Analysis of aroma components in honey by HS-SPME-GC-TOFMS

Product used: Mass Spectrometer (MS)

Introduction

Honey is produced by bees collecting flower nectar, mixing it with their internal enzymes, and ripening it within their hive. The flavor of honey depends on the flower type of nectar source. Honey with multiple nectar sources is called "Mixed flower honey", and honey with one nectar source is called "Single flower honey". In Japan, Astragalus honey and Acacia honey which have mild flavor among single flower honey have been preferred. But Manuka honey and Jarrah honey which have rich flavor have also been attracted attention in recent years due to health consciousness.

In this MSTips, we introduce the results of headspace-solid-phase microextraction-gas chromatography-time-of-flight mass spectrometry (HS-SPME-GC-TOFMS) analysis of aroma components that affect the flavor of honey. 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. Since honey, which is a natural product, is expected to contain components not registered in the NIST database, we used JMS-T2000GC and msFineAnalysis AI, which are capable of AI structural analysis.

Experiment

Three types of commercially available honey (Mixed flower / Acacia / Jarrah) were used as samples. 5 g of each was sealed in a 20 mL headspace vial (Figure 1). HS-SPME extraction was performed at 70℃ for 30 minutes using HT2850T, and EI/FI measurement was performed using JMS-T2000GC (Figure 2). For data analysis, msFineAnalysis AI was used to perform qualitative analysis and difference analysis between samples.

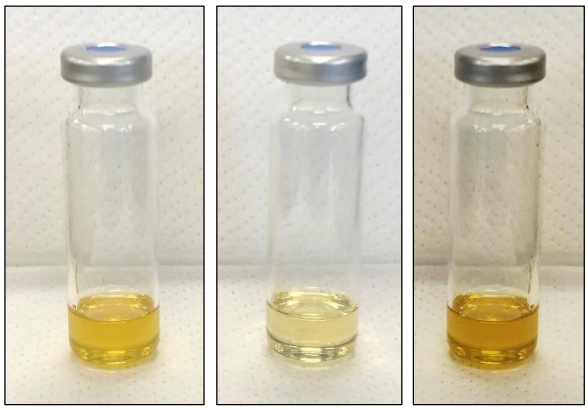

Figure 1 Honey samples (Mixed flower / Acacia / Jarrah)

Figure 2 JMS-T2000GC with HT2850T autosampler

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

Table 1 Measurement conditions

HS-SPME conditions			
Auto-sampler	HT2850T (HTA S.R.L.)	SPME fiber	DVB/CAR/PDMS 2cm (MERCK)
Sample	5g honey in 20mL headspace vial	Extraction	70 °C 30min
Mode	HS-SPME	Desorption	250 °C 5min

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	250 °C (EI)
Oven temperature	50°C - 5°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 three types of honey (Mixed flower / Acacia / Jarrah). Linalool oxide was strongly detected in Mixed flower honey. Linalool oxide was also detected in Acacia honey, but its intensity was about 1/10 of that in Mixed flower honey. Acetoin was strongly detected in Jarrah honey, but linalool oxide was not detected in this honey. Although they were both honeys, they had significant differences in the type and intensity of their aroma components.

