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SUPERCRITICAL CO_2 EXTRACTION OF AROMATIC VOLATILES FROM MANDARIN PEEL *Citrus unshiu*

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INTRODUCTION

Citrus peel is considered as a source rich in volatile compounds and has been extensively studied for aromatic profile, mainly contributed by the presence of terpene hydrocarbons, esters, ketones, aldehydes, and alcohols. Supercritical CO_2 (SC-CO_2) extraction technique showed great potential in obtaining nonpolar and volatile components, being also promising green alternative to conventional methods.
In this study, mandarin peel Citrus unshiu has been extracted at temperature of 40 °C and at two different pressures (100 and 300 bar). The extracts were analysed in detail by gas chromatography/mass spectrometry (GC/MS), and results of quantification analysis were expressed as a percentage in total quantity (%). The obtained results indicated the predominance of limonene (13.16 – 30.65 %) in SC-CO_2 fraction, followed by α-farnesene (5.72 – 10.63 %), germacrene (4.11 – 6.66 %), linalool (1.85 – 2.18 %), and α-terpineol (1.31 – 2.10 %). The content of volatile compounds, mainly including terpene and aldehydes, is commonly used as a commercial index of quality. Therefore, it could be concluded that SC-CO_2 extraction technique is applicable to food by-products processing, such as mandarin peel as it exhibited a strong potential for the industrial development in the production of the extracts rich in bioactive and aromatic compounds.

MATERIALS AND METHODS

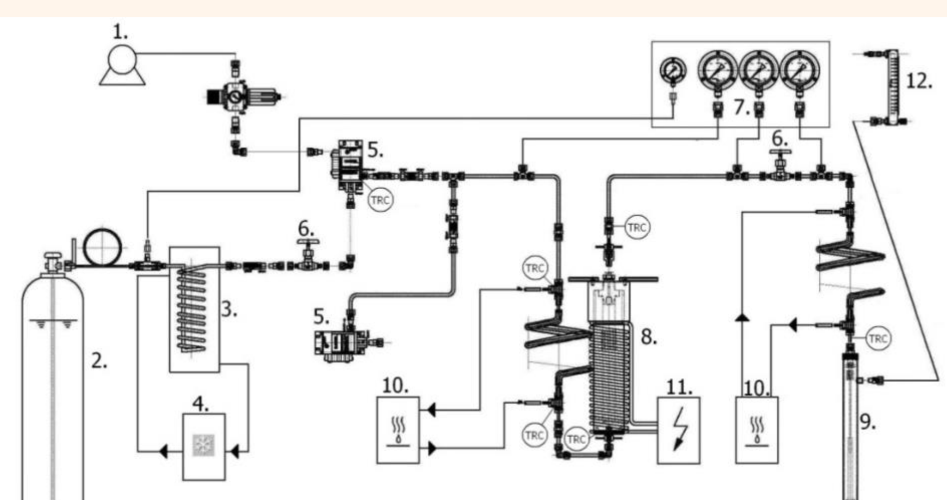
The mandarin peels of *Citrus unshiu* were obtained in November 2019 from small family farm Dalibor Ujević (Opuzen, Croatia). Before extraction, the mandarin peels were dried and milled using laboratory mill (IKA M 20 Universal mill) and sieved applying a vertical vibratory sieve shaker (Retsch AS 200, Germany) for 20 min.

INSTRUMENTATION

- Supercritical fluid extraction system (SFE)
- GC-MS system (Agilent Technologies, USA)
- IKA M 20 Universal mill

SUPERCRITICAL CO_2 EXTRACTION

For each extraction a 100.0 g of grounded mandarin peel *Citrus unshiu* was used. Material was placed into the extractor vessel and the obtained extracts were collected to glass tubes in a separator at 1.5 MPa and 25 °C. A series of extractions was performed in different conditions of pressure (100 and 300 bar) and temperature of 40 °C. The applied extraction time was set to 60 minutes and CO_2 mass flow rate was kept at 2 kg/h.



1. Compressor; 2. CO2 Tank; 3. Stainless steel coil; 4. Cooling bath; 5. Air driven fluid pump Haskel MS-71; 6. Valves (B-HV); 7. Manometers; 8. Extraction vessel; 9. Separator vessel; 10. Water bath; 11. Centralized system glass fiber heater; 12. Flow meter

Figure 1. Supercritical fluid extraction system (SFE) sheme

GC-MS ANALYSIS

An Agilent Technologies (Palo Alto, CA, USA) 7890A gas chromatograph with 5975C mass detector was used for the analysis. The capillary column used was HP-5MS (5%-phenyl-methyl polysiloxane), 30 m x 0.25 mm i. d., coating thickness 0.25 µm. The operation conditions were injector temperature, 250 °C; column temperature programmed at 70 °C isothermal for 2 min, at ramped at 3°C/min to 200 °C and held isothermal for 18 min; Helium was carrier gas at 1 mL/min flow; ionization voltage 70 eV; ion source temperature 230 °C; mass scan range: 45-450 mass units; split ratio 1:50. A part of the extracts (10 mg) was diluted with hexane and diethyl ether (1:1, v/v) and 1 µL of the solution was inserted into the GC injector. The percentage composition was calculated without correction factors from the GC peak areas applying the normalization method. The percentages were calculated as mean values from duplicate GC-MS analyses of all the extracts.

