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Determination of fusel oil content in various types of liquor distributed in Korea

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Abstract

This study was performed to analyze the content of 6 different fusel oils in 9 types of liquor distributed in domestic market. GC-FID method was employed for quantifying fusel oil (1-propanol, iso-butanol, 1-butanol, 2-butanol, iso-amyl alcohol, active amyl alcohol) levels in 260 liquor samples of liquor. Relative standard deviations (%) of intra- and interday measurements were under 1.56 and 2.44%, respectively, while recovery rates (%) were 98.22-105.26% and 98.53-107.15%, respectively. Pretreatment method (filtering and centrifugation) of *Takju* did not affect analytic results. The average of total fusel oil contents in *Yakju* (39 types) and fruit wines (30 types) were 497.6 and 151.9 mg/L, showing *Yakju* contains more fusel oils than *Takju* or fruit wines. In fruit wines, iso-amyl alcohol was the major fusel oil component (at 6.8-249.0 mg/L). The highest content of fusel oil was found in foreign brandy, whereas the diluted *Soju* did not contain fusel oils. However, the average of total fusel oil contents was high at 764.5 mg/L in the three types of distilled *Soju* and iso-amyl alcohol content ranged from 114.2 to 421.0 mg/L. Domestic and foreign beers were similar in terms of their fusel oil compositions and contents. In conclusion, excluding the diluted *Soju*, the contents of total fusel oils ranged from 114.8 to 1447.3 mg/L in the monitored liquors.

Key words : fusel oil content, gas chromatograph, flame ionization detector, liquor, monitoring

Introduction

Alcohols other than ethanol, aldehydes, organic acids, esters, and carbonyl compounds can be produced during the course of liquor fermentation. Of the higher alcohols, iso-amyl alcohol (3-methyl-1-butanol), active amyl alcohol (2-methyl-1-butanol), iso-butanol (2-methyl-1-propanol), 1-propanol, 1-butanol, and 2-butanol are main components of fusel oils, and have been reported to be derived from specific amino acids. Generally, high fusel oil concentrations negatively affect the flavors liquors while, when present at low concentrations, fusel oils can improve the flavors of certain liquors. In addition, it is known that if fusel oils are consumed in large quantities, they can have adverse consequences on health (1-3).

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To analyze fusel oil levels in liquors, gas chromatograph equipped with a flame ionization detector (FID) has been used by the Alcohol and Tobacco Tax and Trade Bureau (TTB) in the USA (4) and by the Commission of the European Communities in Europe (5). In most cases, the major compounds of fusel oil in liquor are 1-propanol, iso-butanol, 1-butanol, 2-butanol, iso-amyl alcohol, and active amyl alcohol. Although there is a colorimetric method given by the Korea National Tax Services, it is required to perform an instrumental analysis for quantification of fusel oil in liquors. Until now, limited results have been issued on fusel oil contents of various types of liquor distributed in Korea. Furthermore, it is needed to study a pretreatment method for determining fusel oil levels in liquor which contains suspended matters such as *Takju.*

In this study, an internal standard curve was prepared and linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ) were determined. In addition, recovery rates with respect to ethanol concentrations (5, 10, and 20%) and different sample matrices (*Takju, Yakju, Cheongju,* beer, fruit wine, brandy, and whiskey) were investigated. Finally, we determined the contents of fusel oil in various types of liquor currently being sold in Korean market.

Materials and Methods

Materials and reagents

The fusel oil standards used in the study, that is, 1-propanol, iso-butanol, 1-butanol, 2-butanol, iso-amyl alcohol, and active amyl alcohol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Also, 3-pentanol used as internal standard was purchased from Sigma-Aldrich. Takju, which was used to check sample pretreatment recovery rates (i.e., filtering and centrifugation), and eight types of liquors (*Takju, Yakju, Cheongju*, beer, fruit wine, brandy, and whiskey), which were used to check recovery rates from different sample matrices, were purchased from the supermarkets in Seoul. To monitor the content of fusel oils, commercially available *Takju* (72 types), *Yakju* (39 types), fruit wines (30 types), diluted Soju (10 types), distilled Soju (3 types), foreign whiskey (12 types), brandy (9 types), domestic beer (39 types), and foreign beers (46 types) were used.

