Comparison of Volatile Components in Tea Blossom Obtained by Supercritical CO_2, Subcritical CO_2, and Petroleum Ether Extractions

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Abstract: The volatile components of tea blossom were extracted by supercritical CO_2, subcritical CO_2, and petroleum ether medium extractions. Gas chromatography-mass spectrometry was applied to isolate and identify the volatile components in the three extracts. Principal component analysis, using the SPSS software, was adopted to analyze and compare the main flavors of the three extracts. Results showed that there were, in total, 58 volatile components in tea blossom. Of these, 55, 50, and 54 components were identified in the supercritical CO_2, subcritical CO_2, and petroleum ether extracts, respectively. They included 17 fatty hydrocarbons and aromatic hydrocarbons, 4 terpenes, 6 terpenols and terpenones, 4 aromatic alcohols and aromatic ketones, 2 aliphatic alcohols, 6 aliphatic ketones, 2 aldehydes, 5 esters, and 7 acids. The major volatile compounds of tea blossom included 2-hydroxy-2-phenylacetophenone (11.04, 10.23, and 8.41 μg/g, respectively, in the supercritical CO_2, subcritical CO_2, and petroleum ether extracts; same below), phenethylol (11.49, 9.98, and 8.52 μg/g), α-phenethylol (13.91, 12.30, 9.72 μg/g), linalool oxide (5.34, 6.35, and 4.38 μg/g), benzyl alcohol (6.60, 5.34, and 4.43 μg/g), 1,8-dimethyl-4-isopropyl-8,9-epoxy theaspirone (3.68, 2.37, and 1.61 μg/g), and β-ionone (2.67, 1.76, and 1.16 μg/g). The two principal components were deduced based on an analysis by SPSS. The fragrances of the supercritical CO_2 extract were found to be richer than those of the subcritical CO_2 and petroleum ether extracts.

Key words: tea blossom; supercritical; subcritical; extractions; gas chromatography-mass spectrometry; volatile

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超临界 CO_2、亚临界 CO_2 与石油醚萃取的茶树花精油的挥发性成分的对比

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摘要：分别利用超临界 CO_2、亚临界 CO_2 与石油醚萃取茶树花精油，采用 GC-MS 分离和鉴定这 3 种萃取方法得到的茶树花精油的挥发性成分，再运用主成分分析程序分析和比较这三个样品的主要挥发性物质。结果表明：茶树花萃取物中共有 58 种挥发性成分，其中超临界 CO_2、亚临界 CO_2 与石油醚的萃取样品各有 55、50 和 54 种成分。有 17 种脂肪烃与芳香烃，4 种萜烯、6 种萜烯醇与萜烯酮、4 种芳香醇与芳香酮、2 种脂肪醇、6 种脂肪酮、2 种醛、5 种酯、7 种酸。茶树花的主要挥发性成分有苯乙酮（超临界、亚临界与石油醚萃取样品中分别含有 11.04、10.23、8.41 μg/g。以下也按此顺序表示）、苯乙醇（11.49、9.98、8.52 μg/g）、α-苯乙醇（13.91、12.30、9.72 μg/g）、氧化芳樟醇（5.34、6.35、4.38 μg/g）、苯甲醇（6.60、5.34、4.43 μg/g）、1, 8-二甲基-4-异丙基-8, 9 环氧-茶螺酮（3.68、2.37、1.61 μg/g）、β-紫罗兰酮（2.67、1.76、1.16 μg/g）。主成分分析结果显示萃取物样品的挥发性成分共有 2 种主成分。超临界萃取样品的香气成分比亚临界与石油醚萃取的更丰富。

关键词：茶树花；超临界；亚临界；萃取；气质联用；挥发性成分

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1 Introduction

Tea (*Camellia sinensis*) is one of the important farm crops in China and other Asian countries and tea blossom is a by-product of tea plantation. The yield ratio of tea-to-tea blossom is generally about 5:1, depending on the differences in the cultivar and cultivation modes of tea farmers. In China for example, the tea product and tea blossom output in 2010 was about 1,475,069 and 295,014 ton, respectively. However, as a bio-resource, tea blossom has not been utilized appropriately so far.

Tea blossom contains many functional components such as polyphenols, polysaccharides, caffeine, amino acids, and others. Furthermore, it has a floral and fresh fragrance. Based on these characteristics, tea blossom has potential applications in the food industry, daily chemical series products, and even pharmaceutical industry. Moreover, recently, tea blossom has applied in production of tea flower beverage, ice cream, shampoo, and toothpaste with special flavor.

