



## Analysis of Chemical Constituents of the Volatile Oil in the Different Parts of Okra by SPME-GC/MS

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This study reports the chemical constituents of the volatile oil in the okra leaf, flower and seed. The chemical constituents were extracted from the different parts of okra by solid phase micro extraction method (SPME). The components of chemical constituents of the volatile oil were separated and identified by GC-MS. The relative content of each component was determined by area normalization. Sixty eight components obtained from leaf, flower and seed were identified. Among them, 19 common compounds exist in all the three different parts of okra. The leaf and flower contain 30 common compounds, the flower and seed have 22 and the flower and seed contain 23 common compounds. This study is the first report on the chemical compositions of the oil of different parts of okra. The result can provide reference for further research on the composition of okra volatile oil as well as for the exploration and utilization of it.

**Key Words:** Okra, SPME, Leaf, Flower, Seed, Volatile oil, GC-MS.

### INTRODUCTION

Okra, an annual herb, belongs to the malvaceae family. Other synonyms are gumbo, invigorating the kidney straw<sup>1</sup>. Okra, which is one of the favourite vegetables of the African, American and Southeast Asian, originated from Africa and was widely cultivated in P.R. China in the early 1990s<sup>2</sup>. Okra contains abundant protein, unsaturated fatty acid, mineral substance, flavone and polyphenol compounds<sup>3</sup>. With the mild taste and cold-nature, its bright fruit is edible and has high nutritive value like relieving sore-throat and regulating menstruation<sup>4</sup>. So it is as good as ginseng. However, okra is more suitable for daily dietetic invigoration. The fruits also contain viscous substances, such as pectin, polysaccharides, *etc.*, which can motivate gastrointestinal motility, prevent constipation, nourish yin and invigorate yang<sup>5</sup>. So in Japan and Korea, okra is called as green ginseng and in America it is called as vegetal viagra since it has no toxic and side-effect<sup>6</sup>.

In recent years, okra has already been cultivated in Zhejiang, Jiangxi province. The study mainly focused on plantation and cultivation technique. By the literature retrieval, there are no previous reports on the volatile chemical composition of the volatile oil of okra. In this paper, the oil of okra was obtained from its leaves, flowers and seeds by solid-phase microextraction, which uses adsorption and desorption technique

to enrich the volatile and semi-volatile component of the sample<sup>7</sup>. Then couple with other analytical instruments to assay the volatile and semi-volatile organics. Compared to steam distillation extraction method the solid-phase micro extraction has the following advantages<sup>8</sup>: shorter running time, smaller usage amount of sample and no extractive solvent, *etc.* The components of chemical constituents of the volatile oil were separated and identified by GC-MS. The percentage composition of the oil was computed by peak area normalization method. So we expect to provide references for deep processing of the okra.

### EXPERIMENTAL

Varian CP3800/1200L gas chromatography-mass spectrometry series instrument (American Finnigan Corporation), Chromatographic column: HP-5 (30 m × 0.25 mm, 0.25 mm) capillary-tube column, manual solid-phase micro-extraction device (American Supelco Company), 75 mm PDMS the extraction fibre plug (American Supelco Company).

The flowers, leaves and seeds of okra were collected in the arboretum of Zhejiang Forest and Agriculture university. The samples of this plant are mixed and washed clean and then made into powder for later use.

**Extraction of volatile oil:** Weigh 3.0 g of the dried and powdered sample and put it in the 15 mL sample bottle for

solid-phase micro-extraction. Put the solid-phase micro-extraction fibre plug on the sample-feeding mouth of gas chromatography instrument for ageing. Put the 75  $\mu\text{m}$  PDMS fibre plug into the sample bottle through the rubber washer of the bottle cap and extract for 0.5 h at 50  $^{\circ}\text{C}$ . Then take out the extraction plug from the sample bottle and put it immediately into sample-feeding mouth of gas chromatography instrument to get rid of sorption for 3 min under the temperature of 250  $^{\circ}\text{C}$ . Meanwhile start up the instrument to collect the experimental data.

