



Isolation and Characterization of Essential Oil Extracted from Tangerine Peel

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Essential oil was extracted from tangerine peel by supercritical fluid CO₂(SCF-CO₂) and its chemical composition was detected by GC-MS. Based on orthogonal array design test, the optimum extraction conditions were screened as extraction pressure 250 bar, temperature 45 °C, tangerine peel particle size less than 0.35 mm and extraction time 120 min. Thirty nine volatile compounds were identified in the essential oil and the most abundant volatiles were α -farnesene, β -elemene and limonene.

Keywords: *Citrus reticulata*, α -Farnesene, β -Elemene, Limonene, GC-MS.

INTRODUCTION

Citrus essential oil, a natural mixture of volatile compounds composed mainly of monoterpene hydrocarbons, is confirmed to have many bioactive and physiological activities such as germicidal, antioxidant and anticarcinogenic properties¹⁻³. It was reported that high concentration of *d*-limonene, a bioactive component found in citrus peel oil, had anticancer effects in preclinical studies of mammary carcinogenesis⁴. Citrus essential oil has been applied in food staffs, cosmetics, air-fresheners and medicines^{3,4}.

Citrus essential oil is usually extracted from the citrus peel, a by-product during orange juice manufacture. The traditional methods for preparing essential oils from citrus are cold pressing or distillation methods⁵. During cold pressing, citrus essential oil is agitated vigorously with water, resulting in diminution in citral and terpene alcohols contents and creating conditions leading to hydrolysis, oxidation and resinification. When distillation or hydrodistillation is used, the elevated temperatures and prolonged extraction time lead to chemical modifications of the oil components and often a loss of volatile molecules, especially thermolabile compounds^{6,7}. These shortcomings have led to consideration of the use of new techniques such as supercritical fluid extraction to enhance quantity and yield of essential oil⁸. Supercritical fluid extraction was extensively used in separation of active compounds from herbs since it is an environmentally responsible and efficient extraction progress. It is also a flexible process due to the possibility of continuous modulation of the solvent power and selectivity of the supercritical fluid. It does not need the expensive post-processing of the extracts for solvent elimination^{8,9}.

In the present paper, supercritical CO₂ was used to extract essential oil from tangerine peel (*Citrus reticulata* Banco) based on an orthogonal array design and chemical composition of the essential oil was identified by GC-MS method.

EXPERIMENTAL

The tangerine peel (*Citrus reticulata* Banco) purchased from Hangzhou Herbs Ltd. (Hangzhou, China) was dried at 45 °C for 7 h to a moisture content 84 mg kg⁻¹ and then ground using a EUPA TSK-927S grinder (Cankun Kitchen and Electrical Appliances Co., Ltd., Foshan, China), shifted into three different sizes of particle (< 0.35 mm, 0.35-0.80 mm and 0.8-1.25 mm in diameter). The ground samples were sealed in plastic bags and kept at 4 °C till extraction. Liquid CO₂ with 99.5 % purity (food grade) was purchased from Kai-Tian Gases Co., Ltd (Hangzhou, China). Ethyl decylate used as an internal standard reference in GC/MS analysis was purchased from Sigma-Aldrich (St. Louis, MO, USA). The other chemical reagents used were of AR grade (Jinmei Biotech Corporation, Tianjin, China) except where stated otherwise.

Orthogonal array design (OAD): Four test factors including particle sizes (A), temperature (B), pressure (C) and extraction time (D) with three levels each factor were used in the OAD test (Table-1). The OAD test design was carried out on software of the SAS System for Windows version 8.0 (SAS Institute Inc., Cary, NC, USA) according to matrix OA9 (34), with nine treatments in total in the test (Table-2).

Supercritical CO₂ extraction: A model Speed SFE-2 supercritical fluid extractor (Applied Separation Allentown Inc., PA, USA) was used. 25 g of the above ground sample

TABLE-1
TESTED FACTORS AND LEVELS IN
ORTHOGONAL ARRAY DESIGN

Level	Factors			
	A: Particle size (mm)	B: Temperature (°C)	C: Pressure (bar)	D: Time (min)
1	< 0.3	35	150	60
2	0.3-0.8	45	250	90
3	0.8-1.25	55	350	120

and 20 µL internal standard reference ethyl caprate (100 µg µL⁻¹) were placed in a 50 mL extraction vessel of (60 × 15 mm, i.d.). The extraction vessel was preheated for 10 min to reach the designed operating temperature before extraction. The extracted essential oil were collected in glass vial (30 mL) connected to the outlet of CO₂. When the extraction was finished, 5 mL ethyl ether was added to the vial to dissolve the extracted essential oil and then the mixture was transferred to a 10 mL sample vial in which the extracted essential oil were dehydrated with 3 g of Na₂SO₄ for 24 h.

