Analysis of aroma components in Japanese sake by HS-SPME-GC-TOFMS

Product used: Mass Spectrometer (MS)

Introduction

Japanese sake is an alcoholic drink brewed from rice, koji, and water with Japanese traditional manufacturing method. The taste and aroma of sake depend on the raw materials and manufacturing method. For example, honjozo-sake has a mild flavor, and ginjo-sake has a fragrant and fruity flavor. In this MSTips, we measured and compared the aroma components in sake samples. A HeadSpace-Solid Phase MicroExtraction-Gas Chromatography-Time-Of-Flight Mass Spectrometry (HS-SPME-GC-TOFMS) was used for measurement. In HS-SPME, a sample is sealed in a headspace vial, and the SPME fiber is exposed to its gas phase to adsorb volatile components. Highly sensitive analysis is possible by easily extracting and concentrating volatile components. In addition to this HS-SPME, the GC pretreatment autosampler HT2850T (HTA S.R.L.) can also handle liquid injection and HS-gastight syringe injection by replacing the syringe attachment. JMS-T2000GC and msFineAnalysis AI, which are effective for compounds not registered in NIST database, were used as GC-TOFMS and analysis software.

Experiment

Commercially available honjozo-sake and ginjo-sake were used as samples. 10 mL of each was sealed in a 20 mL headspace vial (Figure 1). HS-SPME extraction was performed at 40℃ for 30 minutes using HT2850T, and EI/FI measurement was performed using JMS-T2000GC (Figure 2). EI measurements were repeated three times for each sample, and qualitative analysis and statistical difference analysis were performed using msFineAnalysis AI. Table 1 shows detail of measurement conditions.

Figure 1 Sake samples

Figure 2 JMS-T2000GC with HT2850T autosampler

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Table 1 Measurement conditions

HS-SPME conditions			
Auto-sampler	HT2850T (HTA S.R.L.)	SPME fiber	DVB/CAR/PDMS 2cm (MERCK)
Sample	10mL sake in 20mL headspace vial	Extraction	40 °C 30min
Mode	HS-SPME	Desorption	250 °C 2min

GC conditions		**MS conditions**	
Gas Chromatograph	8890 GC (Agilent Technologies)	Spectrometer	JMS-T2000GC (JEOL Ltd.)
Column	DB-WAXETR 30m x 0.25mm, 0.25µm (Agilent Technologies)	Ion source	EI/FI combination
Injection mode	Splitless	Ionization	EI(70eV), FI
Inlet temperature	250 °C	Ion source temperature	230 °C (EI)
Oven temperature	40°C(2min) - 10°C/min - 250°C(10min)	Mass range	*m/z* 10-800
Carrier flow	He, 1.0mL/min	Analysis software	msFineAnalysis AI

Results

Figure 3 shows the TIC chromatograms of honjozo-sake and ginjo-sake. In honjozo-sake, ethanol, isoamyl acetate (ginjo aroma, melon-like), and phenylethyl alcohol (basic sake aroma, rose-like) were detected. In ginjo-sake, ethyl caproate (ginjo aroma, apple-like) was strongly detected.