**GC-MS analysis condition:** Chromatographic conditions: DB-WAX elastic quartz capillary-tube chromatographic column (30 m  $\times$  0.25 mm, 0.25 mm); temperature-increasing procedure: keep the initial temperature 40  $^{\circ}\text{C}$  for 3 min and increase the temperature to 80  $^{\circ}\text{C}$  at the rate of 6  $^{\circ}\text{C min}^{-1}$  and then increase the temperature to 230  $^{\circ}\text{C}$  at the rate of 10  $^{\circ}\text{C min}^{-1}$  and keep it for 10 min; the temperature at the sample-feeding entrance is 250  $^{\circ}\text{C}$ , the carrier gas was helium and the flowing rate is 0.8 mL  $\text{min}^{-1}$  without any divided sample-feeding.

**MS condition:** Electron energy 70 eV, ionization mode: EI, ion source temperature: 200  $^{\circ}\text{C}$ , mass scan range: 333-500 m/z, voltage of detector: 350 v, Willey and NIST (standard mass spectrogram).

## RESULTS AND DISCUSSION

Taking out 0.2  $\mu\text{L}$  volatile oil extracted by the method of solid phase micro-extraction, we analyze and identify the volatile chemical constituents by gas chromatography-mass spectrometry instrument. The ratio of the compounds is by the method of area normalization. The calculation of the individual peak area is by Hewlett-Packard software treatment system. The calculation of the relative percentage content in each volatile component is by the method of peak area normalization. We analyzed the volatile chemical component in the volatile oil of okra according to the above GC-MS conditions and achieved the total ion (Fig. 1). After scanning each peak value to the total ion Fig. 1 by mass spectrometry, retrieving and contrasting by mass spectrometry data system (NIST and Willey Standard Atlas Data Bank)<sup>9</sup> and consult the relevant information of MS combined with artificial analysis, we identified the chemical constituents of volatile oil in the leaves, flowers, seeds of okra<sup>10</sup>. The result can be seen in Table-1.

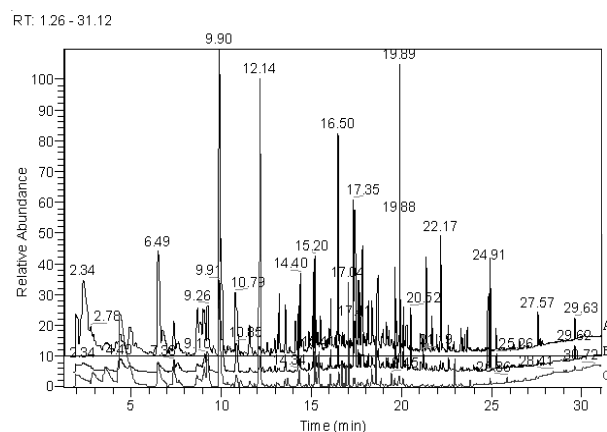


Fig. 1. GC-MS total ion current chromatogram of the volatile oil (A: leave, B: flower, C: seed)

Table-1 showed that 44 components are separated and identified from the of okra's leaves and the main chemical constituents are 2-propenoic acid, butyl ester (10.76 %), 2-hexenal (3.33 %), (-)- $\alpha$ -cedrene (2.51 %) and benzaldehyde (4.07 %).

Forty eight components are separated and identified from the oil of okra's flowers and the major chemical constituents are butyl propanoate (2.11 %), butyl butanoate (2.95 %), 3-bromo-3-methyl butyric acid (2.90 %),  $\alpha$ -farnesene (11.17 %), nonanal (3.11 %), butyl ester of 2-propenoic acid (14.92 %).

Thirty two components are separated and identified from the oil of okra's seeds, the main chemical constituents are acetic acid, butyl ester (3.15 %), 1-butanol (3.21 %), 2-propenoic acid, butyl ester (11.27 %), butanoic acid, 2-methyl-, 2-methyl-butyl ester (22.87 %) and ethyl acetate (4.26 %).