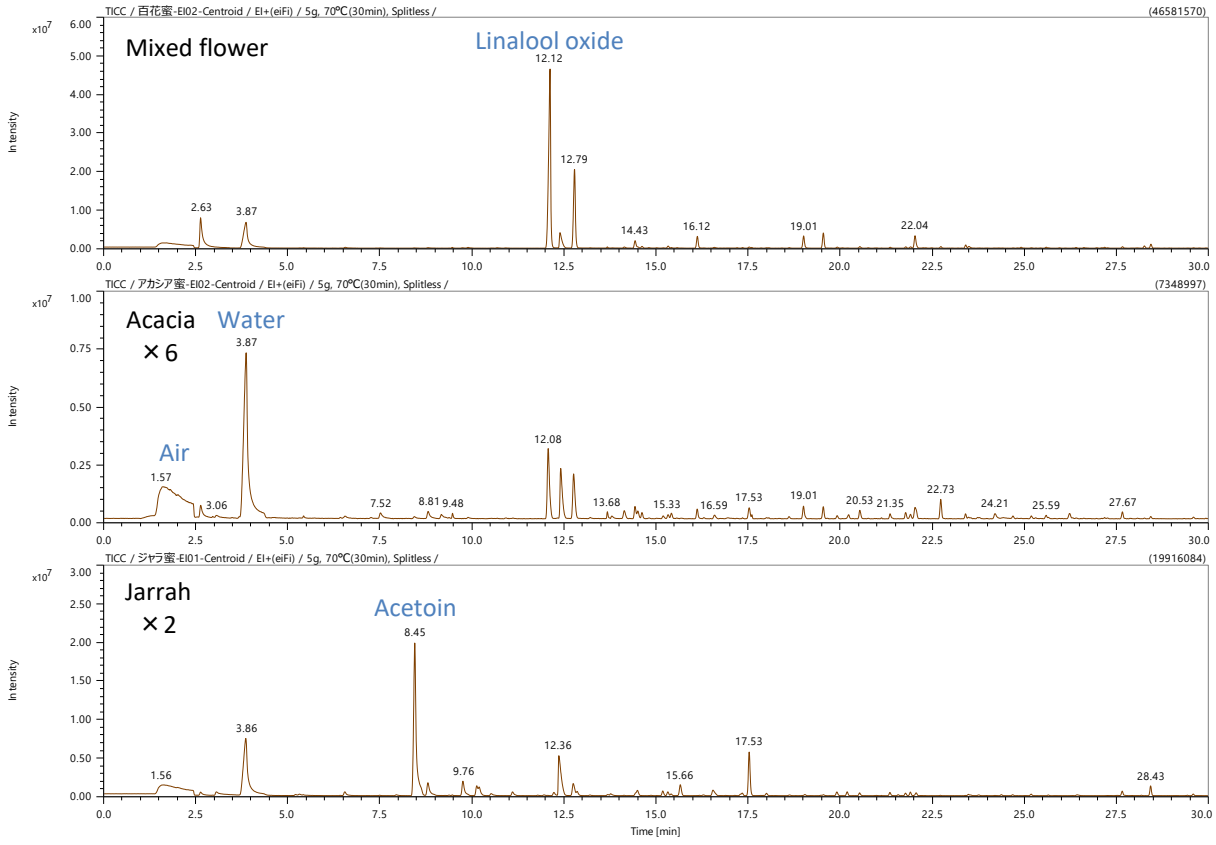

Figure 3 TIC chromatograms of three types of honey

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

Figure 4 shows the results of a difference analysis between Mixed flower honey and Acacia honey using msFineAnalysis AI. In 18 peaks with an intensity ratio of up to 1% to the maximum peak, 12 peaks were strongly detected from Mixed flower honey, 1 peak was strongly detected from Acacia honey.

