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Application News

No. AD-0166

Beer Analysis / HS GC-FID

Quantitative Analysis of Flavor in Beer by Static Headspace GC-FID

❑ Introduction

Beer is made of grains by the fermentation process. The flavor of beer is an important quality, which is affected by wort composition, fermentation conditions, yeast strain etc. [1]. To achieve desirable and consistent flavors in production, monitoring of chemical compositions of wort and finished products is essential. The analysis method using gas chromatography (GC) coupled with static headspace (HS) described is to quantify seven flavor compounds which are commonly contained in beer, i.e. acetaldehyde, n-propanol, ethyl acetate, isobutanol, isoamyl alcohol, 2-methyl-1-butanol, and isoamyl acetate. The results demonstrate the applicability of HS-GC-FID system and the method in characterization of beer flavor through quantitative analysis of targeted flavor compounds.

❑ Experimental

Materials and Analytical Conditions

The analysis was carried out on GC-FID coupled with headspace autosampler. The separation of the flavor compounds and internal standard (IS) was done with SH-Rtx-1 column. The Labsolutions workstation was utilized for data acquisition and processing. The detailed analysis conditions are shown in Table 1 (A & B). Mixture of flavor compounds, i.e. acetaldehyde, n-propanol, ethyl acetate, isobutanol, isoamyl alcohol, 2-methyl-1-butanol and isoamyl acetate was prepared and spiked with n-butanol (IS).

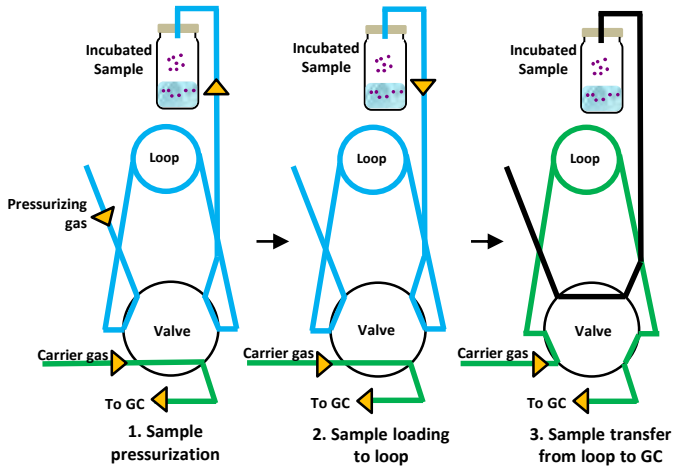

Figure 1: Static headspace technique

Headspace and GC Operation

Each calibration mixed standard was added to a headspace vial and capped immediately. The static headspace technique allowed the volatile flavor compounds to be extracted into vapor by heating the beer sample in the gas-tight vial under a controlled temperature (see Table 1A). The extracted flavor vapor was eventually transferred to a sample loop inside the headspace autosampler, after which the GC carrier gas was allowed to carry the sample vapor from the loop to GC for subsequent analysis. Refer to Figure 1 for static headspace technique.

Table 1(A): Static headspace (HS) analysis condition

Incubation temp	40 °C
Equilibrating time	10 min
Sample line temp	150 °C
Transfer line temp	150 °C
Shaking level	Off
Pressurizing time	1 min (equilibrium time: 0.1 min)
Load time	0.5 min (equilibrium time: 0.1 min)
Injection time	1 min
Needle flush time	1 min
Pressurizing Pressure	60 kPa

Table 1(B): GC analysis condition

Injection mode	Split
Split Ratio	5
Carrier gas	He
Flow control mode	Linear velocity
Linear velocity	50 cm/s
Column	SH-Rtx-1 (30 m L, 0.32 mm ID, 5 μm df)
Column temp program	40°C (3 min) → rate: 4°C/min → 90°C → rate 20°C/min → 180°C (2 min)
Equilibration time	1 min
Detector	FID
Detector temp	200 °C

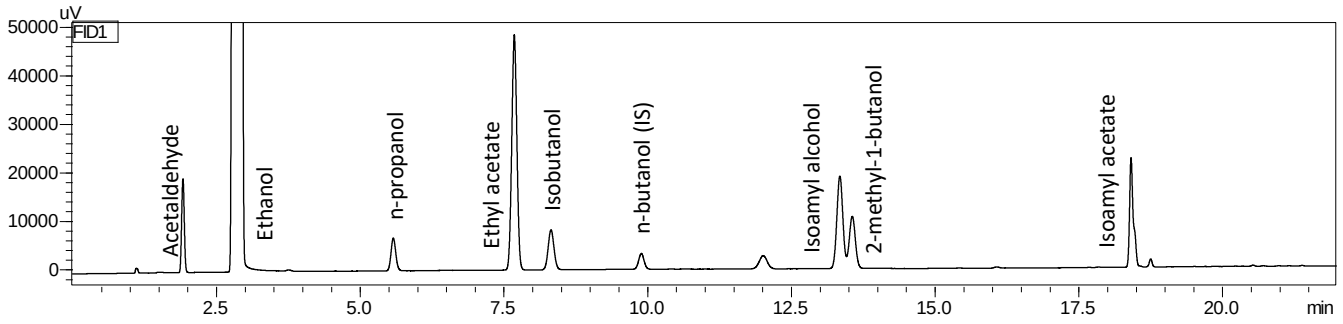

Figure 2: Chromatogram of mixed standard of seven flavor compounds and IS

❑ Results and Discussion

GC chromatogram: As shown in Figure 2, the seven flavor compounds and the internal standard (n-butanol) were separated by SH-Rtx-1 column (30 m length, 0.32 mm ID, 5 µm df) using the temperature program described in Table 1(A) within 20 minutes.