Although many studies have been carried out with a focus on tea and tea leaves, only a few studies focused on tea blossom. Lin *et al*. (2003) have carried out research on the determination of tea polyphenols and caffeine in tea blossom and their hydroxyl radical scavenging and nitric oxide suppressing effects [1]. Yang *et al*. (2007) have studied the antioxidant activity of tea blossom [2]. Joshi *et al*. (2011) have investigated the biochemical attributes of tea blossom at different stages of development in the Kangra region of India [3]. The components reported in this study including polyphenols, catechins, flavanoids, proanthocyanidins, proteins, some enzymes, and glycoside bound volatile components. Joshi et al. (2011) used simultaneous distillation extraction and supercritical fluid extraction to isolate the volatile flavor components of tea blossom, while the analysis and comparison were performed by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), and headspace analysis [4].

A fluid becomes supercritical when it is compressed to a pressure and elevated to a temperature greater than that of its critical point. A supercritical fluid has a high absorption capacity [5]. On the other hand, a fluid is in a subcritical state when its temperature is higher than its boiling point but the temperature and pressure all lower than the critical temperature and pressure; subcritical fluid extraction (SFE), also known as pressurized low-polarity fluid extraction [6], is a popular and efficient extraction method. Supercritical and subcritical fluids are widely used for the extraction of active ingredients such as essential oils.

In the present research, three methods, viz. supercritical CO_2 extraction, subcritical CO_2 extraction, and petroleum ether medium extraction, were employed to obtain different tea blossom extracts. The volatile components in these tea blossom extracts were identified and quantified by GC-MS. Principal component analysis was performed to compare the aromas of the three isolated extracts.

2 Experimental

2.1 Tea blossom sample preparation

Tea blossom was collected from the tea plantation of the Tea Research Institute, Guangdong Academy of Agricultural Sciences. After dehydration, by air-drying at 60 ℃, to about 11% moisture content, the dried flower was ground into 10 mesh size and subjected to supercritical CO_2, subcritical CO_2, and petroleum ether medium extractions.

2.2 Chemicals

Analytical grade petroleum ether and chromatographically pure chloroform were used, while analytical grade ethyl caprate was used as the internal standard for GC analysis.

2.3 Preparation of teablossom extract by supercritical CO_2

The parameters of supercritical CO_2 extraction were set at pressure 35 MPa, temperature 48 ℃, and extraction time 2 h; 43% (*m/m*) ethanol was added as the entrainer. The extracts were concentrated using a rotary vacuum evaporator and subsequently vacuum dried at 40 ℃. The yield of tea flower extract was calculated by equation 1 and is denoted as Y_{super}.

$$Y(\%) = \frac{m}{M} \times 100\% \quad (1)$$

Note: Y- yield of tea flower extract, in %; m- weight of tea flower extract, in g;M - weight of dry flower used for extraction, in g.

2.4 Preparation of tea flower extract by subcritical CO_2

The parameters of subcritical CO_2 extraction were set at pressure 20 MPa, temperature 25 ℃, and extraction time 2 h; 43% (*m/m*) ethanol was added as the entrainer. The extracts were concentrated using a rotary vacuum evaporator and subsequently vacuum dried at 40 ℃. The yield of the tea blossom extract was calculated by equation 1 and is denoted as Y_{sub}.

2.5 Preparation of tea blossom extract by petroleum ether medium

Tea blossom was extracted at 60 ℃, with petroleum ether at a ratio of 1:8 [blossom weight (g)/ petroleum ether (mL)]. The entire extraction was performed twice; the first extraction took 2 h and the second extraction took 1 h. The extracts were concentrated using a rotary vacuum evaporator and subsequently vacuum dried at 40 ℃. The yield of tea blossom was calculated by equation 1 and is denoted as Y_{petro}.

2.6 Preparation of internal standard solution

In this step, 0.1 mL ethyl caprate was accurately poured into a 100 mL volumetric flask; subsequently, chloroform was pipetted into the flask and the volume was made up to 100 mL. Thus, the concentration of the ethyl caprate standard solution was fixed at 0.865 mg/mL; this solution was stored in a refrigerator at 4 ℃.

2.7 Sample preparation for GC-MS analysis

For GC-MS analysis, 1.000 g of each sample of the tea blossom extracts, obtained by the three extractions, was accurately weighed and loaded into a 100 mL volumetric flask. The ethyl caprate standard solution was then pipetted into the flask and the volume was made up to 50 mL; the prepared solutions were stored in a refrigerator at 4 ℃.

2.8 GC-MS analysis

GC-MS analysis was performed using 6890 GC (Agilent) and 5975 MS (Agilent) equipped with a capillary column (HP-INNOWax Polyethylene Glycol, 30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Thermal desorption of the compounds from the column was performed in a GC splitless injector at 230 ℃. The oven temperature was programmed to increase from 70 to 100 ℃ at 5 ℃/min; thereafter, the temperature was increased to 230 ℃ at 4 ℃/min. The volatile compounds were analyzed by the MS, which was equipped with a 70 eV quadripolar filter (electronic impact (EI)). The ion beam temperature was 230 ℃ and the mass range for this acquisition was 30~350 amu. All compounds were identified by comparison with the NIST library spectral data bank. The compounds with similarity higher than 80% were reported; those with similarity lower than 80% were also listed because they are reported to be the characteristic fragrances of tea blossom.