Pre-treatment of samples

For GC analysis of carbonated Takju and beer, carbonic acid was removed by repeated pipetting until no bubbles were generated. *Takju* was centrifuged (Hanil, HA-1000-3, Hanil Science Co., Daejeon, Korea) for 10 min at 3,000 rpm to remove suspended matter. Separately, 3-pentanol (50 mg) was placed in a 100 mL volumetric flask and then 10% ethanol solution was added to prepare a 3-pentanol (internal standard, IS) solution of 500 mg/L. Two milliliter of the supernatant of *Takju* obtained after centrifugation was mixed with the same volume of 3-pentanol solution (500 mg/L), and then it was vortexed for 1 min. This mixture was then filtered through a 0.50 μm syringe filter (PTFE, DISMIC-13JP, Tokyo Roshi Kaisha, Tokyo). In order to determine how filtering and centrifugation affected analytical results, we compared the values before and after the addition of each fusel oil standard with 100 mg/L concentration in *Takju*.

GC analysis of fusel oil

Samples were injected into a GC (Younglin 6100, Younglin, Anyang, Korea) combined with a flame ionized detector (FID). Separation of fusel oils was performed on the DB-624 column (60 m×0.25 mm×1.4 μm, Agilent Technologies, Santa Clara, CA, USA). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. The oven temperature program was as follows: initial temperature of 40℃ for 5 min, increased by 10℃/min to final temperature of 250℃ and held for 10 min. The detector temperature was 280℃. The injector was set at 250℃ with split ratio of 100:1.

Preparation of calibration curves

Fusel oil contents were quantified by dividing the GC peak areas of six fusel oil standard materials at 10, 50, 100, 250, and 500 mg/L by areas of added internal standards (3-pentanol, 250 mg/L). Calibration curves of six fusel oil standards were plotted by setting the ratios between peak areas of fusel oils and peak areas of internal standard materials on the Y axis, and concentrations of fusel oil standards (10, 50, 100, 250, and 500 mg/L) on the X axis. Ratios of peak areas were obtained by averaging the results of five independent experiments.

Linearity, precision, and accuracy

The fusel oil standards (1-propanol, iso-butanol, 1-butanol, 2-butanol, iso-amyl alcohol, and active amyl alcohol) and the internal standard (3-pentanol) were dissolved in the 10% ethanol solution.

Intraday accuracy was determined by conducting experiments for three times in one day using the same conditions, and interday accuracy was obtained by conducting experiments on three separate days using the same conditions. Fusel oil standard materials were prepared by dissolving six types of fusel oils standards in the 10% ethanol aqueous solution to a concentration of 100 mg/L, whereas the internal standard was made up at 250 mg/L. Relative standard deviation (RSD%) was defined as shown below, and results are presented as averages and standard deviations.

RSD (%)=the standard deviation of the response/mean×100.

The recovery rates (%) were calculated. Results are the average values of independent experiments performed in triplicate. Recovery rate was defined as:

Recovery rate (%)=C_F-C_U/C_A×100 (6)
C_F=Concentration of analyte measured in fortified test sample
C_U=Concentration of analyte measured in unfortified test sample
C_A=Concentration of analyte added to fortified test sample

Limit of detection and Limit of quantitation

GC chromatograms were obtained by injecting the blank

(10% ethanol aqueous solution). Peak areas were then obtained by integrating areas near the retention time of each fusel oil standard material and average of peak areas was obtained after five repetitions. Limits of detection (LOD) were obtained by multiplying average peak areas by three, while limits of quantitation (LOQ) were set as ten times average peak areas. LOD and LOQ values were then calculated using gradients and standard deviations using the equations below (6).