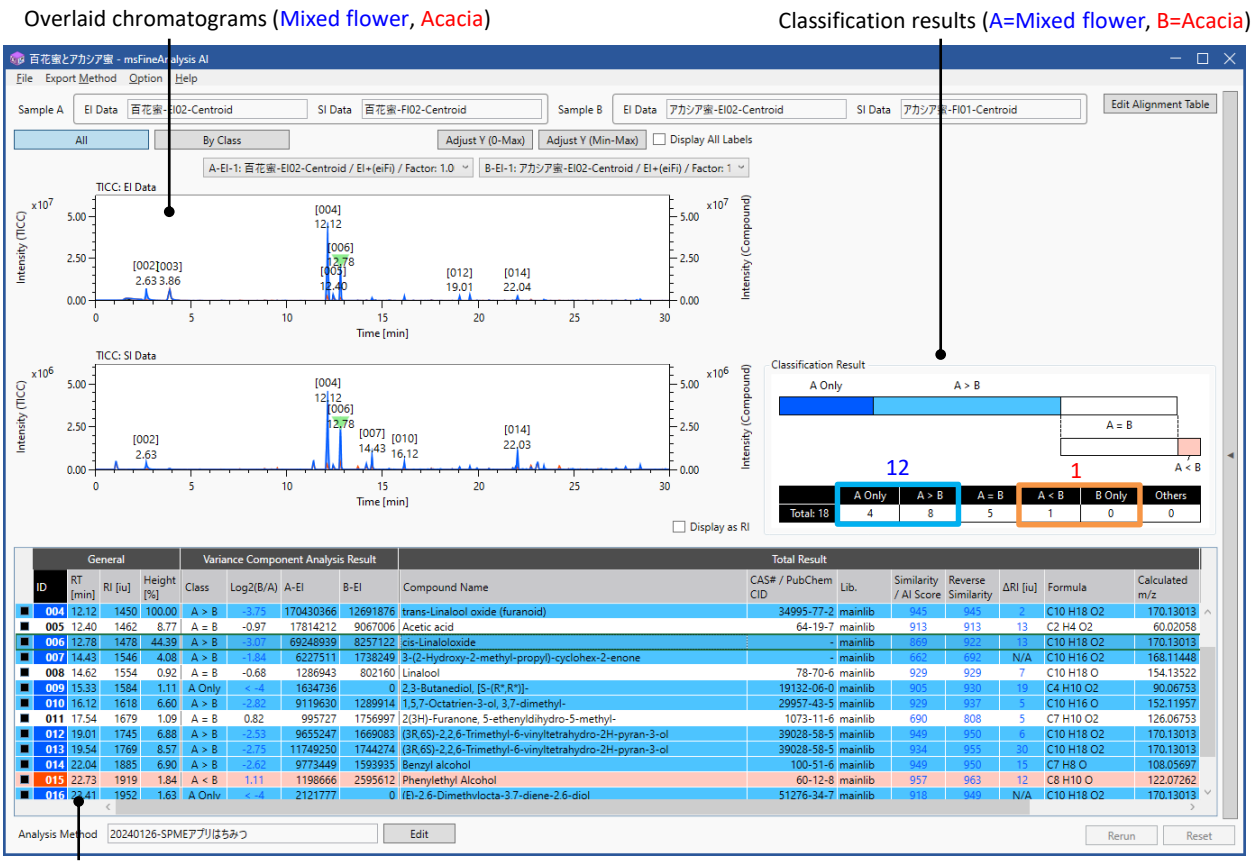

Figure 4 Difference analysis result between Mixed flower honey an Acacia honey

Figure 5 shows the results of a difference analysis between Mixed flower honey and Jarrah honey using msFineAnalysis AI. In 37 peaks with an intensity ratio of up to 1% to the maximum peak, 12 peaks were strongly detected from Mixed flower honey, 19 peak were strongly detected from Jarrah honey.

Figure 5 Difference analysis result between Mixed flower honey and Jarrah honey

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

Table 2 shows a combined peak list of two difference analysis results (Mixed flower and Acacia honey, Mixed flower and Jarrah honey). The difference analysis function of msFineAnalysis AI can compare only two samples, but it is possible to compare three or more samples by combining peak lists. This peak list includes area values and can be easily graphed by exporting to spreadsheet software.

In addition, 4 of 38 peaks were not registered in the NIST database, but the compound name and structural formula could be obtained by AI structure analysis. Some of these peaks were relatively strong, and obtained information were important to understand the characteristics of the sample.