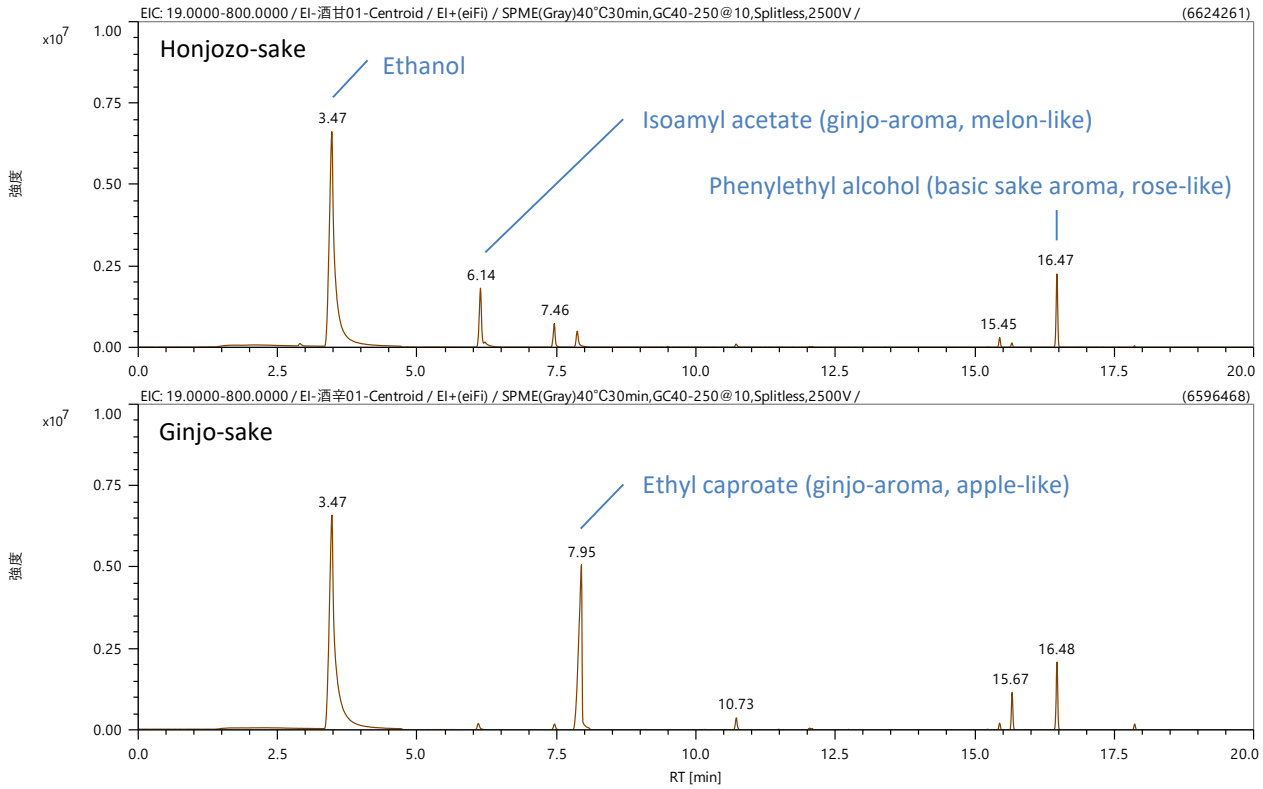

Figure 3 TIC chromatograms of honjozo-sake and ginjo-sake

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Figure 4 shows the results of a difference analysis between honjozo-sake and ginjo-sake using msFineAnalysis AI. In 22 peaks with an intensity ratio of up to 0.1% to the maximum peak, 5 peaks were strongly detected in honjozo-sake, and 11 peaks were strongly detected in ginjo-sake. From the volcano plot obtained by statistical difference analysis, it was possible to visually confirm the characteristic components of each sample.

