GC--MS analysis of Japanese Banana Flower without pollen and Pollen

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Abstract: The Japanese banana flower and pollen were analyzed by gas chromatography-mass spectrometry (GC-MS) technique, coupled with head-space solid micro-extraction (HS-SPME). The relative percentage of compounds were measured by retention index and peak area normalization method. A total of 48 volatiles were identified in Japanese banana flower without pollen. Forty volatiles were identified in Japanese banana pollen.There were 20 compounds we found in both flower and pollen, accounting for 34.35% and 49.99% of thetotal volatiles, respectively. It can be seen the compounds between flowers andpollen were different apparently. This is the first ever report revealing the differences of volatile components between the Japanese banana flower without pollen and pollen.

Introduction

Musa basjoo Sieb. et Zucc. is originating in Japan, the Ryukyu Islands. In China, it is mainly distributed in subtropical regions, such as Guizhou,Guangdong, Guangxi,Hainan,Sichuan,Yunnan and Taiwan[1]. Japanese banana flower is the flower of *Musa basjoo* Sieb.et Zucc. In Yunnan, Japanese banana flower is one of the more popular food and many people like to cook with Japanese banana flower [2-3]. Modern researches show that the dried Japanese banana flower have apparent effective to treat cerebral hemorrhage[4]. Both Japanese banana flowers and pollen all have aromatic smell, but the smell of pollen is stronger than flower's. The reason why the aromatic smell of pollen is stronger than flower's is unexplained, and there is no any relevant reports about that.Volatile oil is a key of aromatic smell, so the research on volatile components is very important.In this paper, the flower and pollen of Japanese banana were analysis by gas chromatography–mass spectrometry (GC–MS) with solid-phase micro extraction (SPME). Then, the comparison among the flower and pollen of Japanese banana was made. A comparative analysis on why the aromatic smell of pollen is stronger than flower's of Japanese banana also evaluated. This study could provide a scientific basis for the further development and utilization of the flower and pollen of Japanese banana.

Materials and methods

Plant materials

The flower without pollen and pollen of Japanese banana were collected from Yunnan in May, 2015 and further identified by Dr. X.P. Wang, Department of Pharmacognosy Guiyang College of Traditional Chinese Medicine, P. R. China. All voucher specimens were deposited in the Department of Pharmacognosy, Guiyang College of Traditional Chinese Medicine, P.R. China. The pollen was separated from the flowers,and they were placed in a cool, dry environment (20 °C) to dry naturally.

Solid-phase micro extraction procedure

The flower without pollen small pieces and pollen of Japanese banana were accurately weighed (6.0 g) and placed into 25-mL sample vials from Supelco (Bellefonte, USA), respectively. Then, a

2cm-50/30um DVB-CAR-PDMS Stable Flex fiber (Bellefonte, USA) was used to the headspace above the samples for extracting 40 min under about 100 °C. then the extraction head was removed from sample vials and immediately inserted onto the GC injection port. The SPME fiber head was hung over the vial for 3 min and then directly desorbed and analyzed.

Gas Chromatography–Mass Spectrometry

The analyses of gas chromatography was performed on a Hewlett-Packard 6890GC-5973C MSD (Agilent, Palo Alto, CA, U.S.A.) using a ZB-5MSi (5%phenyl-95%dimethylpolysiloxane) fused silica capillary column (30m×0.25mm×0.25 mm). The oven temperature was programmed as follows: held at 40°C for 2 min, adjusted to 270°C at a rate of 5°C/mi,run 48 min,The injector temperature was 250°C. High-purity helium (99.999%) was used as the carrier gas at a flow rate of 1.0 ml/min with splitless injection.

The mass spectrometer was fitted with an electron ionization source operated at 70 eV. The source temperature was 230°C, and the interface temperature was 280°Cwith a solvent delay of 1.5 min. The emission current was 34.6μA, and the multiplier voltage was 1294V . Mass spectra were recorded from m/z 29–450 amu in the full scan mode. Volatile compounds were identified by comparison of mass spectra of the analytes with those of authentic standards from the NIST2005 and Wiley275 libraries. The instrument Chemstation data processing system was used to determine the relative concentrations of the analytes by the peak area normalization method.

Determination of Retention Index

After mixing the chromatography on n-alkanes (C_6 -C_{26}) and the same amount from each sample , according to the above GC chromatographic analysis conditions, the retention time was measured for each sample and n-paraffins,and then the retention indices(KI) was calculated by formula($KI = 100n + 100(t_x - t_{n)} / (t_{n+1} - t_n))$[5]. T_x represented the retention time of the test substance , n represented the corresponding n-alkane carbon atom, t_n and t_{n+1} are the retention time of n-alkane whose number of carbon atoms in a difference of 1($t_n < t_x < t_{n+1}$). Kovats retention indices can improve the accuracy of identification results .In the identification of aromatic oils complex component , this method got generally accepted and widely used in the international arena.

