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Technical Report

Simplified Analysis of Flavor in Shiikuwasha Products Using Monolithic Silica Adsorbents "MonoTrap" and GC-MS

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Abstract:

Flavor components in a processed tangerine product were concentrated and analyzed by GC-MS using a simplified procedure involving a MonoTrap monolithic silica adsorbent in combination with an OPTIC-4 multimode inlet. GC-MS analysis of flavor components collected from a tangerine (*Citrus depressa* Hayata, or shiikuwasha in Japanese) vinegar extract by the headspace method using a MonoTrap adsorbent resulted in detection of flavor components originating from the tangerine. The ability to preserve the collected flavor components by freezing the MonoTrap adsorbent presumably helped improve analysis efficiency.

Keywords: Shiikuwasha (*Citrus depressa* Hayata), flavor components, MonoTrap, OPTIC-4, GC-MS

1. Introduction

The *Citrus depressa* Hayata fruit (or shiikuwasha in Japanese) is a small tangerine native to Okinawa with a distinctive strong aroma and sour flavor that weighs about 20 to 50 grams. Production of related products has increased sharply due to the discovery that they contain nobiletin and other polymethoxy flavones, which are known to offer a wide variety of health benefits, and also due to the recent boom in the popularity of Okinawa in general. The juice of the shiikuwasha fruit is mainly used for processed products, but due to the approximately 50 % juice extraction efficiency, dealing with the large quantities of extraction byproducts is becoming a problem. Therefore, the authors have been engaged in developing applications for the byproducts remaining after juicing, which has resulted in using the shiikuwasha flavor to develop yogurt and vinegar extract products.

Conventional methods available for analyzing flavor components, such as solvent extraction, distillation extraction, and solid phase extraction, all require complicated extraction operations. In contrast, headspace method enables analysis under conditions that most closely resemble the conditions under which humans actually smell things and the analysis procedure is simple to perform. That makes it well-suited to simplified analysis, but high-sensitivity analysis requires first concentrating samples using an adsorbent. This report describes results from using a MonoTrap monolithic silica adsorbent to collect flavor components from a shiikuwasha vinegar extract by the headspace method and analyzing the components by GC-MS.

To prevent chromatogram peak broadening in thermal desorption analysis involving an adsorbent, a cryotrap is normally used to initially cold-trap samples injected into the column inlet before rapid thermal desorption. However, liquid nitrogen is required for cooling cryotraps. Therefore, in an environment where liquid nitrogen is not available whenever needed, collected samples must be stored until they can be analyzed at one time. Consequently, we also evaluated the ability to store the MonoTrap adsorbent containing the collected flavor components in a frozen state.

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2. Procedure

2-1. Measurement Sample Preparation

The peel was obtained by removing the seeds and segment wall matter from material remaining after shiikuwasha juice was extracted by Okinawa Sogo Nosan Kako K.K. (Higashi-mura, Okinawa Prefecture). The fruit peel was processed by adding fermented vinegar (Heinz white vinegar) and pulverizing. Then the vinegar extract was obtained by removing insoluble components by centrifugal separation and filtering.

2-2. Analytical Conditions

To collect flavor components, we used a monolithic silica adsorbent for thermal desorption (MonoTrap RGPS TD from GL Sciences) that contains graphite carbon and is coated with a polydimethyl siloxane (PDMS) phase and end-capped.

To analyze the peel, 0.10 g of ground sample material was placed in a 40 mL glass vial and sprayed with 8 mL of 0.1 % n-hexanol (special grade from Wako Pure Chemical) as an internal standard. To analyze the vinegar extract, 1.0 mL of sample was placed in a vial and 10 mL of 0.1 % cyclohexanol (special grade from Kanto Chemical) was added as an internal standard. We placed the MonoTrap adsorbent in the MT holder (GL Sciences) (Fig. 1) and sealed the vial (Fig. 2) and kept it heated to 50 °C for one hour to collect flavor components. Then, we placed the MonoTrap adsorbent containing the collected flavor components in a glass tube specialized for thermal desorption systems (MonoTrap TD Liner for OPTIC/LINEX from GL Sciences), and sealed it with a CDC liner cap (GL Sciences). Next, we placed the TD Liner in a 15 mL sample tube and stored it frozen at -20 °C until it was analyzed.