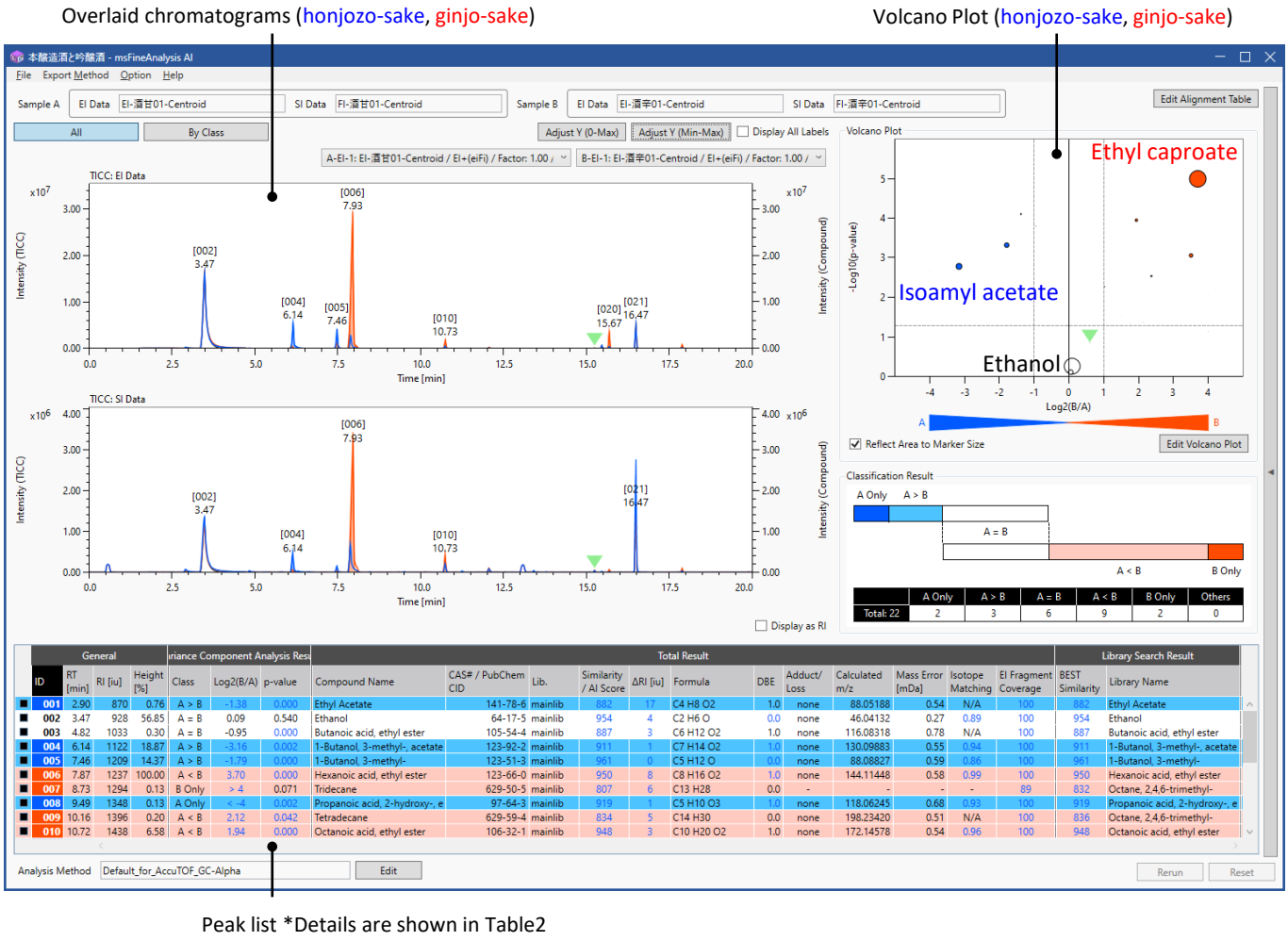

Figure 4 Difference analysis result between honjozo-sake and ginjo-sake

Table 2 shows the peak list. The background color reflects the intensity differences between samples. Although ID018 was compound not registered in NIST database, its compound name and structural formula were able to obtain by AI structural analysis.

Table 2 Peak list of difference analysis result

(blue : strong in honjozo-sake, red : strong in ginjo-sake, white : almost same intensity)

