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Determination of Ethanol in Low-Alcohol Beer by Headspace GC-FID : 2013 BCOJ Collaborative Work

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CONCLUSIONS

1. Relative repeatability standard deviation (RSD_r) and repeatability limit (r_{95}) for determination of ethanol content using the headspace GC-FID method ranged from 0.6 to 2.3% and from 0.0003 to 0.0209v/v%, respectively, and were judged acceptable.

2. Relative reproducibility standard deviation (RSDR) and reproducibility limit (R95) for determination of ethanol content using the headspace GC-FID method ranged from 1.5 to 4.3% and from 0.0006 to 0.0809v/v%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. It was concluded that the headspace GC-FID method is capable of determining ethanol content in low alcohol beer containing 0.005–1.0v/v% ethanol.

2. The subcommittee recommends that the Headspace GC-FID method be adopted for inclusion in *the Method of Analysis of BCOJ*.

3. Discharge the subcommittee.

ABSTRACT

Recently, there is an increased consumer's interest regarding low alcohol beer category in Japan. Japanese major brewing companies have launched some low alcohol beer brands labeled "alcohol 0.00v/v%". "Alcohol 0.00%" means that a concentration of alcohol is less than 0.005%. Therefore it is needed to develop an analytical method for accurate determination of 0.005% alcohol.

This subcommittee was charged with evaluating the headspace GC-FID method for analysis of ethanol in low alcohol beer.

PROCEDURE

Each sample or calibration standard solution of 10mL (final conc. 0.005, 0.01, 0.025, 0.05, 0.1v/v% of ethanol) was placed in auto sampler vial. If the ethanol concentration in the sample excess 0.1v/v%, the sample should be diluted to 10 times with distilled water. Internal standard solution of 0.1mL (10v/v% of 2-propanol) was added and capped the vial immediately. Then the samples were injected to headspace GC-FID systems. Each analysis was carried out in duplicate.

The GC method was modified from the analysis methods by the National Tax Agency Japan(4). GC analysis was performed under the following conditions (Table I).

Table I
Headspace GC-FID Condition

Headspace Sampler Condition
Oven Temp.; 60℃
Sample Loop Temp.; 110℃
Loop-fill Time; 0.03min
Loop-equilibrium Time; 0.20min
Transfer Line Temp.; 120℃
GC Cycle Time; 25min
Sample Vial Equilibrium Time; 20min
Injection Time; 0.50min
GC-Condition
Column; Agilent DB-1, 30 m * 0.53 mm ID, 3μm FT or equivalent
Carrier Gas; He, 6mL/min (constant flow)
Injection Temp.; 120 ° C
Split Ratio; 1:30
Colum Oven Temp.; 50° C (isothermal)
FID-Condition
Detection Zone Temp.; 250° C
Hydrogen Gas Flow; 30mL/min
Air Flow; 400mL/min
Mode; constant column + make up (30mL/min)
Make Up Gas; He or N2

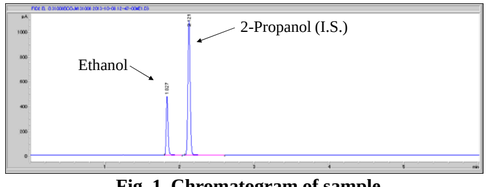

Fig. 1 Chromatogram of sample

The results were processed according to JIS Z 8401:1999 guidelines(2) and statistical analysis for the processed data was performed according to JIS Z 8402-2:1999 guidelines(3) and AOAC International Guidelines(1).

RESULTS AND DISCUSSION

The results for ethanol content are shown in Table II. All of the samples were checked for outliers using Mandel's *h* and *k* statistics, and Cochran and Grubbs outlier test, and outliers were excluded from the statistical analysis (1), (3). The statistical summary of results is shown in Table III.

Each of the calculated analytical values ranged as follows: RSDr ranged from 0.6 to 2.3%; r95 ranged from 0.0003 to 0.0209v/v%, respectively; RSDR ranged from 1.5 to 4.3%; and R95 ranged from 0.0006 to 0.0809v/v%, respectively, and were judged acceptable.

