Differential Analysis of Chocolates with Different Cacao Content by using a HS-SPME-GC-QMS and msFineAnalysis iQ

Product used: Mass Spectrometer (MS)

Introduction

Food flavor components are known to be an important element of good taste, and off-flavor components, such as rancid odors, are also an important element of food quality. Gas chromatography-mass spectrometry (GC-MS) is often used to analyze these food flavor components due to the high volatility of flavor components and the complex composition of many components.

Qualitative analysis by GC-QMS is generally performed by searching the library database (DB) using the measurement data of the Electron Ionization (EI). However, if qualitative analysis is performed using only the similarity with the library spectrum as an index, several significant candidates may be obtained depending on the compound, or erroneous candidates may be selected as the identification result. In such cases, confirmation of molecular ions by the soft ionization (SI) such as the photoionization (PI) is effective. In 2021, we released msFineAnalysis iQ, an integrated qualitative analysis software that automatically combines the analysis results of EI and SI methods measured by GC-QMS. The details of this software are introduced in MSTips No. 347 and 348. In this MSTips, we will introduce an analysis example of differential analysis of chocolates with different cacao content using msFineAnalysis iQ.

Experimental

Two types of chocolates (approximately 70% and 95% cacao content) were used as samples, and the sample weight was 5 g. A GC-QMS (JMS-Q1600GC UltraQuad™ SQ-Zeta, JEOL Ltd.) was used for the measurement. The SPME mode of the HT2850T autosampler (HTA S.R.L.) was used as the sample pretreatment device, and volatile components in the headspace area of the vials were targeted for the measurement. We performed HS-SPME-GC-QMS measurements using both EI and photoionization (PI) modes with a combination EI/PI ion source. The qualitative data processing was performed with msFineAnalysis iQ (JEOL Ltd.). Detailed measurement conditions are shown in Table 1.

JMS-Q1600GC UltraQuad™ SQ-Zeta

HT2850T

Table 1 Measurement condition

SPME	
SPME Fiber	DVB/CAR/PDMS 2mm (Merck)
Sample amount	5 g
Extraction temp.	60 °C
Extraction time	30 min
Desorption time	3 min

GC	
Column	ZB-WAX (Phenomenex) 30 m×0.25 mm I.D., df=0.25 µm
Inlet	300℃, EI=Split 20:1, PI=Splitless
Oven	40℃ (2 min) →10℃/min→250℃/min (1min)
Carrier flow	He, 1.0 mL/min (Constant Flow)

MS	
Ion Source	EI/PI combination ion source
Ionization mode	EI+ (70 eV, 50 µA), PI+ (D2 lamp, 8~10 eV)
Mass range	*m/z* 33-500 (Scan mode)

Results and Discussion

TICC and differential analysis result

Figure 1 shows TICC of EI data and Figure 2 shows volcano plot of differential analysis result (Blue: 70% cacao, Red: 95% cacao). Volcano plots made it possible to visualize common and different components in each sample. We were able to extract 11 components characteristic of 70% cacao, 19 components characteristic of 95% cacao, and 21 common components. Compounds known as chocolate flavor such as Acetic acid , Butanoic acid, 3-methyl- and Vanillin ware detected as the common components of each sample (Figure 1). The results of the integrated qualitative analysis of the characteristic components of each sample are described in the next section.