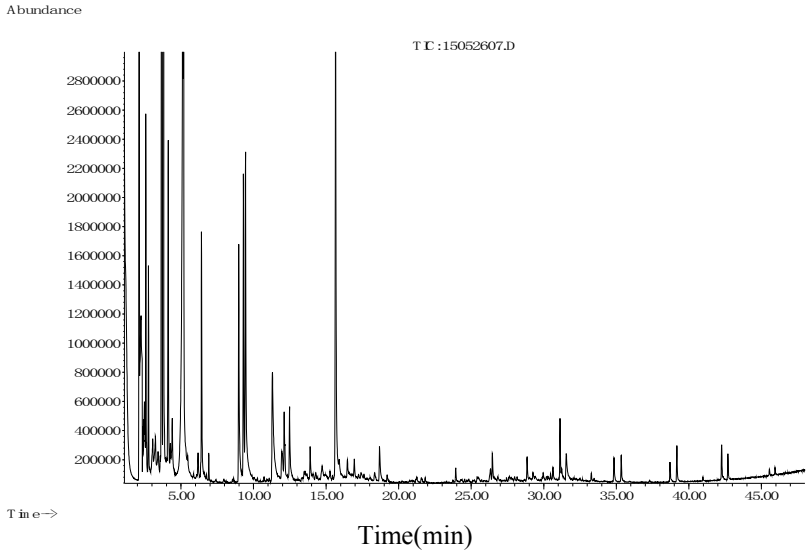

Fig1. TIC of volatile components extracted from Japanese banana flower without pollen

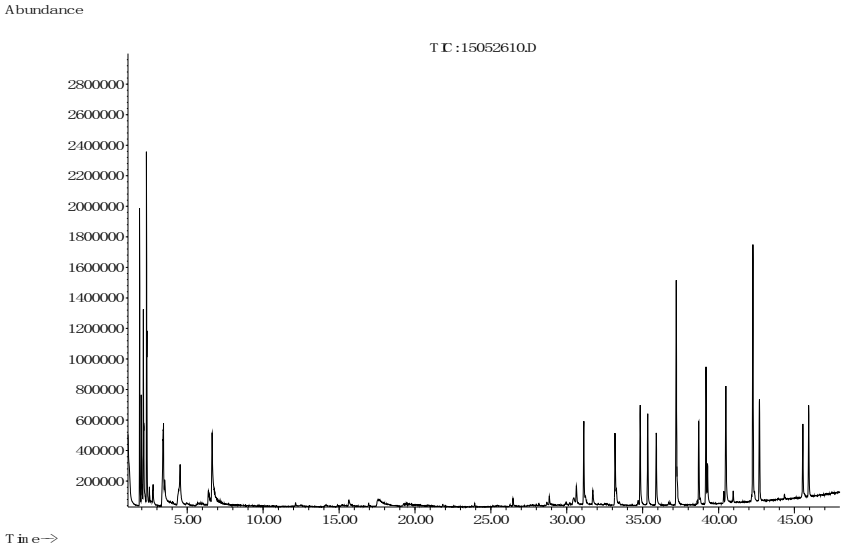

Fig 2. TIC of volatile components extracted from Japanese banana pollen

Table1.Percentages of volatile components extracted from Japanese banana flower without pollen and pollen

No.	Retention time(min)	Compound	Percentage(%)	
			Flower	Pollen
1	1.97	Ethanal	—	2.336
2	2.10	EtOH	—	4.691
3	2.25	Methanol	5.494	—
4	2.31	Dimethyl sulfide	2.19	8.103
5	2.48	Acetone	0.46	—
6	2.50	2-Methyl-propanal	1.789	0.312
7	2.62	Methyl acetate	0.065	—
8	2.75	2,3-Butanedione	—	0.614
9	3.05	2-Butanone	0.342	—
10	3.42	2-Methyl-propanol	0.282	—
11	3.43	Acetic acid	—	4.672
12	3.52	2-Methylbutanal	17.753	1.542
13	3.66	3-Methylbutanal	9.907	—
14	4.12	2-Pentanone	3.506	—
15	4.40	2-Pentanol	0.539	—
16	4.53	3-Hydroxy-2-butanone	—	3.45
17	5.11	3-Methyl-1-butanol	10.653	—
18	5.18	2-Methyl-1-butanol	6.826	—
19	5.46	2-Hydroxy-3-butanone	0.154	—
20	6.18	2-Hexanone	0.358	—
21	6.36	Octane	0.081	—
22	6.41	Hexanal	2.679	—
23	6.64	2,3-Butanediol	—	5.324
24	6.75	2-Octene	0.047	—
25	8.98	2-Heptanone	3.42	—
26	9.31	Heptanal	3.626	—
27	9.45	2-Heptanol	5.771	—
28	11.30	Benzaldehyde	3.374	—
29	12.11	2-Amylfuran	0.759	—
30	12.48	Octanal	1.106	—
31	13.91	trans-beta-Ocimene	0.551	—