GC-MS analysis of essential oil: One mL of the above essential oil was diluted with 5 mL of ethyl ether and 1 µL of the diluted essential oil was injected into GC-MS (Model HP6890GC/5973MSD, Applied Separation Allentown Inc., PA, USA) and analyzed under the following conditions: column: HP-Innowax fused capillary column(60 m × 0.32 mm id, 0.5 µm film thickness); injection port temperature at 250 °C; column temperature being held at 50 °C for 5 min and then increased to 210 °C at 3 °C min⁻¹, being held at 210 °C for 10 min and finally increased to 230 °C at 3 °C min⁻¹; detector temperature at 230 °C; total carrier gas being helium (99.99 % purity) at a flow rate 1 mL min⁻¹; ionizing energy 70 eV.

The identification of the volatile components was done by comparing their Kovats GC retention indices and mass

spectra with those of authentic compounds or reported data¹⁰. Relative concentrations of the detected volatile were expressed as the ratio of the peak height of the tested volatile to the peak height of internal reference ethyl caprate.

Data analysis: The tests in this paper were carried out in triplicate and the data analysis was done on software of the SAS System for Windows (version 8.01; SAS Institute Inc, Cary, NC, USA). The data was expressed as mean ± SD (standard deviation).

RESULTS AND DISCUSSION

Optimized conditions for supercritical CO₂ extraction:

There were significant differences in essential oil levels between treatments, ranging from 5.13 mg g⁻¹ (treatment 7) to 12.44 mg g⁻¹ (treatment 3) (Table-2). According to the range value (the difference between factor maximum and factor minimum), particle size was the most important factor (range = 5.85 ± 0.07) and temperature was less important, with pressure and time in between (Table-2). Analysis of variance (ANOVA) shows that the four tested factors had significant influence on the yield of essential oil (Table-3). Based on these results, the optimum conditions for supercritical fluid CO₂ (SCF-CO₂) extraction of essential oil from tangerine peel were: particle size < 0.3 mm, temperature 45 °C, pressure 350 bars and extraction time 120 min.

ANOVA results show that there were significant differences between levels of particle size, temperature, pressure and extraction time (Table-3). In general, decrease in particle size will increase specific surface area and decrease diffusion path in the solid matrix. These will result in a smaller intra-particle resistance to solute diffusion and benefit extraction. Table-2 shows that essential oil yield increased from 5.28 ±

TABLE-2
ORTHOGONAL ARRAY DESIGN MATRIX OA₉(3⁴) AND ESSENTIAL OIL YIELD (MEAN ± SD, mg g⁻¹)^a

Treatment No.	A: Particle size	B: Temperature	C: Pressure	D: Time	Yield
1	1	1	1	1	9.73 ± 0.15 C
2	1	2	2	2	11.21 ± 0.18 B
3	1	3	3	3	12.44 ± 0.07 A
4	2	1	2	3	7.47 ± 0.41 D
5	2	2	3	1	6.96 ± 0.13 E
6	2	3	1	2	5.96 ± 0.30 F
7	3	1	3	2	5.13 ± 0.21 G
8	3	2	1	3	5.40 ± 0.09 G
9	3	3	2	1	5.60 ± 0.03 G
K1 ^b	11.13 ± 0.13 A	7.44 ± 0.26 B	7.04 ± 0.18 B	7.32 ± 0.10 B	
K2 ^b	6.80 ± 0.28 B	7.87 ± 0.13 A	7.98 ± 0.21 A	7.43 ± 0.23 B	
K3 ^b	5.28 ± 0.11 C	7.89 ± 0.13 A	8.18 ± 0.14 A	8.45 ± 0.19 A	
Range ^c	5.85 ± 0.07	0.45 ± 0.13	1.14 ± 0.07	1.13 ± 0.13	

^aSD: standard deviation, n = 3. Data marked with different letters in a same column were significantly different at *p* = 0.01

^bK1: mean at level 1 of a tested factor; K2: mean at level 2 of a tested factor; K3: mean at level 3 of a tested factor

^cRange: difference between factor maximum and factor minimum

TABLE-3
ANOVA ANALYSIS

Source	Degree of freedom	Sum of squares	Mean square	F value	<i>p</i>
A: Particle size	2	16.57	8.29	1915.94	0.0001
B: Temperature	2	0.11	0.06	12.97	0.0003
C: Pressure	2	0.66	0.33	76.15	0.0001
D: Time	2	0.70	0.35	80.51	0.0001
Error	18	0.77	—	—	—

0.11 mg g⁻¹ to 11.13 ± 0.13 mg g⁻¹ when particle size decreased from 0.8-1.25 mm to <0.30 mm. Similar results were observed in SCF-CO₂ extraction of isoflavones from soybean¹⁰. However, excessive decrease in particle size will lead to inhomogeneous extractions due to fluid channeling effect in the fixed bed¹¹.

Pressure was an important factor influencing essential oil yield. The higher the pressure, the higher the yield of essential oil (Table-2). This is because high pressure increases the CO₂ fluid density, which leads to enhanced solubility of the solutes at a given tested temperature. The mean yields of essential oil at pressures 250 bar and 350 bar were significantly higher than that at 150 bar, while no significant difference in essential oil yield was observed between 250 bar and 350 bar (Table-2). This suggests that the increase in packing density of material particles induced by excessively high pressure counteracted the positive effect of the pressure. According to result in Table-2, 250 bar of extraction pressure is recommended.