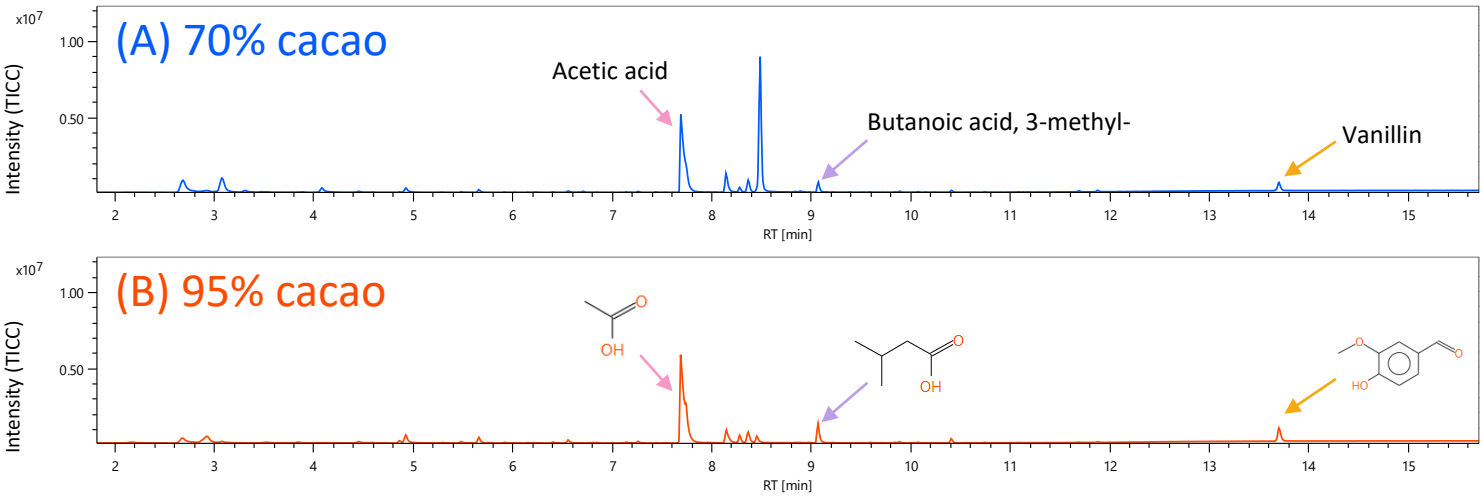
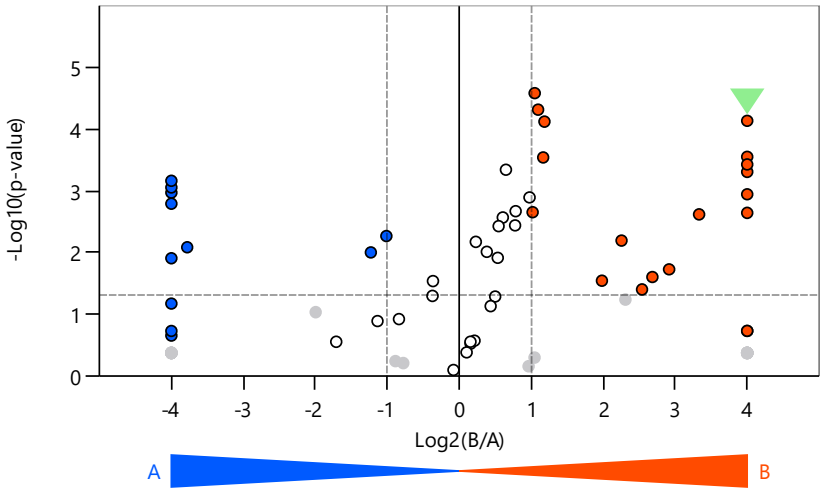


Figure 1 Total ion current chromatograms of EI



Differential analysis conditions
If p-value≦0.05, intensity ratio is 2 times or more, it is determined that the component is characteristic of each sample.

Figure 2 Volcano plot of variance component analysis result

Integrated qualitative analysis result of characteristic components of each sample

Table 2 and Table3 shows the integrated qualitative analysis result of each sample (Table 2: 70% cacao, Table 3: 95% cacao). In integrated qualitative analysis, the quality of the qualitative results can be confirmed by the background color of the table.

Blue level: high-level library matching and verifying information

Yellow level: medium-level library matching; missing or poor verifying information

In this study, most of the qualitative results showed blue levels and a high degree of accuracy. In each sample, components that could be derived from chocolate flavor components, such as aldehydes, esters, carboxylic acids and pyrazines, were detected.

The mass spectra of the component with the highest relative intensity for each sample is shown in Figure 3. The ID 040 with the highest relative intensity in the 70% cacao was estimated to be Propylene glycol. Propylene glycol is a component used as a solvent and emulsifier for food flavors and colorants. The low cacao content of this sample suggests that Propylene glycol is used as a flavoring solvent. The ID 032 with the highest relative intensity in the 95% cacao was estimated to be Pyrazine, tetramethyl-. Pyrazine, tetramethyl- is an aroma produced by roasting cacao, and is thought to be strongly detected in this sample with the high cacao content. Although this component eluted at a similar time to the common component Acetic acid in both samples, the peak could be detected using the deconvolution peak detection function of msFineAnalysis iQ (Figure 4).