32	14.10	1-Ethyl-2-formyl pyrrole	—	0.317
33	14.89	2,6-diethyl-Pyrazine	—	0.124
34	15.21	3-Ethyl-2,5-dimethylpyrazine	—	0.263
35	15.27	2-Nonanone	0.099	—
36	15.65	Nonanal	7.844	0.421
37	17.58	2,3-Dihydro-3,5-dihydroxy-6-Methyl-4H-pyran-4-one	—	1.987
38	18.69	Decanal	0.677	—
39	19.21	beta-Cyclocitral	0.111	—
40	21.26	Tridecane	0.063	—
41	23.93	Tetradecane	0.184	0.105
42	26.33	beta-ionone	0.275	—
43	26.46	Pentadecane	0.489	0.272
44	28.69	1-Hexadecene	—	0.104
45	28.85	Hexadecane	0.3	0.285
46	30.63	8-Heptadecene	0.152	0.885
47	31.12	Heptadecane	0.755	2.94
48	31.24	Pristane	0.167	0.387
49	31.72	Methyl myristate	—	0.648
50	33.18	Ethyl myristate	—	2.926
51	33.27	Octadecane	0.088	0.681
52	33.46	Phytan	0.029	
53	34.70	(Z)-9,17-Octadecadienal	—	0.208
54	34.84	1-Nonadecene	0.368	3.837
55	35.33	Nonadecane	0.381	3.21
56	35.89	Methyl palmitate	—	3.025
57	37.21	Ethyl palmitate	—	8.87
58	38.69	10-Heneicosene	0.246	2.925
59	39.17	Heneicosane	0.484	4.79
60	39.27	Methyl linolenate	—	1.618
61	40.34	Ethyl linoleate	—	0.446
62	40.48	Ethyl linolenate	—	5.078
63	40.97	Docosane	0.05	0.403
64	42.26	(Z)-9-Tricosene	0.451	9.3
65	42.69	Tricosane	0.33	3.717
66	44.34	Tetracosane	—	0.158
67	45.55	Z-12-Pentacosene	0.071	2.657
68	45.94	Pentacosane	0.087	3.274

Results and Discussion

The volatile components were isolated and identified by experimental methods and experimental conditions. The relative percentage of compounds were measured by retention index and peak area normalization method.The total ion chromatogram of Japanese banana flower without pollen and pollen of are shown in Fig. 1 and Fig. 2, and the corresponding volatile compounds are listed in Table 1 . A total of 68 volatile components were identified from Japanese banana flower without pollen and pollen by GC-MS technology. It is shown that 48 volatile components were identified from the Japanese banana flower without pollen in Table 1.There were some components with high concentrate relatively, for example, 2-methylbutanal (17.753%), 3-methyl-1-butanol(10.653%), nonanal(7.844%), 2-heptanol(5.771%),etc. It was also shown that 40 volatile components were identified from the Japanese banana flower without pollen in Table 1.There are some components with high concentrate relatively, such as ethyl palmitate(8.87%), dimethyl sulfide(8.103%), 2,3-butanediol(5.324%), ethyl linolenate(5.078%)and so on.

As shown in Table 1, 20 components were found both in flower and pollen, accounting for 34.35% and 49.99%, respectively. Decanal and cyclocitral were unique to flowers and pyrrole and

pyrazine were pollen-specific. The content of aldehydes were higher in flower and esters had a higher content in pollen.

It was found that 2-methylbutyraldehyde has a chocolate flavor[6]. In food industry, nonanal is a food additive, and in terms of chemical process it can be used as intermediates in organic synthesis, spices raw materials, and rubber accelerator [7]. Maybe nonanal and 2-methylbutyraldehyde are the reason why Japanese banana flower without pollen have aroma. Ethyl palmitate is the main volatile components in pollen. It showed a weak wax, fruity and cream aroma[13].Therefore,the ethyl palmitate is likely to be a major contributor to the aroma of pollen.In addition, it was found ethyl palmitate may be an active ingredient in tagging Plutella[14].

Conclusion

In this paper, a total of 68 volatile components from Japanese banana flower without pollen and pollen were isolated and identified by headspace solid phase microextraction and gas chromatography – mass spectrometry. In Japanese banana flowers without pollen,48 components were identified, and the main components were 2-methylbutanal, 3-methyl-1-butanol,nonanal, 2-heptanol . In Japanese banana pollen only 40 components were identified,and the main components were ethyl palmitate, dimethyl sulfide, 2,3-butanediol, ethyl linolenate. It was found that 20 components were both in flower without pollen and pollen,but the main components from them were different.It can be seen the compounds between flowers and pollen were different apparently.The difference of the main components between flowers and pollen was possible leading to different flavor.This work could provide a reference for the further development of Japanese banana flower and pollen .

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