ANOVA results show that the average yield of essential oil extracted for 120 min was significantly higher than those extracted for 60 and 90 min (Table-2). Extension of extraction time is in favor of the contact between solvent and sample particles and also the diffusion of solutes. The present study shows that the extraction time for SCF-CO₂ extraction of essential oil from tangerine peel should not be less than 120 min.

Increase in extraction temperature is beneficial to the penetration of solvent into particle interior and diffusion of the solutes. As shown in Table-2, the mean extraction yield increased significantly as temperature increased from 35 to 45 °C. However, no significant difference was observed between 45 °C and 55 °C. This might be that the increase in temperature will counteract the effect of pressure. The optimum temperature is considered to be 45 °C in view of energy-saving.

Volatile composition of essential oil: SCF-CO₂ extraction conditions affected not only on extraction yield, but also on volatile composition of the extracted essential oil. Thirty eight volatiles were detected in sample of the essential oil extracted under conditions of treatment No. 3, while twenty volatiles were detected in sample of essential oil extracted under conditions of treatment No.7 (Table-4). Volatiles α-farnesene, β-elemene and limonene were the most abundant volatiles in the essential oil extracted from the tangerine peel. Samples of treatment No. 3 and No. 7 showed a same trend (Table-4).

Volatile α-farnesene is reported to be an important insect semiochemical and it acts as alarm pheromone in termites and aphid¹² or food attractants for the apple tree pest codling moth¹³. The alarm pheromone release not only affects the immediate responses of insects in the presence of predators, but it also has longer-term consequences for insect colony composition and dispersal. Limonene is also an alarm pheromone and a potential predator kairomone¹⁴. These suggest that α-farnesene and limonene can be used as an insect repellent, which will be very interesting for the crop pest control management. It was reported that β-elemene had antitumour capability^{15,16}. β-Elemene was effective in the treatment of hyperplastic and proliferative disorders such as prostatic hypertrophy, hystero myoma and neoplasms^{16,17}. The present study shows that tangerine peel was a good source of α-farnesene, β-elemene and limonene (Table-4), suggesting the extraction

TABLE-4
COMPOSITION OF ESSENTIAL OILS
FROM TREATMENTS No. 3 AND No. 7

Volatiles	I _k ^a	Relative concentration ^b	
		Treatment No. 3	Treatment No. 7
Limonene	1352	1.55	0.33
γ-Terpinene	1373	0.21	0.01
α-Terpinolene	1395	0.02	ND
β-Linalool	1407	0.16	0.02
Pyranone	1446	0.50	0.00
α-Terpineol	1474	0.08	ND
Acetic acid, hexyl ester	1490	0.01	ND
Bicyclo[7.1.0]decane	1502	0.06	ND
1,4-Pentadiene	1532	0.02	ND
Hexadecanal	1556	0.03	ND
3-Methylene-1,5,5-trimethyl-cyclohexene	1573	ND	0.01
δ-Elemene	1576	0.66	0.15
β-Citronellene	1587	0.04	0.01
cis-Geranyl acetate	1595	0.19	0.04
Copaene	1600	0.15	0.03
α-Myrcene (8Cl)	1607	0.07	0.02
β-Geranyl acetate	1608	0.20	0.04
Germacrene D	1610	0.10	0.03
β-Elemene (6Cl)	1613	1.44	0.39
Ethyl caprate (internal standard)	1617	1.00	1.00
Hexadecanal	1623	0.07	ND
α-Cubebene	1634	0.03	0.14
Santolina triene	1638	0.17	0.04
β-Caryophyllene	1650	0.19	0.05
γ-Caryophyllen	1665	0.02	ND
β-Cubebene	1668	0.62	0.13
allo-Aromadendrene	1674	0.02	0.01
2,5-Dimethyl-3-methylene-1,5-heptadiene	1677	0.11	ND
α-Blulnesene	1683	0.07	ND
α-Farnesene	1688	2.34	0.68
α-Elemol	1710	0.08	ND
α-Caryophyllene	1713	0.02	ND
β-Cadinene	1695	0.34	0.10
Octanoic Acid	1837	0.01	ND
Aristoleadiene	1877	0.01	ND
Tetradecanoic acid	1941	0.39	ND
n-Hexadecanoic acid	1946	0.61	ND
(Z,Z)-9,12-Octadecadienoic acid	2018	0.11	ND
2-Methyl-Z,Z-3,13-octadecadienol	2127	0.11	ND
Total		11.81	3.23

^aI_k, Kovats retention index, I_k = 100*n + 100*(t_x-t_n)/(t_{n+1}-t_n); n, the number of carbon atoms in the alkane; t_x, retention time of aimed component; t_{n+1} and t_n, the retention time of alkane with 'n + 1' and 'n' carbon atoms.

^b20 μL internal standard reference ethyl caprate (100 μg μL⁻¹) was extracted with the sample and the relative concentrations were expressed as the ratio of the peak height of detected volatile to that of ethyl caprate.

of essential oil from the by-product of tangerine will be prospective industry

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