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Aroma components analysis of sake using internal standard by ball SAW gas chromatograph

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1. Introduction

There are various needs for on-site analysis of many types of volatile organic compounds (VOCs). For example, there is a demand for on-site analysis of changes in aroma components in the production of alcoholic beverages and products in the cultivation and development of yeast. [1)] Gas chromatograph (GC) is effective for analyzing a wide variety of gases, but GC is generally large and require many utilities, making it difficult to apply it on-site.

In contrast, we have developed an ultra-compact ball SAW GC by applying a ball surface acoustic waves (SAW) sensor that uses SAW making multiple turns on the surface of a spherical element. [2,3)] In this report, concentration measurement using an internal standard was investigated as a method for analyzing the aroma components of sake using a ball SAW GC.

2. experimental method

Fig. 1 shows the ball SAW GC (Sylph SY-402) used this time. It is equipped with a gas supply hydrogen canister, pressure reducer, concentrator, gas separation column, ball SAW sensor, valve manifold, and drive circuit, and can be connected to a PC via USB for analysis. The column used was a 30 m metal capillary Ultra-Alloy® wax column coated with polyethylene glycol (PEG) as the stationary phase wound in a solenoid shape.

We referred to the analysis method prescribed by the National Tax Agency for the aroma components of sake. Using a 20 mL vial bottle, 1 mL of the internal standard solution and 9 mL of the sample were mixed to prepare a 10 mL solution, and the 10 mL headspace was concentrated to 5 mL by the dynamic method. **Table 1** summarizes the analysis method prescribed by the National Tax Agency[1)] and the proposed analysis method.

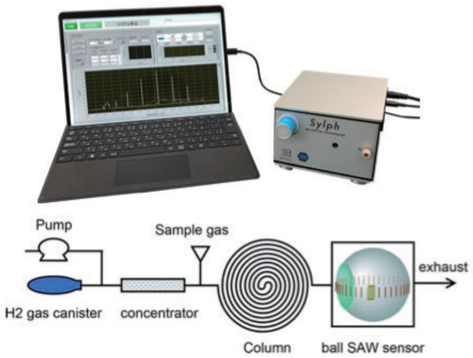

Fig.1 Ball SAW GC

For the test procedure, put 9 mL of the five types

Table 1 Analysis method

		Specified analytical method	Sylph On-site analysis
GC		GC-FID	Ball SAW GC
Vial		10 mL	20 mL
Sample	Specimen	0.9 mL	9 mL
	Internal STD	0.1 mL	1 mL
Collection condition	Headspace method	Static HS	Dynamic HS
	Volume	1 mL	5 mL
	Temperature	50 °C	20 °C

of aroma component standard solutions and 1 mL of the internal standard solution in a 20-mL glass vial, stopper tightly, and mix. After 30 minutes at 20°C, inject 5 mL into the Sylph by the dynamic headspace method. The area ratio (R) is calculated by the following formula from the peak areas of the obtained aroma components and the internal standard.

$$\text{Area ratio}(R) = \frac{\text{peak areas of the aroma components}}{\text{peak areas of the internal standard.}}$$

The internal standard for isoamyl alcohol was normal amyl alcohol, ethyl acetate, isoamyl acetate, and methyl caproate for ethyl caproate. **Table 2** shows the aroma component standard solution and the internal standard solution.

Obtain the area ratio for each concentration of aroma component standard solution, create a calibration curve between each concentration of

Table 2 Aroma component standard solution and internal standard solution

Aroma component standard solution

	ST0 (mg/L)	ST1 (mg/L)	ST2 (mg/L)	ST3 (mg/L)	ST4 (mg/L)
Ethyl acetate	0	37.5	75	112.5	150
Propanol	0	25	50	75	100
Isobutanol	0	37.5	75	112.5	150
Isoamyl alcohol	0	75	150	225	300
Isoamyl acetate	0	2.5	5	7.5	10
Ethyl caproate	0	3.75	7.5	11.25	15

Internal standard solution

	(mg/L)
Methyl caproate	50
Normal amyl alcohol	2000

aroma component and the area ratio, then treat 9 mL of sample in the same manner to calculate the area ratio. Then, the amount of each aroma component in the specimen is determined using the calibration curve.

3. Experimental results and discussion

Fig. 2 shows the chromatogram of STD1. where 1 is air, 2 is Ethyl acetate, 3 is Ethyl alcohol, 4 is Propanol, 5 is Water, 6 is Isobutanol, 7 is Isoamyl alcohol, 8 is Isoamyl acetate, and 9 is Ethyl caproate. In addition, i1 is the internal standard Methyl caproate, and i2 is Normal amyl alcohol. It was observed that each peak was clearly separated.