Limit of detection (LOD)=3.3×SD/S
Limit of quantitation (LOQ)=10×SD/S
Where, S= the slope of the standard curve, SD= the standard deviation of the response

Recovery rates according to ethanol concentration and sample matrices

Since ethanol concentrations of the liquors differed, recovery rates were investigated using aqueous ethanolic solutions of different concentrations. The six fusel oil standard materials (25 mg each) were placed into 25 mL flasks, and volumes were adjusted with ethanol to a fusel oil standard concentration of 1,000 mg/L. Also, 62.5 mg of internal standard material (3-pentanol) was added to a 25 mL flask and its concentration adjusted to 2,500 mg/L with ethanol. The prepared six fusel oil standards and the 3-pentanol solution were taken as 2 mL into 25 mL vial, respectively, and 6 mL of ethanol was added to prepare diluted fusel oil standard solutions. Meanwhile, 5%, 10%, and 20% ethanol solutions were prepared and mixed with the fusel oil standard solutions to obtain a fusel oil standard concentration of 100 mg/L, and an internal standard concentration of 250 mg/L.

Recovery rates were determined for eight different types of liquors (*Takju, Yakju, Cheongju*, beer, fruit wine, wine, brandy, and whiskey). The six fusel oil standards at a concentration of 1,000 mg/L and 3-pentanol at 2,500 mg/L were prepared in flasks. Standards were aqueous 10% ethanol solutions. The prepared standard solutions of fusel oils (2 mL) were mixed with 2 mL of internal standard solution. These were then diluted to a standard material concentration of 100 mg/L, and an internal standard concentration of 250 mg/L. These standard materials were spiked into the eight liquor types and their recovery rates were determined. The experiment was repeated twice and recovery rates were calculated using:

Total fusel oil value-sample fusel oil value/added fusel oil value by spiking×100

Monitoring of fusel oils in liquors

Analysis was carried out for *Takju* (72 types), *Yakju* (39 types), fruit wines (30 types), diluted *Soju* (10 types), distilled *Soju* (3 types), foreign whiskey (12 types), brandy (9 types), domestic beers (39 types), and foreign beers (46 types). Liquors containing more than 20% ethanol content (such as whiskey, brandy, and distilled *Soju*) were appropriately diluted before analysis.

Statistical analysis

Analysis was carried out twice and averages and standard deviations were calculated. The significances of differences were determined using Duncan's test in SAS Ver. 9.2 (SAS Institute Inc., Cary, NC, USA), and statistical significance was accepted for $p<0.05$.

Results and Discussion

Effect of pre-treatment (filtering and centrifugation) on results

Samples containing suspended matter (*Takju*) and fusel oil standard solution were filtered through a polytetrafluorethylene (PTFE) syringe filter. The results obtained are presented in Table 1. The contents of fusel oils in *Takju* before filtering were 82.83 mg/L (1-propanol), 96.48 mg/L (iso-butanol), 198.98 mg/L (iso-amyl alcohol), and 50.03 mg/L (active amyl alcohol), while values after filtering were 81.51 mg/L (1-propanol), 96.36 mg/L (iso-butanol), 199.80 mg/L (iso-amyl alcohol), and 53.17 mg/L (active amyl alcohol), which showed filtering did not significant affect results ($p>0.05$). The recovery rate of filtered 100 mg/L fusel oil standard solution was in the range of 102.26-109.62%. Because the recovery rates of the fusel oils were not significantly affected by filtering ($p>0.05$), it was considered that filtering as a pre-treatment did not affect fusel oil contents.

Centrifugation is an another method used to remove floating matter present in liquor. Fusel oil contents of *Takju* after centrifugation are presented in Table 1. The contents of fusel oils after centrifugation were 81.5 mg/L (1-propanol), 97.37 mg/L (iso-butanol), 197.47 mg/L (iso-amyl alcohol), and 51.55 mg/L (active amyl alcohol), and these did not differ significantly from results obtained before centrifugation ($p>0.05$). The analytical methods used in foreign countries were designed for liquors with less suspended matter, such as, beer, wine, brandy, and spirit, than *Takju*. Since floating matter causes column and injector problems during GC analysis, reproducibility can be negatively affected. In the present study, neither filtering nor centrifugation of *Takju* to remove suspended matter significantly affected fusel oil content results.