Quantitative analysis was based on the ratio of the peak area of the detected components to the total peak area of all components. Ethyl caprate was the internal standard. The quantities of each component were calculated according to the following equations:

$$W_i = fA_i \tag{2}$$

$$W_i(\mu g) = \frac{865A_i}{A_{std}} \times Y \tag{3}$$

$$f = \frac{W_{std}}{A_{std}} = \frac{C_{std}V_{std}}{A_{std}} = \frac{0.865\,mg/mL \times 1mL}{A_{std}} = \frac{865\mu g}{A_{std}} \tag{4}$$

Note: W - weight of each component, in μg/g, of tea flower; i - number of each peak; f - correction factor; A - area of each peak; A_{std} - area of the internal standard peak; Y - yield of tea flower extracts, in %.

2.9 Principal component analysis

Principal component analysis (PCA) was performed, using SPSS version 17.0 (SPSS Inc., Chicago, USA), to identify the most important volatile compounds in the samples.

3 Results and discussion

3.1 Sparation and identification of volatile components by GC-MS

The yields of tea flower extracts by supercritical CO_2, subcritical CO_2, and petroleum ether medium were 2.785±0.068%, 1.484±0.063% and 2.541±0.082 respectively. The yield of supercritical CO_2 extraction was the highest, followed by petroleum ether medium extraction and the subcritical CO_2 extraction. GC-MS analysis and identification of the composition of the three extracts show the total ion chromatograms as figure 1. The isolated peaks were identified by searching the NIST library and comparison with the correlative references. The identified components of the tea flower extracts are listed in table 1 and the compounds were presented in order of elution.

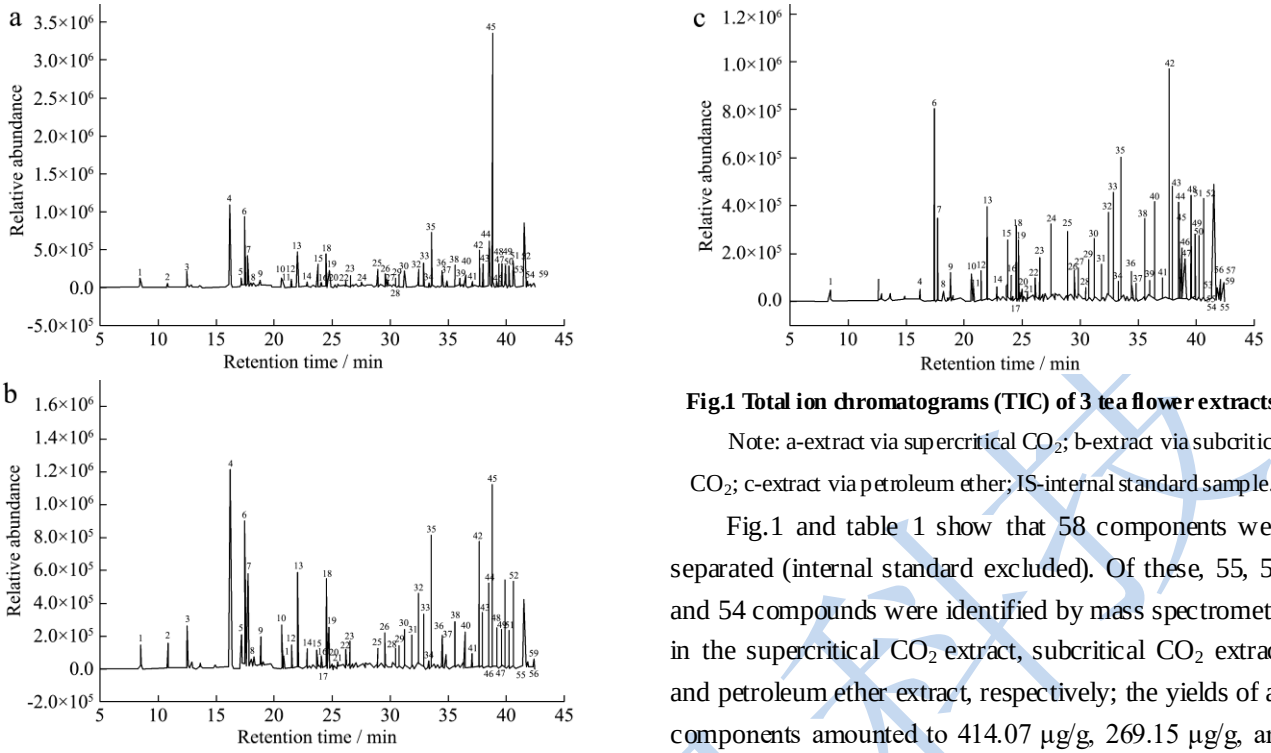

Fig.1 Total ion chromatograms (TIC) of 3 tea flower extracts

Note: a-extract via supercritical CO_2; b-extract via subcritical CO_2; c-extract via petroleum ether; IS-internal standard sample.