Using msFineAnalysis iQ, it was possible to easily extract and qualify the characteristic components of each sample.

Table 2 Characteristic compounds in 70% cacao

	General			Variance Component			Total Result								
	ID	RT [min]	RI [iu]	Height [%]	Class	Log2(B/A)	p-value	Library Name	CAS#	Lib.	Similarity	Lib. RI [iu]	ΔRI [iu]	Formula	DBE	MW
★	040	8.49	1600	100.00	A Only	< -4	0.001	Propylene Glycol	57-55-6	mainlib	921	1600	0	C3 H8 O2	0.0	76
	006	3.08	937	12.76	A > B	-3.78	0.008	Ethanol	64-17-5	replib	699	932	5	C2 H6 O	0.0	46
	012	4.08	1041	4.15	A Only	< -4	0.012	Butanoic acid, ethyl ester	105-54-4	replib	890	1036	5	C6 H12 O2	1.0	116
	003	2.70	895	2.50	A Only	< -4	0.221	Ethane, 1,1-diethoxy-	105-57-7	replib	878	892	3	C6 H14 O2	0.0	118
	007	3.31	962	2.11	A Only	< -4	0.067	Propanoic acid, ethyl ester	105-37-3	replib	883	953	8	C5 H10 O2	1.0	102
	049	11.69	2195	0.82	A > B	-1.01	0.005	Nonanoic acid	112-05-0	replib	892	2170	26	C9 H18 O2	1.0	158
	025	6.71	1340	0.77	A Only	< -4	0.001	Heptanoic acid, ethyl ester	106-30-9	replib	866	1331	9	C9 H18 O2	1.0	158
	041	8.89	1667	0.77	A > B	-1.23	0.010	Butyrolactone	96-48-0	replib	809	1632	35	C4 H6 O2	2.0	86
	008	3.47	979	0.34	A Only	< -4	0.186	n-Propyl acetate	109-60-4	replib	926	973	6	C5 H10 O2	1.0	102
	011	3.89	1022	0.29	A Only	< -4	0.001	α-Pinene	80-56-8	replib	869	1027	5	C10 H16	3.0	136
	021	6.23	1277	0.26	A Only	< -4	0.002	Pyrazine, methyl-	109-08-0	replib	727	1266	11	C5 H6 N2	4.0	94

Table 3 Characteristic compounds in 95% cacao

	General			Variance Component			Total Result								
	ID	RT [min]	RI [iu]	Height [%]	Class	Log2(B/A)	p-value	Library Name	CAS#	Lib.	Similarity	Lib. RI [iu]	ΔRI [iu]	Formula	DBE	MW
★	032	7.75	1486	9.49	A < B	1.02	0.002	Pyrazine, tetramethyl-	1124-11-4	replib	935	1469	16	C8 H12 N2	4.0	136
	039	8.46	1595	4.65	A < B	2.54	0.040	Propanoic acid, 2-methyl-	79-31-2	mainlib	917	1570	25	C4 H8 O2	1.0	88
	005	2.93	921	4.14	A < B	2.25	0.006	Butanal, 3-methyl-	590-86-3	replib	839	918	3	C5 H10 O	1.0	86
	018	5.66	1208	4.04	A < B	1.05	0.000	1-Butanol, 3-methyl-	123-51-3	replib	896	1209	0	C5 H12 O	0.0	88
	023	6.55	1319	2.21	A < B	1.10	0.000	2-Heptanol	543-49-7	replib	902	1320	1	C7 H16 O	0.0	116
	014	4.86	1121	1.18	A < B	3.33	0.002	2-Butanol, 3-methyl-	598-75-4	replib	797	1094	27	C5 H12 O	0.0	88
	017	5.48	1188	1.12	A < B	1.18	0.000	2-Heptanone	110-43-0	replib	912	1182	6	C7 H14 O	1.0	114
	022	6.40	1299	0.75	A < B	1.17	0.000	Acetoin	513-86-0	mainlib	868	1285	14	C4 H8 O2	1.0	88
	004	2.89	917	0.70	A < B	2.92	0.019	di-tert-Butyl dicarbonate	24424-99-5	replib	705	876-1638	0	C10 H18 O5	2.0	218
	010	3.84	1017	0.60	A < B	1.98	0.029	Isobutyl acetate	110-19-0	replib	878	1012	5	C6 H12 O2	1.0	116
	001	2.17	815	0.53	B Only	> 4	0.000	Propanal, 2-methyl-	78-84-2	mainlib	873	819	4	C4 H8 O	1.0	72
	009	3.53	985	0.51	A < B	2.68	0.025	2,3-Butanedione	431-03-8	replib	862	979	6	C4 H6 O2	2.0	86
	026	6.80	1352	0.40	B Only	> 4	0.000	Propanoic acid, 2-hydroxy-, ethyl ester	97-64-3	replib	855	1347	5	C5 H10 O3	1.0	118
	019	5.92	1240	0.37	B Only	> 4	0.186	trans-β-Ocimene	3779-61-1	replib	812	1250	10	C10 H16	3.0	136
	016	5.30	1168	0.35	B Only	> 4	0.001	β-Myrcene	123-35-3	replib	851	1161	7	C10 H16	3.0	136
	027	6.85	1359	0.35	B Only	> 4	0.002	Pyrazine, 2,3-dimethyl-	5910-89-4	replib	883	1344	14	C6 H8 N2	4.0	108
	042	8.98	1681	0.35	B Only	> 4	0.000	Acetophenone	98-86-2	replib	899	1647	35	C8 H8 O	5.0	120
	033	7.96	1518	0.29	B Only	> 4	0.000	2-Nonanol	628-99-9	replib	872	1521	3	C9 H20 O	0.0	144
	048	11.08	2070	0.25	B Only	> 4	0.186	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (±)-	79-50-5	replib	867	2070	0	C6 H10 O3	2.0	130