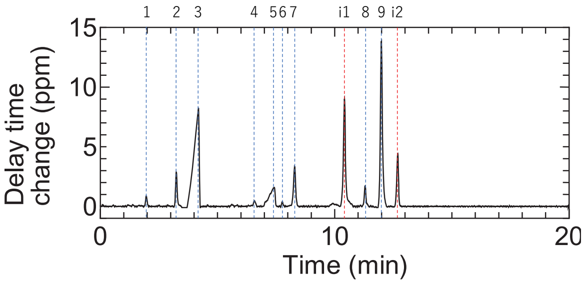

Fig. 2 Chromatogram of STD1

Next, **Fig. 3** shows the calibration curve of Ethyl acetate obtained by using the aroma component standard solution. In this experiment, when the sample vial was connected to the Sylph, the lid was replaced with a stopper for collection, so the calibration curve without the internal standard deviated from the straight line and had large scatter (R2 = 0.989). On the other hand, the calibration curve normalized by the internal standard had an R2 of 0.998. As a result, it was confirmed that the use of the internal standard improved the analytical accuracy.

Fig. 4 shows the chromatograms of four types of commercially available sake, Honjozo-shu, Junmai-

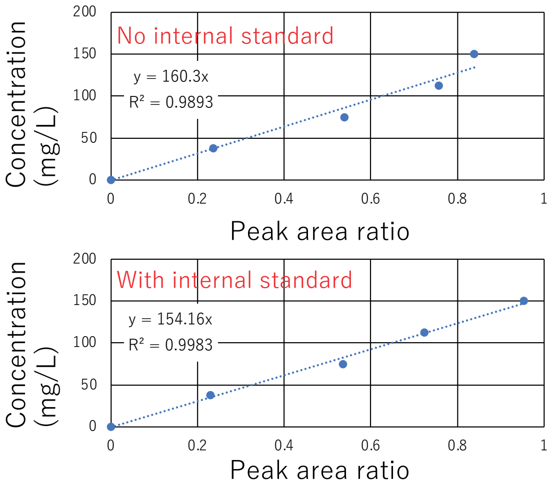

Fig. 3 Ethyl acetate calibration curve

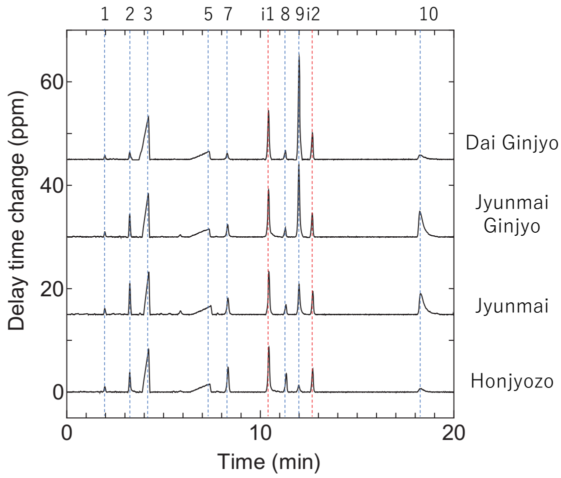

Fig. 4 Chromatogram of commercially available sake

shu, Junmai ginjo-shu, and Dai ginjo-shu. Here 10 was Ethyl caprylate.

Table 3 shows the concentrations of ethyl acetate, isoamyl acetate, isoamyl alcohol, and ethyl caproate in each sake obtained from the calibration curve.

The aromatic components of Junmai ginjo-shu are 47 mg/L for Ethyl acetate, 2.1 mg/L for Isoamyl acetate, 110 mg/L for Isoamyl alcohol, and 3.7 mg/L for Ethyl caproate. It was also rich in Ethyl caprylate, known for its apricot aroma, as well as isoamyl acetate, which characterizes the aroma of Honjozo-shu.

Table. 3 Aroma component concentration of sake

	Honjyozo (mg/L)	Jyunmai (mg/L)	Jyunmai Ginjyo (mg/L)	Dai Ginjyo (mg/L)
Ethyl acetate	53.4	83.4	47.3	17.2
isoamyl acetate	3.7	3.4	2.1	1.1
isoamyl alcohol	222	117	110	89
ethyl caproate	0.4	2.1	3.7	5.5

4. Conclusion

The method using the ultra-compact ball SAW GC presented in this report enables quantitative analysis of the aroma components of sake at the manufacturing site. As a result, it is expected that not only quantitative quality control in the sake brewing process, but also comparison of brewing culture and changes in people's tastes can be grasped over time.

Referents

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2) K. Yamanaka, H. Cho, and Y. Tsukahara: Appl. Phys. Lett. 76, 2729 (2000).
3) T. Iwaya , et al., Jpn. J. Appl. Phys., 61, SG1051 (2022).