Fig.1 and table 1 show that 58 components were separated (internal standard excluded). Of these, 55, 50, and 54 compounds were identified by mass spectrometry in the supercritical CO_2 extract, subcritical CO_2 extract, and petroleum ether extract, respectively; the yields of all components amounted to 414.07 μg/g, 269.15 μg/g, and 316.32 μg/g, respectively.

Table 1 Volatile components and their contents in tea flower extracts by different extraction methods

Peak no.	Components	Weight of each component[a]/(μg/g)		
		Supercritical CO_2 extraction	Subcritical CO_2 extraction	Petroleum ether medium extraction
1	Octyl aldehyde	3.18	2.47	1.16
2	2- Nonanone	1.66	2.57	nd[b]
3	Acetic acid	4.69	3.93	nd
4	Propylene glycol	28.22	20.82	1.31
5	Butyrate	2.72	3.07	nd
6	Ethyl caprate[c]	-	-	-
7	2-Hydroxy-2-phenylacetophenone	11.04	10.23	8.41
8	Terpineol	1.56	1.26	0.50
9	Dioctylmethane	2.62	3.68	4.23
10	Linalool oxide	5.34	6.35	4.38
11	Hexadecane	2.72	3.02	3.58
12	Octadecane	3.38	2.97	4.08
13	α-Phenethylol	13.91	12.30	9.72
14	n-Heptanoic acid	4.59	3.78	2.07
15	Benzil alcohol	6.60	5.34	4.43
16	Nonadecane	4.08	3.07	4.64
17	Menthyl salicylate	nd	0.91	0.76
18	Phenethylol	11.49	9.98	8.52
19	α-Pinene	6.40	4.89	7.61
20	β-Ionone	2.67	1.76	1.16
21	Camphene	nd	1.06	2.07

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22	β-Pinene	4.08	4.79	5.19
23	Eicosane	5.09	3.38	5.59
24	Squalene	2.02	nd	8.57
25	Heneicosane	6.75	2.47	8.62
26	Phytone	7.41	6.10	5.69
27	Caffeine	2.67	nd	5.54
28	1,8-Dimethyl-4-isopropyl-8,9-epoxy theaspirone	3.68	2.37	1.61
29	Globulol	4.49	2.72	5.09
30	Docosane	6.10	4.69	7.71
31	Dibutyl phthalate	nd	3.68	4.38
32	Ethyl palmitate	6.30	8.77	10.83
33	2,3-Dihydro-3,5-dyhydroxyl-6-methyl-4H-pyran-4-one	8.97	6.30	10.13
34	1,2,3-Trimethyl-4-propenylnaphthalene	2.02	1.16	2.77
35	2,6,10,14-Tetramethylhexadecane	20.31	16.23	18.54
36	Stearaldehyde	6.15	4.08	3.43
37	2-methyl-3-butylene-2-ol	3.98	3.33	1.56
38	tetracosane	7.81	5.39	9.88
39	2-cyclopropyl-2-methyl-N-(1-cyclopropyl ethyl)-cyclopropyl formamide	3.23	nd	2.77
40	2-Nonadecanone	6.80	3.98	11.24
41	Triethyl citrate	2.62	2.37	3.63
42	3-Methyl dioctylmethane	12.40	13.81	16.17
43	Eicosylene	3.93	2.97	6.35
44	Propyl octyl phthalate	17.64	10.74	12.90
45	Palmitic acid	89.76	21.37	6.60
46	Linoleic acid	1.86	0.55	2.72
47	Linolenic acid	2.92	0.86	2.47
48	Tricosane	10.48	4.69	12.55
49	Phytol	9.58	8.92	7.76
50	3-Dodecyl-2,5-furandione	3.73	nd	7.81
51	1,2-Dihydro-4-phenyl-naphthalene	9.93	5.70	10.13
52	1-Cyclododecyl-Ethanone	8.97	9.53	11.79
53	N-(4-methoxyl-phenyl)-succinamic acid-p-tolyl ester	2.27	nd	3.93
54	Longipinene	3.28	1.11	2.27
55	6,10-Dimethyl-9-undecenyl-2-one	2.17	1.26	2.17
56	Decalin-8a-ethyl-1,1,4a,6-tetramethyl naphthalene	1.66	nd	2.42
57	2-Methyl-cis-7,8-epoxy nonadecane	0.76	nd	2.32
58	5-(Decalin-5,5,8a-trimethyl-2-methylene-1-naphthyl)-3-methyl-2-pentenoic acid	2.17	nd	nd
59	3,4,5-Trimethoxy-methyl benzoate	3.23	2.37	4.59
	In all	414.07	269.15	316.32