Precision, accuracy, limit of detection, and limit of quantitation

Calibration curves were prepared using the ratios of peak areas of the six standard materials at 10, 50, 100, 250, and 500 mg/L versus the internal standard, and coefficients of correlation (R^2) were calculated. The R^2 values of calibration curves were >0.99 for all six types of fusel oils examined, which is similar to that found for 1-propanol and iso-amyl alcohol at concentration ranges of 5-500 mg/L and 50-3,000 mg/L, respectively in a previous study (7). Therefore, the reliable result was expected in the concentration range 10-500 mg/L. GC-FID detects component with low boiling points first, and components with greater affinity for the packing material (stationary phase) move more slowly through thecolumn. Of the six types of fusel oils examined, 1-propanol eluted first with a retention time of 11.3 min.

Generally, when alcohols are separated by GC, a polar column with crossbond-polyethylene glycol is used, and it is difficult to separate iso-amyl alcohol and active amyl alcohol using such a column. However, DB-624 column (mid-polarity stationary phase, i.e. 6% cyanopropyl phenyl and 94% dimethyl polysiloxane) can improve the separation of these two components. Iso-amyl alcohol (16.0 min) had a slightly smaller retention time than active amyl alcohol (16.1 min). Relative standard deviations (RSD%) were calculated from average values and standard deviations of fusel oils in order to quantify intraday and interday precisions

Table 1. Effect of sample pretreatments (filtering and centrifugation) for analyzing fusel oils in liquor

(unit: mg/L)

Sample	Fusel oils	Before sample pretreatment	After filtering	After centrifugation
Takju	1-Propanol	82.83±4.28	81.51±2.63	81.5±4.06
	Iso-butanol	96.48±1.70	96.36±0.82	97.37±0.98
	1-Butanol	ND[1)]	ND	ND
	2-Butanol	ND	ND	ND
	Iso-amyl alcohol	198.98±2.48	199.80±0.30	197.47±7.12
	Active amyl alcohol	50.03±0.14	53.17±2.11	51.55±1.95
100 mg/L of fusel oil solution	1-Propanol	105.86±2.50	106.02±1.43	-[2)]
	Iso-butanol	109.38±0.20	109.62±0.22	-
	1-Butanol	103.76±5.05	106.16±2.12	-
	2-Butanol	104.18±0.74	105.23±2.47	-
	Iso-amyl alcohol	101.95±9.14	102.26±7.90	-
	Active amyl alcohol	103.02±6.17	103.35±3.80	-

[1)]ND, under limit of detection.
[2)]-, experiment was not conducted.
Analysis was performed in duplicate and no significant differences were found among samples (before and after treatment) from Duncan test.

Table 2. Relative standard deviation (RSD%), recovery rate (RR %), limit of detection (LOD), and limit of quantitation (LOQ) of the fusel oils

Fusel oils	Intraday		Interday		LOD (mg/L)	LOQ (mg/L)
	RSD%	RR (%)	RSD%	RR (%)		
1-Propanol	1.07	98.22	2.44	98.53	2.58	8.65
Iso-butanol	1.39	104.98	1.60	106.62	1.50	5.59
1-Butanol	1.36	98.95	2.20	102.53	1.52	5.70
2-Butanol	0.81	102.94	1.31	102.83	1.54	5.95
Iso-amyl alcohol	0.99	101.55	1.09	103.03	1.47	5.04
Active amyl alcohol	1.56	105.26	1.50	107.15	1.29	4.94

(Table 2). For intraday precisions, the RSDs% of 1-propanol, iso-butanol, 1-butanol, 2-butanol, iso-amyl alcohol, and active amyl alcohol were 1.07, 1.39, 1.36, 0.81, 0.99, and 1.56%, respectively. For interday precisions RSDs% range from 1.09 to 2.44%. In AOAC, a satisfactory RSD is cited to be 3.7% if analyte concentration is 1,000 mg/L, 5.3% for 100 mg/L, and 7.3% for 10 mg/L (6). Recovery rates were also calculated to determine intraday and interday accuracies. Intraday recovery rates ranged from 98.22% (1-propanol) to 105.26% (active amyl alcohol), and interday rates from 98.53% (1-propanol) to 107.15% (active amyl alcohol). Both precision and accuracy were higher than recommendation. The limits of detection (LOD) and limits of quantitation (LOQ) are presented in Table 2 and their LODs and LOQs fell in the ranges of 1.29-2.58 mg/L and 4.94-8.65 mg/L, respectively. Previously the LOD of fusel oils in a distilled liquor called Raki was in the range of 2-5 mg/L for 1-propanol, 2-butanol, and iso-amyl alcohol (7), which are similar to our results.

