This method does not have complex extraction methods and does not require expensive instruments (e.g., a mass spectrometer) in comparison to other existing methods. The method was validated with linearity, accuracy, and precision, which showed good performances. After validation, various kinds of commercial alcoholic beverages were analyzed with the developed method to confirm the suitability of the method and to monitor the levels of VOCs. Using PCA, patterns of VOCs were studied to investigate byproducts that should be carefully monitored regarding each type of alcohol beverage.

In summary, the developed method for the determination of VOCs is suitable for analyzing commercial alcoholic beverages and can contribute to the safe production of alcoholic beverages by monitoring VOC levels.

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