Note: a - Quantitative analysis was based on the ratio of the peak area of a particular component and the total peak area of all components, ethyl caprate was the internal standard, equations 1-3 were used for the calculations; b - nd means not detected; c - The peak of ethyl caprate was excluded from the total peak area.

Table 2 Classifications and contents of volatiles in tea blossom extracts by different extraction methods

Classifications	Supercritical CO_2 extraction			Subcritical CO_2 extraction			Petroleum ether medium extraction		
	No.	Contents /(μg/g)	Perce-ntage /%	No.	Contents /(μg/g)	Perce-ntage /%	No.	Contents /(μg/g)	Percen-tage /%
Fatty hydrocarbons and aromatic hydrocarbons	17	102.06	21.27	14	73.23	24.66	17	128.13	35.29
Terpenes	4	18.24	3.80	5	14.57	4.91	5	22.22	6.12
Terpenols and terpenones	6	30.24	6.30	6	26.76	9.01	6	21.11	5.81
Aromatic alcohols and aromatic ketones	4	43.04	8.97	4	37.85	12.74	4	31.09	8.56
Aliphatic alcohols	2	32.20	6.71	2	24.14	8.13	2	2.87	0.79
Aliphatic ketones	6	32.31	6.73	5	23.64	7.96	5	43.13	11.88
Aldehydes	2	9.32	1.94	2	6.55	2.21	2	4.59	1.26
Esters	5	32.05	6.68	6	28.83	9.71	7	41.01	11.30
Acids	7	108.71	22.65	6	33.57	11.30	4	13.86	3.82
Others	2	5.90	1.23	0	0.00	0.00	2	8.31	2.29
In all	55	414.07	86.28	50	269.14	90.63	54	316.32	87.13

Table 2 shows that among the identified components, there are 17 fatty hydrocarbons and aromatic hydrocarbons, 4 terpenes, 6 terpenols and terpenones, 4 aromatic alcohols and aromatic ketones, 2 aliphatic alcohols, 6 aliphatic ketones, 2 aldehydes, 5 esters, 7 acids and 2 others. The percentages of these identified components in the whole isolated volatiles of the three extracts reach about 86.28%, 90.63% and 87.13% respectively.

Tea flowers are floral, fresh and of fruity odour[4]. The main categories of flavors in tea flower which form the typical tea flower fragrances are considered as aromatic alcohols and aromatic ketones, terpenols and terpenones, aliphatic alcohols, aldehydes and some esters. Moreover, the aliphatic alcohols and menthyl salicylates are of gramineous flavor[5].

On the basis of table 1 and 2, the content of each component was found different, although the classification for these compounds in the three extracts is similar. The contents of the volatiles of tea flower extract by supercritical CO_2 are ranked as acids (22.65%), followed by fatty hydrocarbons and aromatic hydrocarbons (21.27%), aromatic alcohols and aromatic ketones (8.97%), aliphatic ketones (6.73%), aliphatic alcohols (6.71%), esters (6.68%), terpenols and terpenones (6.30%), terpenes (3.80%), aldehydes (1.94%) and others (1.23%). In the supercritical CO_2 extract, the main aromas which contribute to the fresh and sweet flavor of tea flower[7] were found as 2-hydroxy-2-phenylacetophenone (11.04 μg/g, 2.30%), phenethylol (11.49 μg/g, 2.39%), α-phenethylol (13.91 μg/g, 2.90%), linalool oxide (5.34 μg/g, 1.11%) and benzil alcohol (6.60 μg/g, 1.38%). Besides, propylene glycol (28.22 μg/g, 6.82%) and 2-methyl-3-butylene-2-ol (3.98 μg/g, 0.96%) were found to present the gramineous smell of tea flower[7].

In the subcritical CO_2 extract, the contents of different volatiles are ranked as fatty hydrocarbons and aromatic hydrocarbons (24.66%), followed by aromatic alcohols and aromatic ketones (12.74%), acids (11.30%), esters (9.71%), terpenols and terpenones (9.01%), aliphatic alcohols (8.13%), aliphatic ketones (7.96%), terpenes (4.91%) and aldehydes (2.21%) orderly. The important fragrances[4] were found as 2-hydroxy-2-phenylacetophenone (10.23 μg/g, 3.45%), phenethylol (9.98 μg/g, 3.36%), α-phenethylol (12.30 μg/g, 4.14%), linalool oxide (6.35 μg/g, 2.14%) and benzil alcohol (5.34 μg/g, 1.80%) etc. There were plenty of palmitic acids and alkanes both in the supercritical CO_2 and subcritical CO_2 extracts but they exhibited few influence to the major odor of tea flower.