Table 2 Combined peak list of two difference analysis

General			Area (deconvolved peak)			Total Result			
No	RT [min]	RI [iu]	Mixed flower	Acacia	Jarrah	Compound Name	CAS# /	Lib.	Similarity /
001	1.59	632	19285992	24488193	21949877	Nitrogen	7727-37-9	mainlib	713
002	2.63	877	42032218	2952335	2453498	Ethanol	64-17-5	mainlib	963
003	3.07	980	0	0	4887724	2,3-Butanedione	431-03-8	mainlib	939
004	3.87	1054	56436862	59615120	63823518	Water	7732-18-5	mainlib	959
005	6.56	1211	0		2498942	1-Butanol, 2-methyl-	137-32-6	mainlib	890
006	8.46	1297	0		92712123	Acetoin	513-86-0	mainlib	886
007	8.82	1313	0		8289055	2-Propanone, 1-hydroxy-	116-09-6	mainlib	919
008	9.76	1352	0		8672994	3-hydroxypentan-2-one	1290, 59886107	AI	946
009	10.14	1368	0		5340703	2-Hydroxy-3-pentanone	5704-20-1	mainlib	698
010	10.21	1371	0		3811026	2-Pentanone, 4-hydroxy-4-methyl-	123-42-2	mainlib	904
011	11.12	1409	0		2112525	Ethanol, 2-butoxy-	111-76-2	mainlib	877
012	12.12	1450	170430366	12691876	0	trans-Linalool oxide (furanoid)	34995-77-2	mainlib	945
013	12.24	1455	0		1727020	1-methoxy-3,3-dimethylbutan-2-one	11815387	AI	909
014	12.40	1462	17814212	9067006	26224881	Acetic acid	64-19-7	mainlib	913
015	12.76	1477	0		6918092	Furfural	98-01-1	mainlib	926
016	12.78	1478	69248939	8257122	0	cis-Linaloloxide	-	mainlib	869
017	12.87	1481	0		1806709	3-hydroxy-3,4-dimethylpentanal	65985223	AI	893
018	14.43	1546	6227511	1738249	0	3-(2-Hydroxy-2-methyl-propyl)-cyclohex-2-enone	-	mainlib	662
019	14.51	1550	1082775		2351571	Propanoic acid	79-09-4	mainlib	943
020	14.62	1554	1286943	802160	0	Linalool	78-70-6	mainlib	929
021	15.19	1578	0		1943546	Propanoic acid, 2-methyl-	79-31-2	mainlib	929
022	15.33	1584	1634736	0	1549305	2,3-Butanediol, [S-(R*,R*)]-	19132-06-0	mainlib	905
023	15.67	1598	0		5238223	Isophorone	78-59-1	mainlib	956
024	16.12	1618	9119630	1289914	0	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	29957-43-5	mainlib	929
025	16.56	1637	0		2708569	2,5-Hexanedione, 3,4-dihydroxy-3,4-dimethyl-	28123-56-0	mainlib	837
026	17.54	1679	995727	1756997	0	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	1073-11-6	mainlib	690
027	17.54	1680	0		18248374	Butanoic acid, 2-methyl-	116-53-0	mainlib	882
028	19.01	1745	9655247	1669083	0	(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	39028-58-5	mainlib	949
029	19.54	1769	11749250	1744274	0	(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	39028-58-5	mainlib	934
030	19.92	1786	0		1631158	1,4-Cyclohexanedione, 2,2,6-trimethyl-	20547-99-3	mainlib	908
031	20.20	1799	0		1530738	Ethanol, 1-(2-butoxyethoxy)-	54446-78-5	mainlib	909
032	21.36	1854	0		1281361	Hexanoic acid	142-62-1	mainlib	888
033	22.04	1885	9773449	1593935	0	Benzyl alcohol	100-51-6	mainlib	949
034	22.73	1919	1198666	2595612		Phenylethyl Alcohol	60-12-8	mainlib	957
035	23.41	1952	2121777	0	0	(E)-2,6-Dimethylocta-3,7-diene-2,6-diol	51276-34-7	mainlib	918
036	27.67	2172	1065079		1733266	Nonanoic acid	112-05-0	mainlib	914
037	28.27	2205	1722977	0	0	Epoxy-linalooloxide	-	mainlib	728
038	28.44	2215	2958612	0	3696169	(6S)-2,6-dimethyloct-7-ene-1,6-diol	328, 157010350	AI	897

mainlib : The structure formula was obtained from NIST database/ AI=from AI structure analysis

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

Figure 6 shows a graph of the area values of each peak created from Table 2. For the major peaks, aroma type were added as annotations. Mixed flower honey had multiple strong floral aromas, Acacia honey had mild floral aromas and Jarrah honey had rich aromas such as butter and herb-like. These results were reflected the characteristics of each type of honey.