Nineteen common compounds all exist in the three different parts of okra. They mainly are ethanol, ethyl benzene, acetic acid butyl ester, 2-propenoic acid butyl ester, nonanal, (-)- $\alpha$ -cedrene,  $\alpha$ -farnesene. The leaf and flower contain 30 common compounds, which mainly are ethanol, toluene, butyl ester of 2-propanoic acid, hexanal, (-)- $\alpha$ -cedrene,  $\alpha$ -farnesene. The leaf and flower contain 22 components and the major are ethanol, toluene, butyl acetate, 1-butanol, 2-propenoic acid butyl ester. The flower and seed contain 23 and the major are ethanol, butyl acetate, propanoic acid, butyl ester,  $\alpha$ -farnesene, butanoic acid, 2-methyl-2-methylbutyl ester.

Through the analysis of the volatile oil component of okra, we discovered that the content of terpenes are high, especially for sesquiterpenes, accounting for 37.04 % of the total. The relative content of sesquiterpenes was respectively 13.24 %, 22 %, 1.8 % in leaves, flowers and seeds volatile oil. Terpenes and their ramification widely exist in nature and have perfume<sup>11</sup>. The terpenes, as good as the flavone, have doughy physiological activity as flowings: immunoloregulation, resisting neoplasm, sterilizing, relieving fever and easing pain. The sesquiterpenes which possess a high per cent in okra oil have been found that they existed in some medical plants had activity like antianaphylaxis, antiviruses and anticancer<sup>12</sup>.

Solid phase micro extraction a solvent-free sample extraction is a new sample pre-processing technique<sup>13</sup>. In recent years, the extraction matrixes in solid phase micro extraction have been developed to pretreat various biological and vegetal samples. Depending on the principle of like dissolves like, the solid phase micro extraction surface fibers of different coatings of property or thickness were selected in accordance with different compounds. To compare with conventional technique like liquid-liquid extraction, Soxhlet extraction, chromatography and steam distillation, the main advantages of solid phase micro extraction are: high sensitivity, solventless, small sample volume, simplicity and rapidity<sup>14</sup>. Solid phase micro extraction coupling with GC or GC-MS has been applied to extract drugs and chemicals from biological samples, in this experiment the method was made most use of to extract volatile oil from okra and get results effectively.

The sesquiterpenes of the okra oil enhance the efficacy of resisting neoplasm and enhance immunity. Okra can also be treated as a kind of health care vegetable for dietetic invigoration and thus more attention have been paid to its value.