Calibration Curves and Linearity: Three concentration levels were prepared in 4% ethanol for internal standard calibration curves (Table 2). The calibration curves of all compounds with six replicates for each level and their linearity are shown in Figure 3. Excellent linearity of (R^2 > 0.998) was obtained for all calibration curves.

Table 2: Calibration standards concentrations

Compound	Std 1 (mg/L)	Std 2 (mg/L)	Std 3 (mg/L)
Acetaldehyde	1	10	20
n-propanol	2.5	25	50
Ethyl acetate	2	20	40
Isobutanol	2	20	40
Isoamyl alcohol	5	50	100
2-methyl-1-butanol	2.5	25	50
Isoamyl acetate	0.25	2.5	5
n-butanol (IS)	20	20	20

Table 3: Repeatability of the method (n=6)

Compound	Area Ratio RSD* (%)
Acetaldehyde	6.780
n-propanol	5.721
Ethyl acetate	6.818
Isobutanol	2.612
Isoamyl alcohol	3.192
2-methyl-1-butanol	2.646
Isoamyl acetate	5.869

**Calculated from the lowest calibration level*

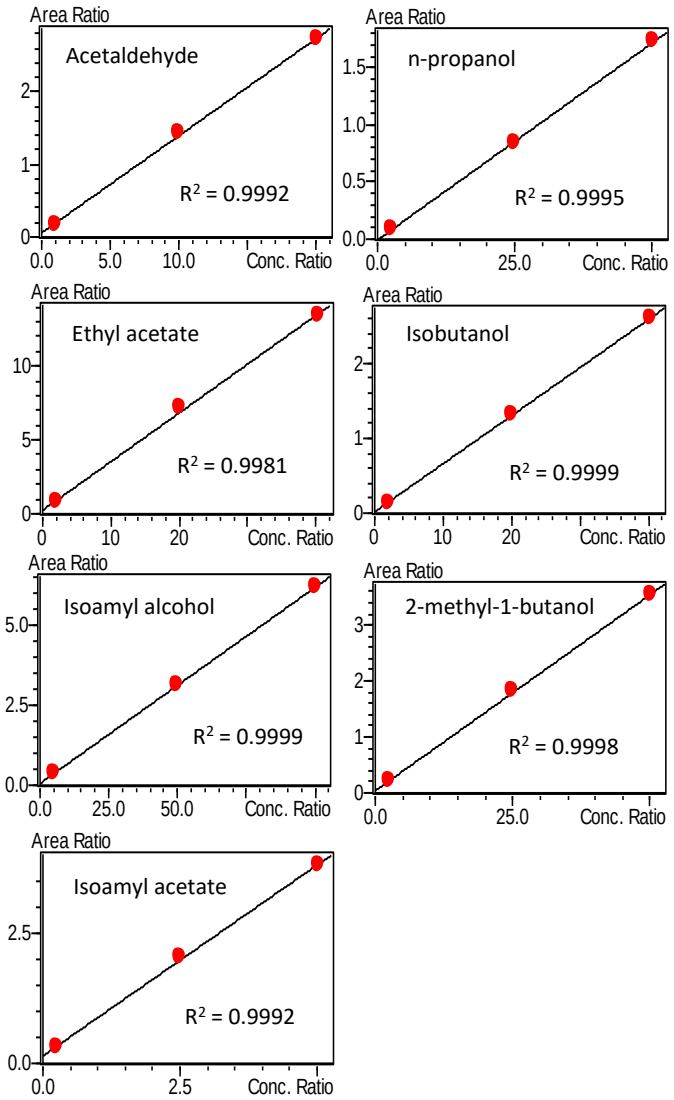

Figure 3: Internal standard calibration curves of seven beer flavor compounds and their linearity

Repeatability: Repeatability of the HS-GC-FID method was determined by calculating the %RSD of peak area ratio (n=6). The repeatability of each compound in the lowest calibration standard was less than 7% (refer to Table 3).