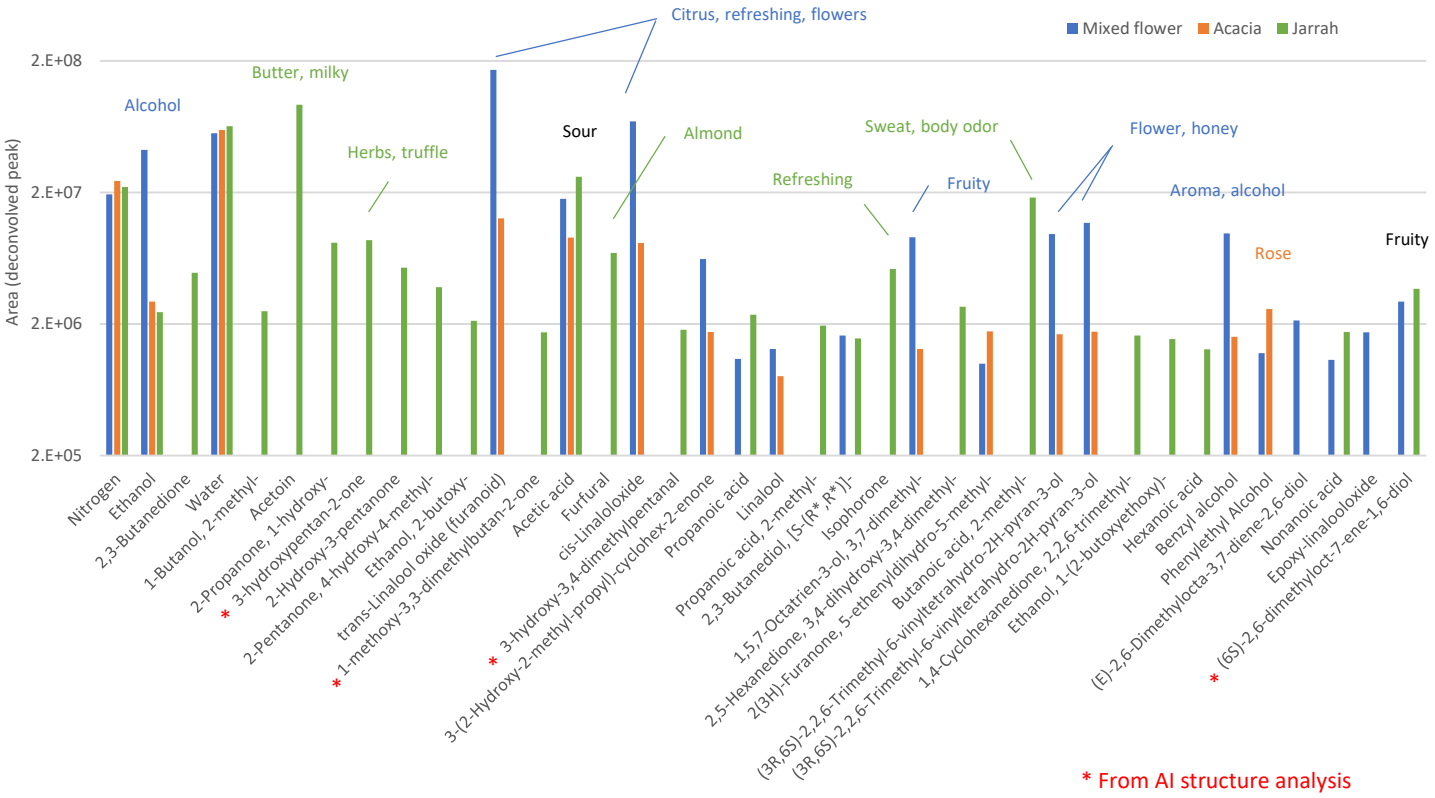

Figure 6 Peak area of each compounds

Conclusion

It was able to detect aroma components in honey with high sensitivity by HS-SPME-GC-TOFMS using HT2850T and JMS-T20000GC. Although some of the detected peaks were not registered in the NIST database, it was able to obtained the compound name and structural formula by AI structural analysis using msFineAnalysis AI. So, it was confirmed that these devices and software are effective for analyzing aroma components in foods.

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