We used the Shimadzu AOC-5000 Plus multifunctional autosampler equipped with a LINEX automated liner exchanger and a CDC (capping/decapping) station for automatic continuous analysis. The flavor components adsorbed to the adsorbent were injected into the GC-MS system using the thermal desorption mode of an OPTIC-4 multimode inlet unit from Shimadzu. Based on the chromatogram data obtained from GC-MS, we used the NIST 11 Mass Spectral Library and FFNSC 2 Flavour & Fragrance Natural & Synthetic Compounds GCMS library (both from Shimadzu) for searching for mass spectra with a similarity score of 90 % or higher to identify the flavor components. The GC-MS analytical conditions are listed in Table 1.

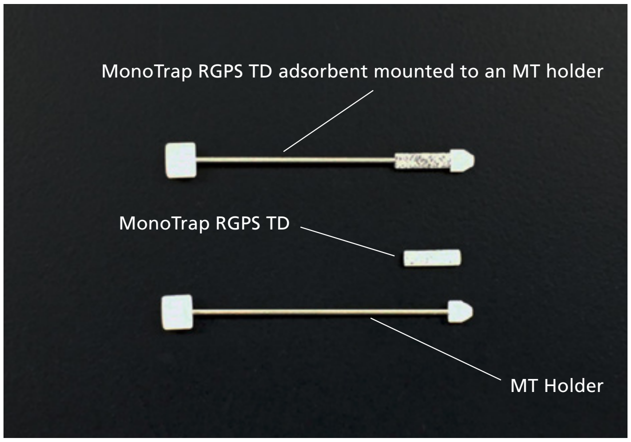


Fig. 1 MonoTrap Adsorbent Mounted to an MT Holder

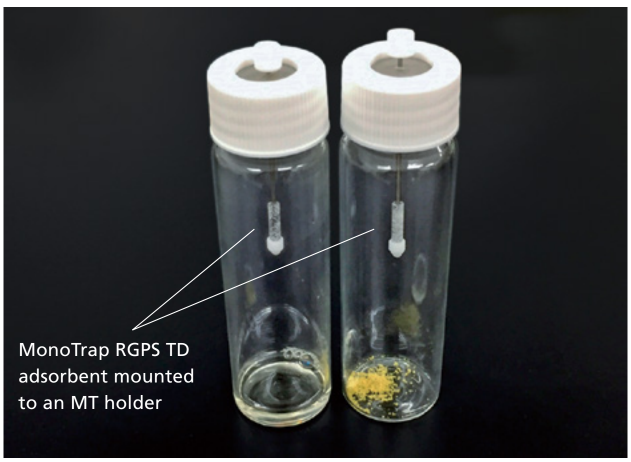


Fig. 2 MT Holders Placed in a Collection Vial
Flavor components were collected without contacting the sample by mounting the MonoTrap adsorbent on an MT holder.

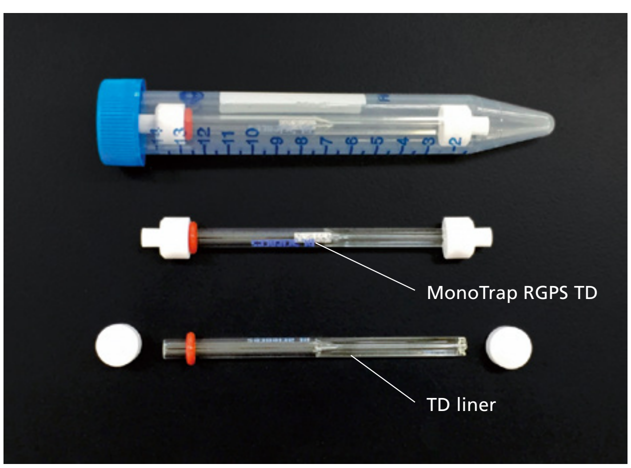


Fig. 3 Storing MonoTrap Adsorbent Frozen
The MonoTrap adsorbent with the collected flavor components was placed in a TD liner, which was capped and placed in a sample tube. Then the sample tube was stored frozen at -20 °C.