General				Variance Component Analysis Result			Total Result											Library Search Result	
ID	RT [min]	RI [iu]	Height [%]	Class	Log2(B/A)	p-value	Compound Name	CAS# / PubChem CID	Lib.	Similarity / AI Score	ΔRI [iu]	Formula	DBE	Adduct/ Loss	Calculated m/z	Mass Error [mDa]	Isotope Matching	EI Fragment Coverage	BEST Similarity	Library Name
001	2.90	870	0.76	A > B	-1.38	0.000	Ethyl Acetate	141-78-6	mainlib	882	17	C4 H8 O2	1.0	none	88.05188	0.54	N/A	100	882	Ethyl Acetate
002	3.47	928	56.85	A = B	0.09	0.540	Ethanol	64-17-5	mainlib	954	4	C2 H6 O	0.0	none	46.04132	0.27	0.89	100	954	Ethanol
003	4.82	1033	0.30	A = B	-0.95	0.000	Butanoic acid, ethyl ester	105-54-4	mainlib	887	3	C6 H12 O2	1.0	none	116.08318	0.78	N/A	100	887	Butanoic acid, ethyl ester
004	6.14	1122	18.87	A > B	-3.16	0.002	1-Butanol, 3-methyl-, acetate	123-92-2	mainlib	911	1	C7 H14 O2	1.0	none	130.09883	0.55	0.94	100	911	1-Butanol, 3-methyl-, acetate
005	7.46	1209	14.37	A > B	-1.79	0.000	1-Butanol, 3-methyl-	123-51-3	mainlib	961	0	C5 H12 O	0.0	none	88.08827	0.59	0.86	100	961	1-Butanol, 3-methyl-
006	7.87	1237	100.00	A < B	3.70	0.000	Hexanoic acid, ethyl ester	123-66-0	mainlib	950	8	C8 H16 O2	1.0	none	144.11448	0.58	0.99	100	950	Hexanoic acid, ethyl ester
007	8.73	1294	0.13	B Only	> 4	0.071	Tridecane	629-50-5	mainlib	807	6	C13 H28	0.0	-	-	-	-	89	832	Octane, 2,4,6-trimethyl-
008	9.49	1348	0.13	A Only	< -4	0.002	Propanoic acid, 2-hydroxy-, e	97-64-3	mainlib	919	1	C5 H10 O3	1.0	none	118.06245	0.68	0.93	100	919	Propanoic acid, 2-hydroxy-, e
009	10.16	1396	0.20	A < B	2.12	0.042	Tetradecane	629-59-4	mainlib	834	5	C14 H30	0.0	none	198.23420	0.51	N/A	100	836	Octane, 2,4,6-trimethyl-
010	10.72	1438	6.58	A < B	1.94	0.000	Octanoic acid, ethyl ester	106-32-1	mainlib	948	3	C10 H20 O2	1.0	none	172.14578	0.54	0.96	100	948	Octanoic acid, ethyl ester
011	11.23	1476	0.10	A Only	< -4	0.006	Furfural	98-01-1	mainlib	907	16	C5 H4 O2	4.0	none	96.02058	0.61	0.80	100		
012	11.49	1495	0.26	A < B	1.77	0.045	Pentadecane	629-62-9	mainlib	865	5	C15 H32	0.0	none	212.24985	0.93	N/A	100		
013	12.05	1540	0.99	A < B	1.02	0.005	Benzaldehyde	100-52-7	mainlib	922	19	C7 H6 O	5.0	none	106.04132	0.49	0.93	100		
014	12.09	1543	0.21	B Only	> 4	0.009	2,3-Butanediol, [S-(R*,R*)]-	19132-06-0	mainlib	931	22	C4 H10 O2	0.0	none	90.06753	0.43	0.90	100		
015	12.74	1595	0.20	A < B	1.50	0.030	Hexadecane	544-76-3	mainlib	808	5	C16 H34	0.0	none	226.26550	0.59	N/A	90		
016	13.39	1649	0.11	A = B	-0.44	0.237	Butyrolactone	96-48-0	mainlib	838	17	C4 H6 O2	2.0	none	86.03623	0.48	0.93	100		
017	13.93	1694	0.13	A < B	1.45	0.032	Heptadecane	629-78-7	mainlib	756	6	C17 H36	0.0	none	240.28115	1.72	N/A	100		
018	15.23	1808	0.36	A = B	0.60	0.138	ethyl 4-hydroxybutanoate	357772	AI	831	-	C6 H12 O3	1.0	none	132.07810	0.52	0.92	100		
019	15.45	1828	2.59	A = B	-0.58	0.010	Acetic acid, 2-phenylethyl ester	103-45-7	mainlib	949	15	C10 H12 O2	5.0	none	164.08318	1.55	0.91	100		
020	15.67	1848	12.95	A < B	3.51	0.001	Hexanoic acid	142-62-1	mainlib	924	2	C6 H12 O2	1.0	none	116.08318	0.32	0.59	100		
021	16.47	1923	18.82	A = B	0.05	0.762	Phenylethyl Alcohol	60-12-8	mainlib	964	16	C8 H10 O	4.0	none	122.07262	0.58	0.96	100		
022	17.87	2059	3.00	A < B	2.37	0.003	Octanoic acid	124-07-2	mainlib	972	0	C8 H16 O2	1.0	none	144.11448	0.34	0.92	100		

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Figure 5 shows the candidate structural formula of ID018 obtained by AI structural analysis. Ethyl 4-hydroxybutanoate, which has a caramel-like aroma, was obtained as a top candidate (2nd /1155 candidates).