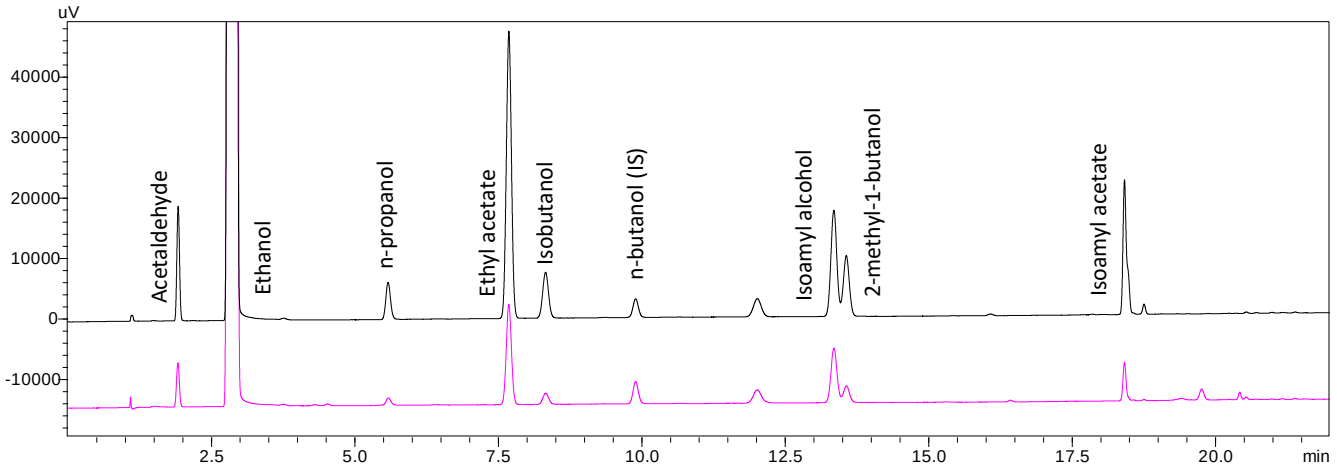

Figure 4: Chromatograms of beer flavor standard (top) and Beer E sample (bottom)

Results of beer samples: Five beer samples labelled as Beer A, B, C, D and E were analyzed using the method established. The same concentration of internal standard (20 mg/L) was spiked into each sample before analysis. Figure 4 shows the chromatogram of Beer E overlaid with a mixed standard.

Quantitation results of the seven flavor compounds in five beer samples are displayed in Table 4 and Figure 5. The beer samples tested in this study were found to contain all the seven flavor compounds with comparable content levels, but their distributions varying from brand to brand.

Table 4: Quantitation results of beer samples

Flavor Compound	Concentration in Beer Samples (mg/L)				
	Beer A	Beer B	Beer C	Beer D	Beer E
Acetaldehyde	1.25	3.79	5.10	2.27	7.55
n-propanol	7.27	10.65	7.41	8.62	9.00
Ethyl acetate	11.05	19.96	14.53	10.06	12.58
Isobutanol	9.00	10.04	6.51	10.69	8.31
Isoamyl alcohol	43.03	50.18	36.19	42.72	44.67
2-methyl-1-butanol	13.15	13.48	9.54	12.09	11.6
Isoamyl acetate	1.57	2.45	1.22	1.29	1.22
Total (mg/L)	86.3	110.6	80.5	87.7	94.9

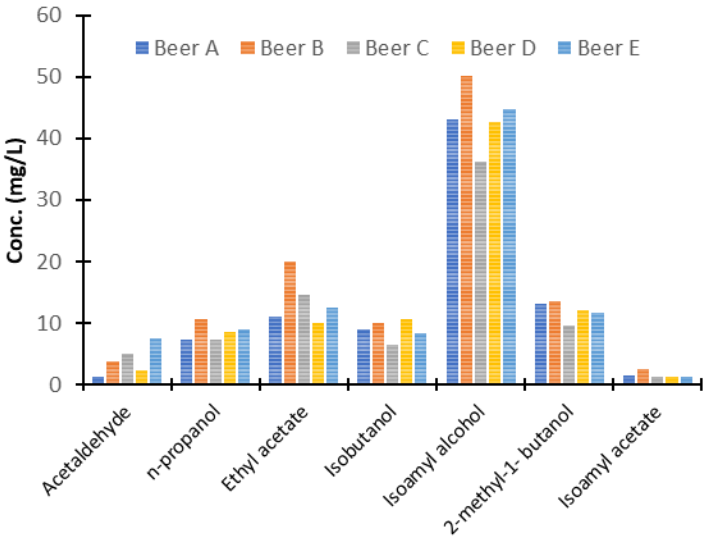

Figure 5: Comparison of flavor compounds in beer samples

❑ Conclusions

A static headspace-GC-FID method was developed for analysis of seven flavor compounds in beer. This simple and fast method could be used for quantitative monitoring of flavor compounds in bear production for quality control (QC) and flavor assessment.

❑ References

1. He, Y., Wort composition and its impact on the flavour-active higher alcohol and ester formation of beer – a review, Journal of the Institute of Brewing, Vol 120, Issue 3, 2014, pp. 157-163.

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Disclaimer: The Shimadzu GC-FID and Headspace system and the data in this Application News are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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