Table 1 GC-MS Analytical Conditions

System Contents	
Multimode inlet	: OPTIC-4 (with Cryotrap)
Autoinjector	: AOC-5000 Plus LINEX + CDC Station
GC-MS	: GCMS-QP2010 Ultra
Software	: GCMSsolution Ver. 4.20 Cycle Composer Ver. 1.6.0 Evolution Workstation Ver. 4.6.3
Libraries	: NIST 11 Mass Spectral Library FFNSC 2 Flavour & Fragrance Natural & Synthetic Compounds GCMS library
GC	
Column	: InertCap Pure WAX ProG 2M, 0.25 mm x 60 m, df=0.25 µm (GL Sciences)
Thermal desorption temperature	: 40 °C- (6.0 °C/sec) -200 °C (300 sec)
Injection mode	: Split (1:50 ratio for peel analysis and 1:20 ratio for vinegar extract analysis)
Cryofocus temperature	: -150 °C (400 sec) - (5.0 °C/sec) -250 °C
Carrier gas	: Helium
Column flowrate	: 1.0 mL/min
Column oven temperature	: 40 °C (5 min) - (6.0 °C/min) -250 °C
MS	
Ion source temperature	: 200 °C
Interface temperature	: 250 °C
Ionization method	: EI
Ionization voltage	: 70 eV
Measurement mode	: Scan
Event time	: 0.30 sec
Scan speed	: 2000
Scan mass range	: *m/z* 30-600

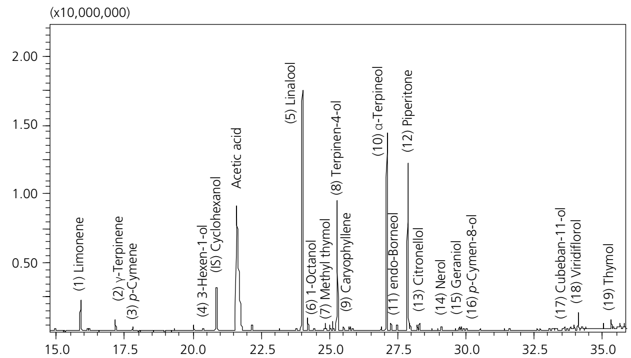
3. Analytical Results

3-1. Flavor Components in Shiikuwasha Vinegar Extract

A library search of GC-MS analysis results for the shiikuwasha vinegar extract successfully identified 19 flavor components originating from the shiikuwasha (Fig. 4). According to reports in the literature, limonene accounts for the largest proportion of hydrocarbon compounds in shiikuwasha flavor components (43 to 45 %), just as with other tangerine varieties, but the limonene content is lower than in sweet oranges (86 %) or yuzu (63 to 68 %). However, shiikuwasha contains higher levels of g-terpinene (28 to 29 %) and p-cymene (8 to 11 %) than other tangerine varieties. Another characteristic of the shiikuwasha is its high content of monoterpene alcohols, such as linalool and terpinen-4-ol, among oxygenated compounds.

Significantly lower levels of limonene and monoterpene alcohols, such as g-terpinene and p-cymene, were detected in the shiikuwasha vinegar extract than reported in the literature, but the relative area value of g-terpinene was 63 % of limonene, which is roughly consistent with literature values. Since monoterpenes appear as major peaks among peel flavor components (Fig. 5), the low extraction rate of monoterpenes, which are lipophilic, was speculated given that vinegar was used as the solvent in aqueous extraction.

Meanwhile, the results show that monoterpene alcohols, such as linalool, terpinen-4-ol, and a-terpineol, were extracted efficiently.