TABLE-1  
ANALYTICAL RESULTS OF CHEMICAL CONSTITUENTS OF VOLATILE OIL

S. No.	Name of components	m.f.	Leave		Flower		Seed	
			RT (min)	Area of A (%)	RT (min)	Area of B (%)	RT (min)	Area of C (%)
1	Borane-methyl sulfide complex	C <sub>2</sub> H <sub>6</sub> BS	2.34	6.94	–	–	–	–
2	Ethanol	C <sub>2</sub> H <sub>6</sub> O	4.38	1.63	4.4	2.60	4.38	18.31
3	<i>n</i> -Butyl ether	C <sub>8</sub> H <sub>18</sub> O	4.95	1.64	4.93	0.43	–	–
4	Toluene	C <sub>6</sub> H <sub>5</sub> CH <sub>3</sub>	6.49	7.43	6.50	1.93	6.48	4.40
5	Butyl acetate	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	7.38	1.42	7.38	1.35	7.37	3.15
6	Hexanal	C <sub>6</sub> H <sub>12</sub> O	7.62	0.43	7.60	1.13	–	–
7	Ethyl benzene	C <sub>8</sub> H <sub>10</sub>	8.71	2.60	8.70	1.07	8.66	1.12
8	1,2-Dimethyl-benzene	C <sub>8</sub> H <sub>10</sub>	8.84	1.68	8.84	0.68	8.81	0.34
9	1,3-Dimethyl-benzene	C <sub>8</sub> H <sub>10</sub>	9.07	1.61	9.01	0.58	9.00	0.40
10	1-Butanol	C <sub>4</sub> H <sub>10</sub> O	9.25	1.59	–	–	9.24	3.21
11	Butyl ester of 2-propenoic acid	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	9.90	10.76	9.90	14.92	9.90	11.27
12	2-Hexenal	C <sub>6</sub> H <sub>10</sub> O	10.79	3.33	–	–	–	–
13	Styrene	C <sub>8</sub> H <sub>8</sub>	11.59	1.22	11.60	0.41	11.60	0.41
14	6-Methyl-5-hepten-2-one	C <sub>8</sub> H <sub>14</sub> O	13.23	1.21	–	–	–	–
15	1-Hexanol	C <sub>6</sub> H <sub>14</sub> O	13.57	1.30	–	–	13.55	0.41
16	(E)-3-Hexen-1-ol	C <sub>6</sub> H <sub>12</sub> O	14.12	0.55	–	–	–	–
17	Nonanal	C <sub>9</sub> H <sub>18</sub> O	14.28	0.69	14.28	3.11	14.26	0.28
18	Tetradecane	C <sub>14</sub> H <sub>30</sub>	14.40	1.50	14.39	0.69	–	–
19	Butanoic acid, 2-methyl-, hexyl ester	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	14.88	0.48	14.88	0.44	14.86	0.48
20	Acetic acid	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	15.20	2.65	15.13	11.15	15.20	1.67
21	O-Acetyl-hydroxylamine	C <sub>10</sub> H <sub>23</sub> NO	16.09	1.15	–	–	–	–
22	Benzaldehyde	C <sub>7</sub> H <sub>6</sub> O	16.50	4.07	16.50	1.11	16.48	0.20
23	1-Chloro-Hexadecane	C <sub>16</sub> H <sub>33</sub> Cl	16.72	0.34	–	–	18.66	0.09
24	(-)- $\alpha$ -Cedrene	C <sub>15</sub> H <sub>24</sub>	17.35	2.51	17.35	1.86	17.34	0.29
25	(+)- $\beta$ -Cedrene	C <sub>15</sub> H <sub>24</sub>	17.42	2.43	17.41	2.08	17.40	0.29
26	$\beta$ -Elemene	C <sub>15</sub> H <sub>24</sub>	17.61	1.04	17.61	0.66	17.60	0.12
27	1-Chloro-octadecane	C <sub>18</sub> H <sub>37</sub> Cl	17.69	0.84	–	–	–	–
28	Thujopsene	C <sub>9</sub> H <sub>10</sub> NOCl	18.18	0.84	18.18	0.68	–	–
29	(E)-1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene	C <sub>15</sub> H <sub>24</sub>	18.68	1.30	–	–	–	–
30	(-)-Alloaromadendrene	C <sub>15</sub> H <sub>24</sub>	18.95	0.43	–	–	–	–
31	(1R,4S,5S)-1,8-dimethyl-4-prop-1-en-2-yl-spiro[4.5]dec-8-ene	C <sub>15</sub> H <sub>24</sub>	19.01	0.34	19.00	0.32	–	–
32	(1a, 4aa, 8aa)-1,2,3,4,4a,5,6,8a-Octahydro-7methyl-4-methylene-1-(1-methylethyl)-naphthalene	C <sub>15</sub> H <sub>24</sub>	19.15	0.44	19.14	0.48	19.13	0.14
33	2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-1H-Benzocycloheptene	C <sub>15</sub> H <sub>24</sub>	19.63	1.31	–	–	–	–
34	$\zeta$ -Elemene	C <sub>15</sub> H <sub>28</sub>	19.70	0.64	–	–	–	–
35	$\alpha$ -Farnesene	C <sub>15</sub> H <sub>24</sub>	19.88	1.90	19.89	11.17	19.86	0.58
36	1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1 $\alpha$ ,4 $\alpha$ ,8 $\alpha$ )]-Naphthalene	C <sub>15</sub> H <sub>24</sub>	20.12	0.61	20.11	1.17	–	–
37	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene	C <sub>15</sub> H <sub>22</sub>	20.28	0.45	20.28	0.57	–	–
38	1,2,3,4-Tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-naphthalene	C <sub>15</sub> H <sub>24</sub>	21.18	0.48	–	–	–	–
39	6,10-dimethyl-, (E)-5,9-Undecadien-2-one	C <sub>13</sub> H <sub>22</sub> O	21.38	1.67	21.38	0.27	–	–
40	Phenylethyl alcohol	C <sub>8</sub> H <sub>10</sub> O	22.17	1.86	22.16	0.52	–	–
41	6,10,14-trimethyl-2-Pentadecanone	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	24.81	0.87	24.80	0.81	–	–
42	8 $\alpha$ H-Cedran-8-ol	C <sub>17</sub> H <sub>28</sub> O <sub>2</sub>	24.91	1.29	24.91	4.21	24.89	0.25
43	5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	27.57	0.53	27.57	0.25	–	–
44	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	29.63	0.39	29.62	0.69	29.60	0.27
45	Dimethyl sulfide	CH <sub>6</sub> SO	–	–	2.34	2.92	–	–
46	Butyl propanoate	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	–	–	9.10	2.11	9.09	1.66
47	Butyl butanoate	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	–	–	10.85	2.95	10.84	1.05
48	Butanoic acid, 2-methyl-, 2-methylbutyl ester	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	–	–	12.14	1.10	12.14	22.87
49	2,6,10,14-tetramethyl-Heptadecane	C <sub>21</sub> H <sub>44</sub>	–	–	15.05	0.34	–	–
50	$\alpha$ -Cubebene	C <sub>15</sub> H <sub>24</sub>	–	–	15.46	0.42	–	–
51	O-Decyl-hydroxylamine	C <sub>10</sub> H <sub>23</sub> NO	–	–	16.09	1.01	–	–
52	Propanoic acid	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	–	–	16.66	0.38	–	–
53	Pentyl-cyclopropane	C <sub>8</sub> H <sub>16</sub>	–	–	17.04	0.53	–	–
54	Cedrene	C <sub>15</sub> H <sub>24</sub>	–	–	17.69	0.59	19.62	0.28
55	3-Methyl-butanoic acid	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	–	–	18.70	1.40	–	–