It was concluded that the method is capable of determining ethanol in low alcohol beer containing 0.005–1.0v/v% of ethanol. The subcommittee recommends that the method should be adopted for inclusion in *the Methods of Analysis of BCOJ*.

Table II
Ethanol Content (v/v%) Determined Using the Headspace GC-FID Method

Collaborator	Sample Pair A	B	Sample Pair C	D	Sample Pair E	F	Sample Pair G	H	Sample Pair I	J
1	0.0050	0.0050	0.0203	0.0203	0.4349	0.4230	0.3513	0.3672	0.7137	0.7211
2	0.0049	0.0047	0.0200	0.0201	0.4320	0.4297	0.3569	0.3561	0.7363	0.7311
3	0.0045	0.0047	0.0198	0.0201	0.4531	0.4368	0.3747	0.3669	0.7562	0.7488
4	0.0048	0.0047	0.0204	0.0203	0.4364	0.4463	0.3662	0.3763	0.7749	0.7819
5	0.0054	0.0051	0.0205	0.0207	0.4374	0.4382	0.3635	0.3671	0.7570	0.7578
6	0.0050	0.0050	0.0203	0.0204	0.4268	0.4272	0.3558	0.3706	0.7421	0.7250
7	0.0049	0.0049	0.0202	0.0203	0.4323	0.4347	0.3645	0.3621	0.7196	0.7258
8	0.0051	0.0051	0.0207	0.0207	0.4337[a]	0.4633[a]	0.3732	0.3674	0.7720[a]	0.7124[a]
9	0.0047	0.0049	0.0196	0.0199	0.4540	0.4488	0.3745	0.3770	0.8091	0.7897
Mean	0.0049	0.0049	0.0202	0.0203	0.4384	0.4356	0.3645	0.3679	0.7511	0.7477
Grand mean	0.0049		0.0203		0.4370		0.3662		0.7494	

a Outliers identified by outlier tests and excluded from the statistical analysis.

Table III
Statistical Summary of Results of the Headspace GC-FID Method

	Sample Pair A/B	Sample Pair C/D	Sample Pair E/F	Sample Pair G/H	Sample Pair I/J
Number of Laboratories	9	9	8	9	8
Grand mean (m)	0.0049	0.0203	0.4370	0.3662	0.7494
Repeatability Standard Deviation (S_r)	0.0001	0.0001	0.0058	0.0062	0.0075
Relative Repeatability Standard Deviation (RSD_r%)	2.3	0.6	1.3	1.7	1.0
Repeatability Limit (r_{95})	0.0003	0.0003	0.0163	0.0174	0.0209
Predicted Relative Repeatability Standard Deviation ($PRSD_r$%)	6.2	5.0	3.1	3.2	2.9
$HorRat_r$ ($RSD_r/PRSD_r$) [a]	0.3[c]	0.1[c]	0.4[c]	0.5[c]	0.3[c]
Reproducibility Standard Deviation (S_R)	0.0002	0.0003	0.0094	0.0077	0.0289
Relative Reproducibility Standard Deviation (RSD_R%)	4.3	1.5	2.2	2.1	3.9
Reproducibility Limit (R_{95})	0.0006	0.0009	0.0264	0.0215	0.0809
Predicted Relative Reproducibility Standard Deviation ($PRSD_R$,%)	9.2	7.5	4.7	4.8	4.3
$HorRat_R$ ($RSD_R/PRSD_R$) [a]	0.5[c]	0.2[c]	0.5[c]	0.4[c]	0.9[b]

a HorRat values were calculated from w/w%* of each ethanol concentration.
*w/w% data not shown
b According to AOAC International Guidelines, HorRat values should be more than 0.5 and less than or equal to 2.0 (1).
c Accurate results although the HorRat values were under 0.5.

LITERATURE CITED

1. AOACI. Guidelines for collaborative study procedures to validate characteristics of a method of analysis. *Official Methods of Analysis of AOAC International* (Appendix D), 18th ed., The Association, Gaithersburg, MD, 2005.
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