As for petroleum ether medium extract, the contents of volatiles are ranked as fatty hydrocarbons and aromatic hydrocarbons (35.29%), followed by aliphatic ketones

(11.88%), esters (11.30%), aromatic alcohols and aromatic ketones (8.56%), terpenes (6.12%), terpenols and terpenones (5.81%), acids (3.82%), aldehydes (1.26%) and aliphatic alcohols (0.79%). The basilic aromas [4, 7] involve in 2-hydroxy-2-phenylacetophenone (8.41 μg/g, 2.32%), phenethylol (8.52 μg/g, 2.35%), α-phenethylol (9.72 μg/g, 2.68%), linalool oxide (4.38 μg/g, 1.21%) and benzil alcohol (4.43 μg/g, 1.22%).

The supercritical CO_2 extract were found containing the highest total content of volatiles. Thereinto, acids, aromatic alcohols and aromatic ketones, terpenols and terpenones, aliphatic alcohols are involved in. The total contents of flavors in the subcritical CO_2 extract are lowest, while the contents of terpenols and terpenones, aromatic alcohols and aromatic ketones, aliphatic alcohols aldehydes are the middle in three extracts. In the petroleum ether extract, the amounts of hydrocarbons, aliphatic ketones and esters are highest, but aromatic alcohols and aromatic ketones, terpenols and terpenones, as the characteristic odours of tea flower, are lower than supercritical CO_2 extract and subcritical CO_2 extract. The quantities of palmitic acid in the supercritical extract are the highest. This may be resulted from the extraction processing. Further rational research is necessary to perform on the higher content of propylene glycol in the supercritical and subcritical samples than that in the petroleum ether extract.

Moreover, some of identified compounds are of low volatility and some are odorless. So the olfactory sensation research also should be conducted in our forward research in order to find out the aroma contribution of various volatiles. Besides, dibutylphthalate is probably a plasticizer contaminant which is from the plastic package materials of tea flowers.

3.2 Volatile comparison by principal component analysis

Table 3 Loading matrix of principal components

components		Loading matrix		Rotated loadings matrix[a]	
		Prin1	Prin2	Prin1	Prin2
AA1	2-Hydroxy-2-phenylacetophenone	0.979	-0.206	0.965	-0.262
AA2	α-Phenethylol	0.993	-0.122	0.984	-0.179
AA3	Benzil alcohol	0.995	0.103	0.999	0.045
AA4	Phenethylol	1.000	0.020	0.999	-0.038
T1	Terpineol	0.973	-0.233	0.957	-0.289
T2	Linalool oxide	0.496	-0.868	0.445	-0.895
T3	α-Pinene	-0.453	0.891	-0.401	0.916
T4	β-Ionone	0.992	0.128	0.998	0.071
T5	Camphene	-1.000	-0.024	-0.999	0.033
T6	β-Pinene	-0.986	-0.170	-0.994	-0.112
T7	Phytone	0.954	0.299	0.970	0.243
T8	1,8-Dimethyl-4-isopropyl-8,9-epoxy theaspirone	0.987	0.162	0.995	0.105
T9	Globulol	-0.254	0.967	-0.197	0.980
T10	Phytol	0.989	-0.146	0.979	-0.203
T11	Longipinene	0.456	0.890	0.507	0.862
FA1	Propylene glycol	0.970	-0.241	0.955	-0.297
FA2	2-methyl-3-butylene-2-ol	0.969	-0.248	0.953	-0.304
AL1	Octyl aldehyde	0.987	-0.159	0.976	-0.216
AL2	Stearaldehyde	0.954	0.299	0.970	0.243
A1	Palmitic acid	0.933	0.359	0.953	0.304
H1	2,6,10,14-Tetramethylhexadecane	0.423	0.906	0.475	0.880
H2	3-Methyl dioctylmethane	-0.991	0.134	-0.982	0.191
H3	Tricosane	-0.264	0.964	-0.208	0.978
Z1	Propyl octyl phthalate	0.664	0.748	0.706	0.708

Note: a - The rotating method of the principal components was varimax and the number of rotating iterations was 3.

Principal component analysis was performed for interpreting the characteristic aroma and explaining the differences in the supercritical CO_2, subcritical CO_2, and petroleum ether extracts. Table 3 lists the 24 major components of the three extracts analyzed using SPSS, including the typical tea blossom fragrances and some high quantity compounds.