S. No.	Name of components	m.f.	Leave		Flower		Seed	
			RT (min)	Area of A (%)	RT (min)	Area of B (%)	RT (min)	Area of C (%)
56	à-Cubebene	C <sub>15</sub> H <sub>24</sub>	–	–	19.54	0.30	–	–
57	2,6,6-trimethyl-1,3-Cyclohexadiene-1-carboxaldehyde	C <sub>10</sub> H <sub>14</sub> O	–	–	18.47	0.23	–	–
58	Longifolene-(V4)	C <sub>15</sub> H <sub>24</sub>	–	–	19.64	1.41	–	–
59	1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-,[2R-(2à,4aà,8aá)]-naphthalene	C <sub>15</sub> H <sub>24</sub>	–	–	19.69	0.80	–	–
60	1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1S-(1à,4aá,8aà)]-Naphthalene	C <sub>15</sub> H <sub>24</sub>	–	–	20.11	1.17	–	–
61	3-Bromo-3-methylbutyric acid	C <sub>5</sub> H <sub>9</sub> BrO <sub>2</sub>	–	–	20.52	2.90	–	–
62	1-Methyl-4-(1,2,2-trimethylcyclopentyl)-(R)-Benzene	C <sub>15</sub> H <sub>22</sub>	–	–	21.07	0.33	–	–
63	Acetic acid, methyl ester	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	–	–	–	–	2.89	2.53
64	Ethyl acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	–	–	–	–	3.56	4.26
65	Butyl caprylate	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	–	–	–	–	16.94	0.69
66	2-Methylbutyl heptanoate	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	–	–	–	–	17.04	3.38
67	à-Caryophyllene	C <sub>15</sub> H <sub>24</sub>	–	–	–	–	18.88	0.38
68	Germacrene D	C <sub>15</sub> H <sub>22</sub>	–	–	–	–	19.45	0.46

The flowers of okra were hoped to be used as a new material to extracting sesquiterpenes.

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