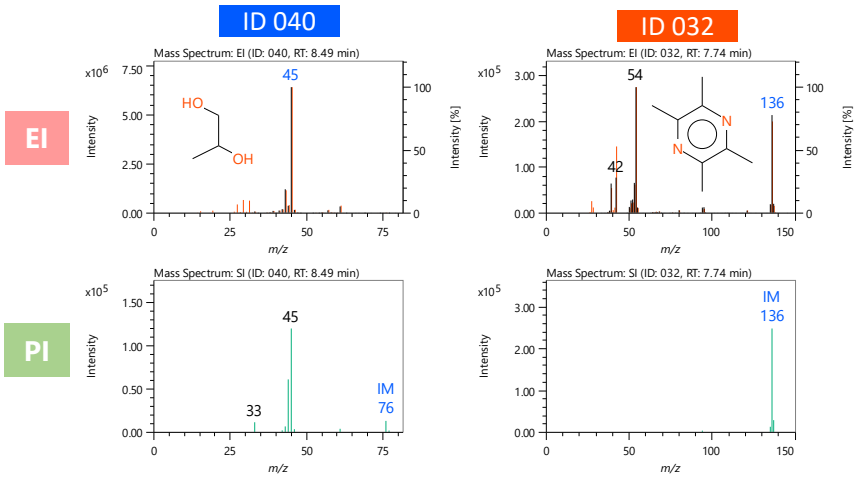


Figure 3 Mass spectra of ID 040 and ID 032

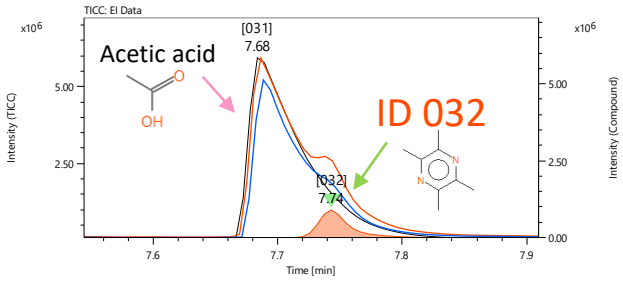


Figure 4 Deconvolution chromatogram of ID 032

Conclusions

In this MSTips, we introduced an example of difference analysis of chocolates with different cacao content using the differential analysis function of msFineAnalysis iQ. This function made it possible to easily extract differential and common components among samples and to easily qualify each component. This software is expected to improve the qualitative accuracy and efficiency of qualitative analysis using GC-QMS.

JEOL Ltd.
3-1-2 Musashino Akishima Tokyo 196-8558 Japan Sales Division Tel. +81-3-6262-3560 Fax. +81-3-6262-3577
www.jeol.com ISO 9001 • ISO 14001 Certified
▼ Local office
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