The loading matrices of the 24 volatile compounds with respect to the two principal components are shown in table 3. In order to simplify the principal components, the loadings were increased with the rotating method by adjusting the relative positions of the factor axes. The rotation showed no impact on the fitting of the data, while the value of loading showed correlation with the principal component; higher the absolute value the greater the correlation. The rotated cumulative variance contribution rate of the first two principal components was found to reach 100%, wherein the first and second principal components account for 73.702% and 26.298%, respectively (table 4).

Table 4 Initial and rotated eigenvalues of principal components

Principal components	Factors loadings			Rotated factors loadings		
	Eigenvectors	Variance contribution rate /%	Cumulative variance contribution rate /%	Eigenvectors	Variance contribution rate /%	Cumulative variance contribution rate /%
1	17.727	73.863	73.863	17.689	73.702	73.702
2	6.273	26.137	100.000	6.311	26.298	100.00

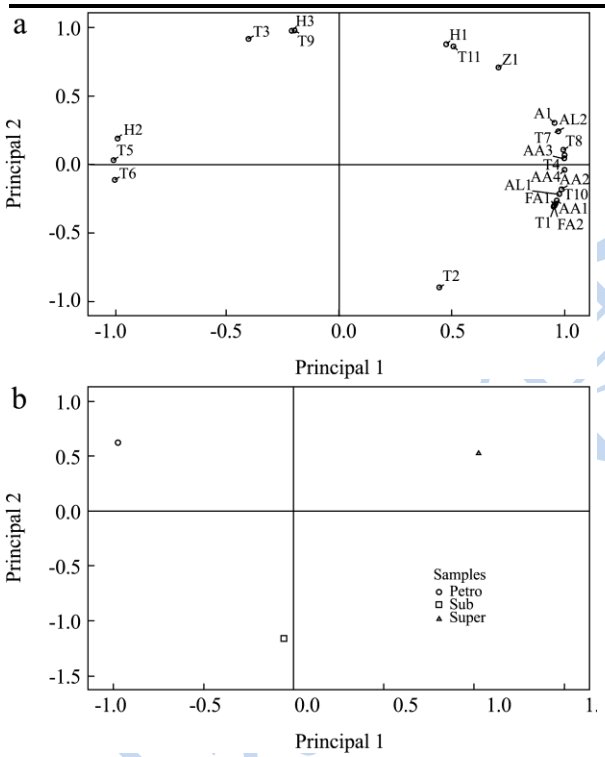

Fig.2 Loading and scores scatter plots by principal component analysis

Note: a-Loading plot of the principal components; b-Score scatter plot of the samples defined by the two principal components The letter plus the number indicate the code of compounds corresponding to the same code in table 4. Samples identification: Petro-Petroleum ether medium extraction; Sub-Subcritical CO_2 extraction; Super-Supercritical CO_2 extraction.

The relationship between the 24 compounds and two principal components is illustrated in fig.2a. The aromatic compounds that exhibited a positive correlation with prin1 were phenethylol (AA4, loading 0.999), benzyl alcohol (AA3, loading 0.999), β-ionone (T4, loading 0.998), 1,8-dimethyl-4-isopropyl-8,9-epoxy theaspirone (T7, loading 0.995), α-phenethylol (AA2, loading 0.984), and 2-hydroxy-2-phenylacetophenone (AA1, loading 0.965). These aromatic compounds determine the fresh and sweet flavor of tea blossom (Wang, 2008). On the other hand, camphene (T5, loading -0.999) and β-pinene (T6, loading -0.994) also exhibited a close relationship with prin1, although the loading was negative, which might result in the wood- and turpentine-like flavor of the terpene compounds. As for prin2, globulol (T9, loading 0.980) and tricosane (H3, loading 0.978) showed a close relationship with the principal component. Globulol has been reported to be a major constituent of the essential oils of some flowers and trees such as eucalyptus flower [8], *Melodorum fruticosum* flower [9], and eucalyptus [10], suggesting that it has a herbal and wood-like smell. Tricosane is also found in the essential oils of rose [11], *Polygonum bistorta* flower [12], and virgin olive oil [13].

In fig.2b, the score of the supercritical CO_2 samples is located on the positive axes of both principal components, while the score of the subcritical CO_2 samples is located on the negative axis of prin2. The score of the petroleum ether extraction samples is located on the negative axis of prin1 as well as the positive axis

of prin2. From these results, it can be inferred that the fragrances of the supercritical CO_2 extract are richer than those of the subcritical CO_2 and petroleum ether extracts.