No.	Compounds	R.T.	Relative Area Value
1	Limonene	15.91	24.35
2	γ-Terpinene	17.17	15.39
3	*p*-Cymene	17.80	8.05
4	3-Hexen-1-ol	20.38	6.12
5	Linalool	24.01	206.69
6	1-Octanol	24.21	12.31
7	Methyl thymol	25.13	18.79
8	Terpinen-4-ol	25.29	140.28
9	Caryophyllene	25.51	3.13
10	α-Terpineol	27.11	193.84
11	endo-Borneol	27.24	18.20
12	Piperitone	27.88	260.01
13	Citronellol	28.29	6.53
14	Nerol	28.96	2.99
15	Geraniol	29.76	6.09
16	*p*-Cymen-8-ol	29.82	9.83
17	Cubeban-11-ol	33.61	1.79
18	Viridiflorol	34.11	7.79
19	Thymol	35.32	18.28

Note: Indicates relative area values (%) that are relative to the area for internal standard substance cyclohexanol.

Fig. 4 TIC of Shiikuwasha Vinegar Extract

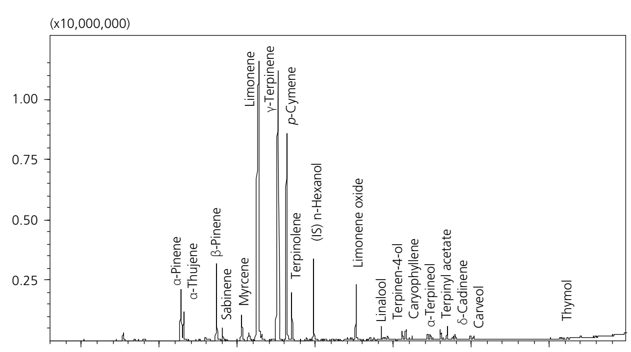


Fig. 5 TIC of Shiikuwasha Peel

3-2. Frozen Storage of Flavor Components Collected in Adsorbent

Results from GC-MS analysis performed immediately after collecting the flavor components in the MonoTrap adsorbent were compared to results from GC-MS analysis performed after storing the adsorbent frozen for one day to four weeks. Fig. 6 shows that there was no difference between TIC results due to storage of the adsorbent. A comparison of the relative area values for the 19 components detected indicated a small coefficient of variation (CV) for each component, between 0.01 and 0.11 (Table 2), and no significant differences in analytical values depending on whether frozen storage of the adsorbent was done or depending on the length of the storage period (verified by the Tukey-Kramer method).