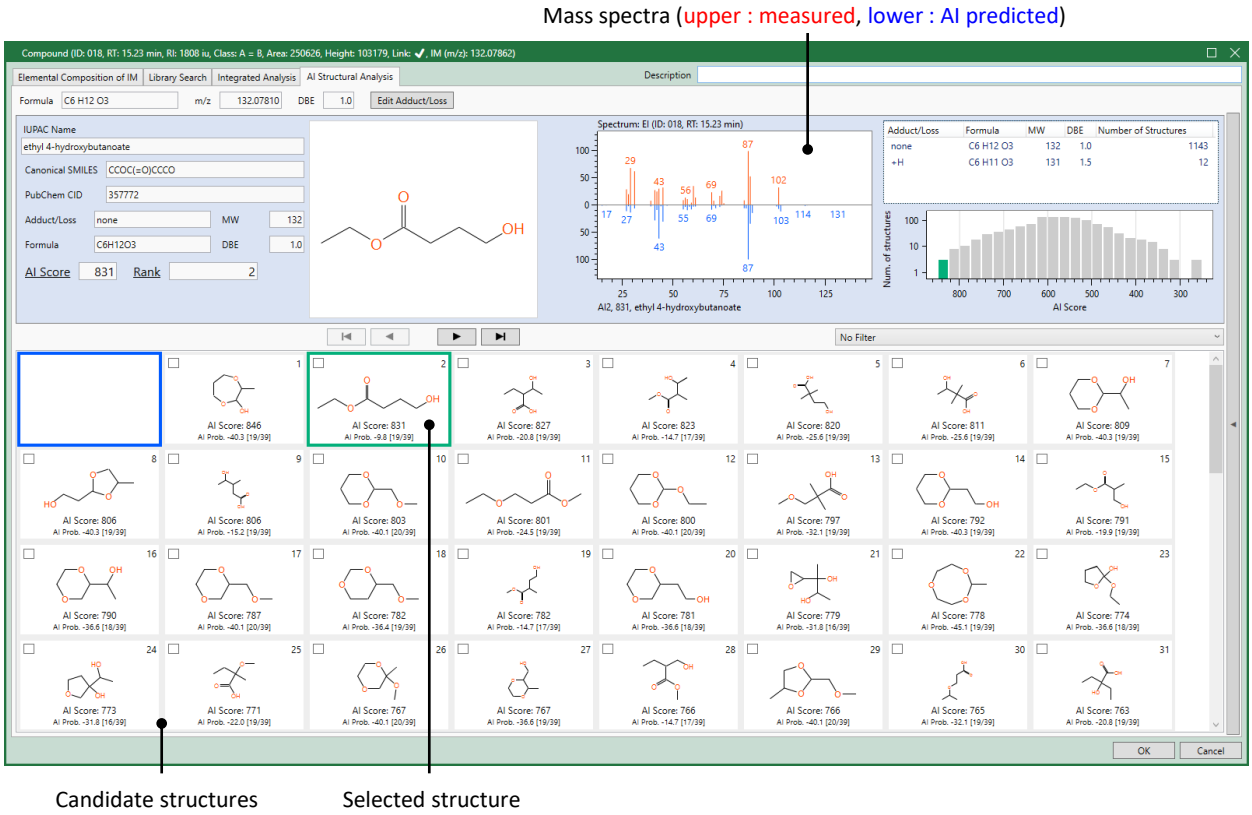

Figure 5 AI structure analysis result of peak ID018

Conclusion

It was able to detect aroma components in sake with high sensitivity by HS-SPME-GC-TOFMS using HT2850T and JMS-T20000GC. And it was able to visually confirm the characteristic components of each sample by volcano plot using msFineAnalysis AI. Although some components were not registered in NIST database, it was able to obtain the compound name and structural formula by AI structural analysis. So, it was confirmed that these devices and software are effective for analyzing aroma components in foods.

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