4 Conclusions

4.1 Gas chromatographic separation and mass spectrometric identification show that there are, in total, 58 volatile components in tea flower. Of these, 55, 50, and 54 compounds were identified in the supercritical CO_2 extract, subcritical CO_2 extract, and petroleum ether extract, respectively. These components include 17 fatty hydrocarbons and aromatic hydrocarbons, 4 terpenes, 6 terpenols and terpenones, 4 aromatic alcohols and aromatic ketones, 2 aliphatic alcohols, 6 aliphatic ketones, 2 aldehydes, 5 esters, and 7 acids. In the supercritical CO_2 extract, the fresh and sweet aromas are due to 2-hydroxy-2- phenylacetophenone, phenethylol, α-phenethylol, linalool oxide, and benzyl alcohol. The gramineous flavor is due to propylene glycol and 2-methyl-3- butylene-2-ol. The main odor components of the subcritical CO_2 extract were 2-hydroxy-2 -phenylacetophenone, phenethylol, α-phenethylol, linalool oxide, and benzyl alcohol. For the petroleum ether extract, the major fragrance compounds are 2-hydroxy-2-phenylacetophenone, phenethylol, α-phenethylol, linalool oxide, and benzyl alcohol.

4.2 The cumulative variance contributions of the two principal components (prin1 and prin2) are 73.702% and 26.298%, respectively. Phenethylol, benzyl alcohol, β-ionone, 1,8 - dimethyl- 4- isopropyl- 8,9 - epoxy theaspirone, α- phenethylol, and 2-hydroxy -2-phenylacetophenone show a positive relationship with prin1, while globulol and tricosane show a close relationship with prin2.

References

[1] Lin Y S, Wu S S, Lin J K. Determination of tea polyphenols and caffeine in tea flowers (*Camellia sinensis*) and their hydroxyl radical scavenging and nitric oxide suppressing effects [J]. Journal of Agricultural and Food Chemistry, 2003, 51(4): 975-980

[2] Yang Z, Xu Y, Jie G. Study on the antioxidant activity of tea flowers (Camellia sinensis) [J]. Asia Pacific Journal of Clinical Nutrition, 2007, 16(Suppl.1): 148-152

[3] Joshi R, Poonam A Gulati. Biochemicalattributes of teaflowers (*Camellia sinensis*) at different developmental stages in the Kangraregion of India [J]. Scientia Horticulturae, 2011, 130(1): 266-274

[4] Joshi R, Poonam R, Saini S, et al. Characterization of volatile components of tea flowers (*Camellia sinensis*) frowing in Kangra by GC/MS [J]. Natural Product Communications, 2011, 6(8): 1155-1158

[5] Leslie Harrison, Tracie Mustain, Bryan Williams. Supercritical CO_2 extraction [EB/OL]. 1997-03-07 [2013-10-10]. http://www.calpoly.edu/~ceenve/enve/ supercrit. html

[6] Liu Zeng-gen, Mei Li-juan, Wang Qi-lan, et al. Optimization of subcritical fluid extraction of seed oil from *Nitraria tangutorum* using response surface methodology [J]. LWT-Food Science and Technology, 2014, 56(1): 168-174

[7] Wang L L. Study on aroma components of tea (*Camellia sinensis*) flowers [D]. College of Food Scienee & Biotechnology Engineering, vol. Master (pp. 33-42). 2008, Hangzhou, China: Zhejiang Gongshang University

[8] Bamoniri A, Mazoochi A, Mirjalili B B F, et al. Survey of the bioctive and fragrant constituents separated by nano scale injection of eucalyptus camaldulensis Var. obtusa cultivated in Kashan Area [J]. Digest Journal of Nanomaterials and Biostructures, 2009, 4(4): 603-606

[9] Pripdeevech P, Chukeatirote E. Chemical compositions, antifungal and antioxidant activities of essential oil and various extracts of *Melodorum fruticosum L.* flowers [J]. Food and Chemical Toxicology, 2010, 48(10): 2754-2758

[10] Elaissi A, Rouis Z, Mabrouk S, et al. Correlation between chemical composition and antibacterial activity of essential oils from fifteen eucalyptus species growing in the Korbous and Jbel Abderrahman Arboreta (North East Tunisia) [J]. Molecules, 2012, 17(3): 3044-3057

[11] Verma R S, Padalia R C, Chauhan A. Chemical investigation of the volatile components of shade-dried petals of damask rose (rose damascena mill) [J]. Archives of Biological Sciences, 2011, 63(4): 1111-1115

[12] Iskender N Y, Gulec C A, Yucel M, et al. Analysis of the essential oil from the flower of polygonum *bistorta L.* subsp carneum (Koch) [J]. Asian Journal of Chemistry, 2011, 23(5): 1940-1942

[13] Baccouria B, Temimea S B, Campeolb E, et al. Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars [J]. Food Chemistry, 2007, 102(3): 850-856

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