Table 1. The most significant volatile compounds of SC-CO_2 mandarin peel *Citrus unshiu* extracts

No.	Compound	100 bar 40 °C (%)	300 bar 40 °C (%)		No.	Compound	100 bar 40 °C (%)	300 bar 40 °C (%)
1.	α-Pinene	0.03	0.09		41.	Undecanal	0.09	0.06
2.	Sabinene	-	0.04		42.	δ-Elemene	0.31	0.4
3.	β-Pinene	0.03	0.1		43.	p-Mentha-1,8-dien-1,2-diol	-	-
4.	Hexanoic acid	0.05	0.03		44.	α-Cubebene	0.11	0.08
5.	β-Myrcene	0.13	0.54		45.	Citronellyl acetate	0.24	0.15
6.	Octanal	-	0.04		46.	(E)-9-Hydroxylinalool	-	-
7.	α-Terpinene	-	0.04		47.	Neryl acetate	0.43	0.28
8.	p-Cymene	0.04	-		48.	α-Copaene	1.46	0.89
9.	**Limonene**	13.16	30.65		49.	Geranyl acetate	0.72	0.45
10.	Benzyl alcohol	-	-		50.	β-Cubebene	1.26	0.78
11.	(Z)-β-ocymene	0.02	0.05		51.	β-Elemene	0.99	0.86
12.	γ-Terpinene	1.75	3.69		52.	Dodecanal	0.23	0.13
13.	cis-Sabinene hydrate	0.11	0.16		53.	trans-Caryophyllene	0.9	0.54
14.	Octan-1-ol	-	0.01		54.	α-Guaiene	0.38	0.24
15.	cis-Linalool oxide	-	-		55.	α-Humulene	1.58	0.95
16.	Heptanoic acid	-	-		56.	Germacrene D	6.66	4.11
17.	α-Terpinolene	0.17	0.32		57.	Valencene	0.51	0.3
18.	trans-Linalool oxide	-	-		58.	Bicyclogermacrene	1.06	0.62
19.	Linalool	2.18	1.58		59.	α-Muurolene	0.63	-
20.	Nonanal	0.07	0.06		60.	Eremophilene	6.7	3.99
21.	2-Phenylethanol	-	-		61.	α-Farnesene	10.63	5.72
22.	trans-p-mentha-2,8-dien-1-ol	-	0.02		62.	γ-Cadinene	2.21	1.38
23.	cis-p-Mentha-2,8-dien-1-ol	-	-		63.	Elemol	0.09	0.11
24.	trans-Limonene oxide	0.02	0.02		64.	Germacrene B	1.11	0.69
25.	Citronellal	0.11	0.1		65.	Dodecanoic acid	0.34	0.32
26.	Terpinen-4-ol	0.22	0.16		66.	t-Muurulol	0.07	0.06
27.	Octanoic acid	0.08	0.05		67.	3-Oxo-α-ionol	-	-
28.	α-Terpineol	2.1	1.31		68.	α-Sinensal	0.08	0.05
29.	Decanal	0.53	0.37		69.	Tetradecanoic acid	2.42	1.56
30.	trans-Carveol	0.17	0.11		70.	Neophytadiene	-	-
31.	β-Citronelol	0.19	0.13		71.	Nootkatone	0.14	0.13
32.	Carvone	0.03	0.02		72.	Hexahydrofarnesyl acetone	-	-
33.	trans-Geraniol	0.08	0.05		73.	6,7-Dimethoxy-coumarin	-	-
34.	Isopiperitenone	-	-		74.	Hexadecanoic acid	8.39	8.6
35.	Perilla aldehyde	0.43	0.3		75.	Octadecan-1-ol	0.18	0.17
36.	Nonanoic acid	0.08	0.06		76.	Heptadecanoic acid	0.19	0.4
37.	p-Mentha-1,8-dien-9-ol	0.23	0.15		77.	Linoleic acid	15.44	19.04
38.	Thymol	0.09	0.08		78.	Oleic acid	2.87	-
39.	Perilla alcohol	-	-		79.	Octadecanoic acid	1.27	1.38
40.	Carvacrol	0.19	0.12					