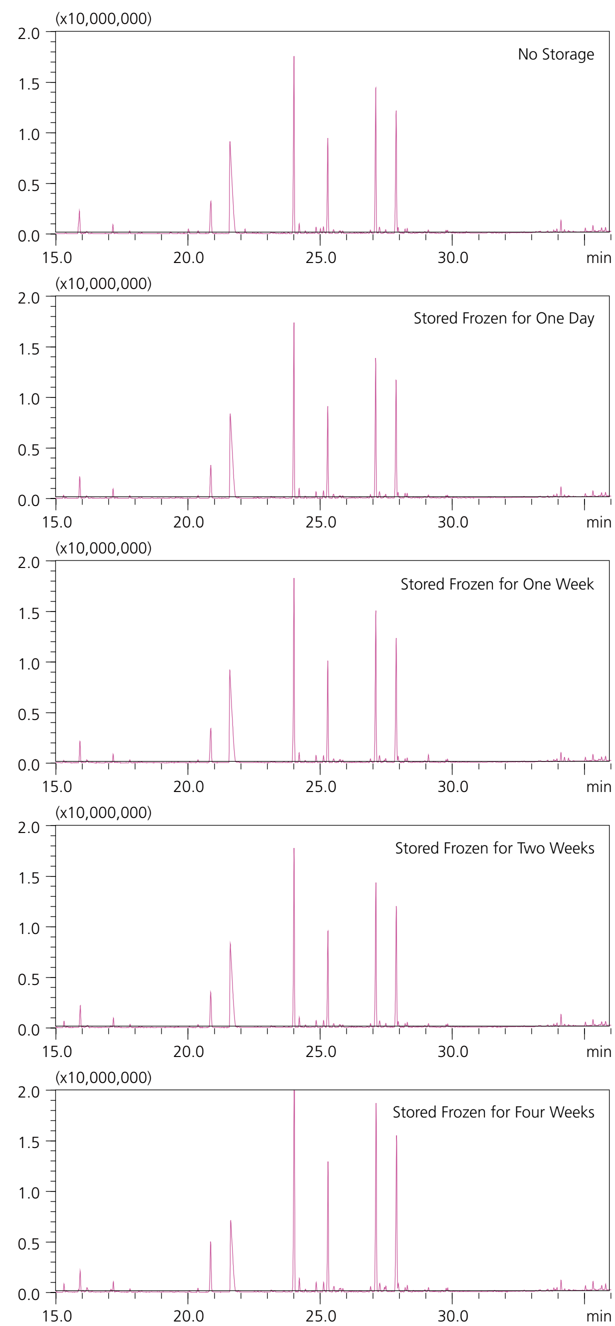


Fig. 6 Comparison of TICs from GC-MS Analysis of MonoTrap RGPS TD Adsorbent Stored Frozen

Table 2 Effect of Frozen Storage of Flavor Components Collected in MonoTrap RGPS TD Adsorbent

No.	Compounds	R.T.	No Frozen Storage	Samples Stored Frozen				Average	Standard Deviation	Coefficient of Variation
				One Day	One Week	Two Weeks	Four Weeks			
1	Limonene	15.91	24.35	23.63	22.24	23.10	21.01	22.87	1.15	0.05
2	γ-Terpinene	17.17	15.39	14.93	12.56	15.00	14.73	14.52	1.00	0.07
3	*p*-Cymene	17.80	8.05	7.61	8.65	8.65	7.74	8.14	0.44	0.05
4	3-Hexen-1-ol	20.38	6.12	5.89	6.10	5.81	6.07	6.00	0.12	0.02
5	Linalool	24.01	206.69	213.90	208.99	208.89	220.80	211.85	5.06	0.02
6	1-Octanol	24.21	12.31	12.34	11.08	11.47	11.91	11.82	0.49	0.04
7	Methyl thymol	25.13	18.79	22.83	18.40	20.97	22.67	20.73	1.87	0.09
8	Terpinen-4-ol	25.29	140.28	143.59	143.34	141.76	147.73	143.34	2.50	0.02
9	Caryophyllene	25.51	3.13	3.51	2.87	3.36	3.60	3.29	0.26	0.08
10	α-Terpineol	27.11	193.84	197.91	196.60	191.63	201.83	196.36	3.50	0.02
11	endo-Borneol	27.24	18.20	18.35	18.08	17.87	18.67	18.23	0.27	0.01
12	Piperitone	27.88	260.01	266.20	261.30	252.87	259.59	259.99	4.27	0.02
13	Citronellol	28.29	6.53	6.48	6.07	6.14	6.16	6.28	0.19	0.03
14	Nerol	28.96	2.99	2.66	2.97	2.79	2.88	2.86	0.12	0.04
15	Geraniol	29.76	6.09	5.58	5.69	5.59	5.57	5.70	0.20	0.03
16	*p*-Cymen-8-ol	29.82	9.83	9.51	10.04	10.37	9.58	9.87	0.31	0.03
17	Cubeban-11-ol	33.61	1.79	1.68	1.65	1.72	1.59	1.69	0.07	0.04
18	Viridiflorol	34.11	7.79	6.97	6.28	7.29	5.73	6.81	0.73	0.11
19	Thymol	35.32	18.28	18.62	19.72	18.62	20.55	19.16	0.85	0.04

Note: Indicates relative area values (%) that are relative to the area for internal standard substance cyclohexanol.

4. Conclusion

GC-MS analysis of flavor components collected using a MonoTrap monolithic silica adsorbent from a shiikuwasha vinegar extract enabled a simple analytical procedure for detecting flavor components originating from the shiikuwasha. The results also showed that flavor components collected in a MonoTrap adsorbent placed in a TD Liner sealed with a CDC liner cap can be stored frozen for four weeks at -20 °C.

Acknowledgments

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Reference

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