Evaluation of recovery rates

Since various liquor types containing different ethanol concentrations are available, effect of the recovery rate in relation to the concentration of ethanol need to be investigated. Particularly, for the liquors with higher concentration of ethanol, these were diluted to around 20% for the analysis. The recovery rates of the six types of fusel oils with respect to ethanol concentration are presented in Table 3. Notably, the recovery rates for all fusel oils in the presence of 5, 10, and 20% ethanol were not significantly different ($p>0.05$).

The recovery rate range of the fusel oils in 5% ethanol was 99.45-104.68%, in 10% ethanol was 99.36-105.44%, and in 20% ethanol was 99.75-105.24%. RSDs (%) were in the range of 0.37-1.63%. In case of 2-butanol, the average recovery rate was 105.12% with a RSD of 0.37%, which was somewhat higher than that of other fusel oil components. These results show that recovery rates of fusel oils were unaffected by ethanol concentrations in the 5-20% range. Recovery rates were also investigated for the six types of fusel oil for different sample matrices. For this purpose, we chose *Takju*, *Yakju*, *Cheongju*, beer, obtained before and after spiking with fusel oil standard solution are presented in Table 4. fruit wine, wine, brandy, and whiskey. The recovery rates Recovery rates in brandy was 99.57% for active amyl alcohol, and 92.67% for 1-propanol. The recovery rates of the other liquor types ranged from 106.91% (iso-amyl alcohol in beer) to 93.55% (iso-amyl alcohol in *Takju*).

Monitoring of fusel oil in the liquors sold in Korea

The compositions of fusel oils in domestically available

Table 3. Recovery rate (%) and relative standard deviation (RSD%) of fusel oil solutions with different ethanol concentration

Fusel oils	Recovery rate (%)				
	5% Ethanol	10% Ethanol	20% Ethanol	Mean	RSD%
1-Propanol	101.09±2.93	101.94±1.99	99.75±0.70	100.93±1.11	1.10
Iso-butanol	102.15±0.72	100.77±2.36	102.34±0.20	101.75±0.86	0.85
1-Butanol	99.45±0.88	99.36±1.19	100.92±1.05	99.91±0.88	0.88
2-Butanol	104.68±1.43	105.44±0.14	105.24±0.85	105.12±0.39	0.37
Iso-amyl alcohol	104.43±3.95	102.22±1.39	101.36±0.22	102.67±1.59	1.54
Active amyl alcohol	101.73±3.95	99.50±1.51	102.72±0.44	101.32±1.65	1.63

Table 4. Recovery rate (%) of fusel oils from different types of liquor

Liquor	1-Propanol	Iso-butanol	1-Butanol	2-Butanol	Iso-amyl alcohol	Active amyl alcohol
Takju	102.68	102.33	106.70	102.58	93.55	105.37
Yakju	96.50	97.56	101.21	98.43	96.80	103.28
Cheongju	94.56	97.11	102.28	101.48	104.96	103.94
Beer	100.21	100.74	106.75	100.77	106.91	103.90
Fruit Wine	105.23	99.35	105.87	101.01	100.12	98.88
Wine	95.12	102.43	102.13	104.75	101.82	104.51
Brandy	92.67	98.36	99.21	98.80	97.56	99.57
Whiskey	93.59	93.75	101.18	99.82	95.06	100.97

Takju (72 types), *Yakju* (39 types), fruit wines (30 types), diluted *Soju* (10 types), distilled *Soju* (3 types), foreign whiskey (12 types), foreign brandy (9 types), domestic beers (39 types), and foreign beers (46 types) were analyzed (Table 5). The average of the content of total fusel oils (1-propanol, iso-butanol, 1-butanol, 2-butanol, iso-amyl alcohol, and active amyl alcohol) in *Takju* was 299.3 mg/L. Twenty-three types of sterilized *Takju* (average 265.9 mg/L) had lower fusel oil contents than 49 types of ordinary *Takju* (on an average 315.0 mg/L) (data not shown). Notably, iso-amyl alcohol (45.8-345.5 mg/L) and iso-butanol (ND-429.0 mg/L) were higher as compared to others. In the case of *Takju*, as rice fermentation progresses, iso-amyl alcohol content increased as compared with other alcohols, and it contributes a flavor to the *Takju* (8). The average of total fusel oil contents in *Yakju* (39 types) and fruit wines (30 types) were 497.6 and 151.9 mg/L, showing *Yakju* contains more fusel oils than *Takju* or fruit wines. In fruit wines, iso-amyl alcohol was the major fusel oil component (6.8-249.0 mg/L). Also, 1-propanol and iso-butanol were also present at maximum levels of 125.9 and 143.1 mg/L, respectively. In a previous report, iso-amyl alcohol content was found to be relatively high in *Bokbunja* wines (9).