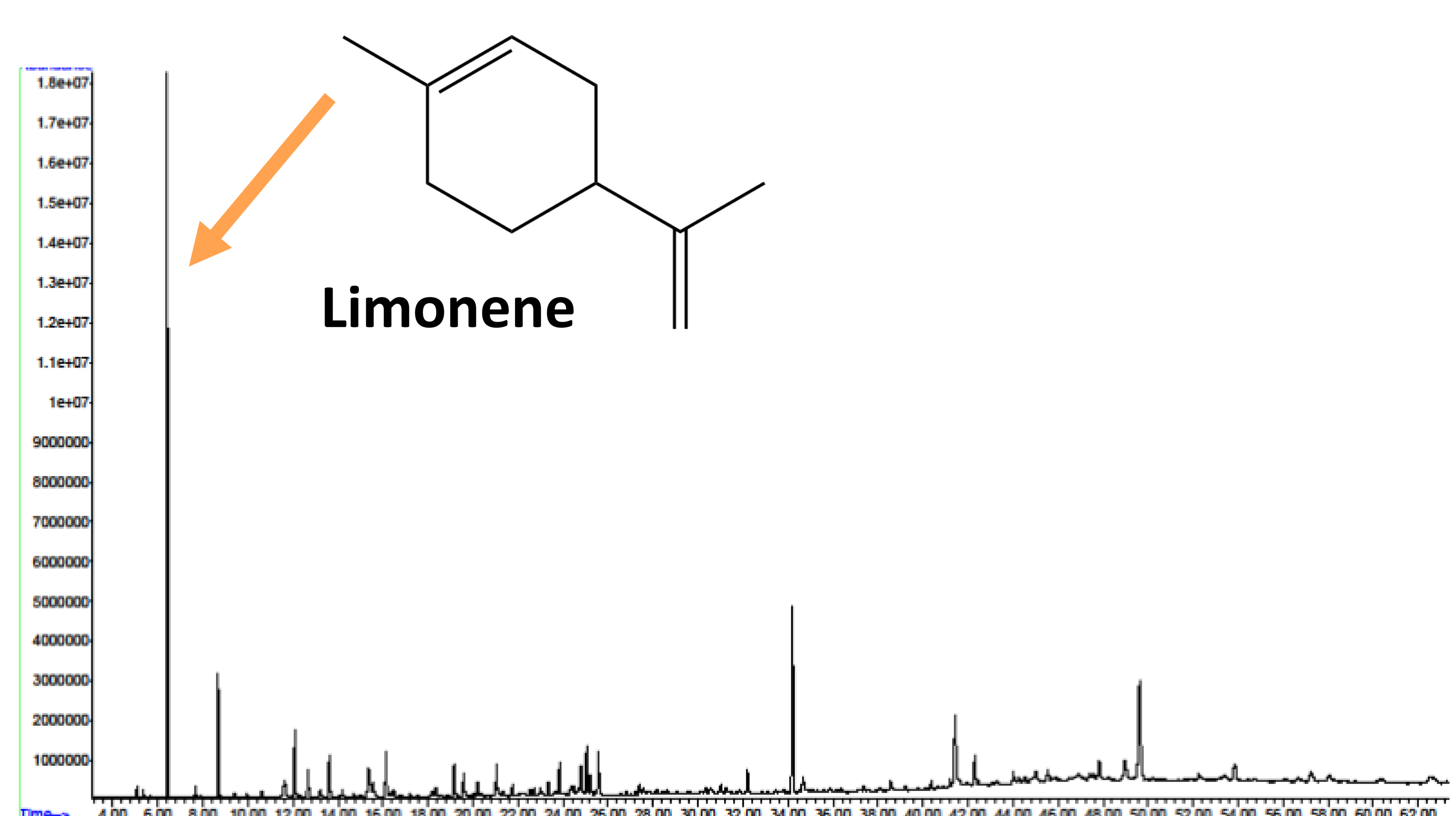


Figure 2. Chromatographic separation of volatile compounds from mandarin peel *Citrus unshiu* (300 bar; 40 °C) by GC-MS with limonene (RT 6.411) as the most abundant compound

CONCLUSIONS

The limonene predominance (13.16 – 30.65 %) was found in samples of mandarin peel *Citrus unshiu*, followed by α-farnesene (5.72 – 10.63 %), germacrene (4.11 – 6.66 %), eremophilene (3.99 – 6.7 %), and α-terpineol (1.31 – 2.10 %). Another abundant monoterpene was linalool (1.58 – 2.58 %), which is the principal oxygenated terpene of citrus peels and is considered to be a prominent constituent of good-quality peel oil.
In addition, sesquiterpene hydrocarbons found in the extracts which have been reported to contribute to the characteristic citrus flavor were α-humulene (0.95–1.58 %) and γ-cadinene (1.38–2.21 %), and trans-caryophyllene (0.54–0.9 %).

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