Meanwhile, the averages of total fusel oil contents in domestic beers (39 types) and foreign beers (46 types) were similar at 121.1 mg/L and 114.8 mg/L, respectively, and iso-amyl alcohol was the major fusel oil component. Beers contained lower fusel oil levels than other liquor types. In a previous report, iso-butanol concentration in beer was 5.5-22 mg/L, and the propanol concentration was 8.7-23 mg/L (10). In another report (11), 2-methyl- and 3-methyl-l-butanol (active amyl and iso-amyl alcohol) contents in beers were in the range of 51-62 mg/L. Major fusel oil components were similar to that of the present study.

Meanwhile, the six types of fusel oil components were not detected at all in diluted *Soju*. However, the average of total fusel oil contents was high at concentration of 764.5 mg/L among three types of distilled *Soju* and iso-amyl alcohol content ranged from 114.2 to 421.0 mg/L. In case of the distilled liquors produced from sugarcane, fusel oil component concentrations, such as, those of n-propyl alcohol (1-propanol) and iso-amyl alcohol, were markedly dependent

Table 5. Concentration ranges of the content of fusel oils in liquors obtained from the market

(unit: mg/L)

Liquor	Number		1-Propanol	Iso-butanol	1-Butanol	2-Butanol	Iso-amyl alcohol	Active amyl alcohol	Average of total fusel oil contents
Takju	72	Min	24.1	ND[1]	ND	ND	45.8	13.0	299.3
		Max	106.8	429.0	9.4	ND	345.5	136.3	
Yakju	39	Min	19.1	26.3	ND	ND	90.9	23.4	497.6
		Max	239.0	273.9	13.4	2.5	302.8	120.2	
Domestic beer	39	Min	11.7	3.8	ND	ND	27.8	9.5	121.1
		Max	47.2	48.5	ND	ND	104.6	34.3	
Foreign beer	46	Min	13.3	9.5	ND	ND	33.3	12.3	114.8
		Max	28.5	42.3	5.8	ND	84.0	32.0	
Fruit wine	30	Min	ND	ND	ND	ND	6.8	ND	151.9
		Max	125.9	143.1	ND	ND	249.0	69.5	
Diluted *Soju*	10	Min	ND	ND	ND	ND	ND	ND	ND
		Max	ND	ND	ND	ND	ND	ND	
Distilled *Soju*	3	Min	51.6	79.2	ND	ND	114.2	40.6	764.5
		Max	197.2	391.9	11.8	ND	421.0	129.5	
Foreign brandy	9	Min	84.2	127.1	ND	ND	424.0	116.0	1447.3
		Max	240.6	609.1	37.8	13.0	1213.8	323.7	
Foreign whisky	12	Min	109.4	217.5	ND	ND	167.3	73.1	1137.9
		Max	350.1	637.2	21.6	ND	1026.3	492.6	

[1]ND, under limit of detection.
Analysis was performed in duplicate.

on liquor type (12), which is probably caused by different grain feedstocks and fermentation conditions. The averages of total fusel oil contents were very high in whiskey (1,137.9 mg/L) and brandy (1,447.3 mg/L), which to some extent might be associated with the unique flavors of these drinks during the aging (13). Notably, iso-amyl alcohol concentrations were much higher than those of other fusel oil components (brandy up to 1,213.8 mg/L, and whiskey up to 1,026.3 mg/L). In all liquor types examined, 1-butanol and 2-butanol contents were much lower than other fusel oil components.

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