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SUPERCRITICAL CO_2 EXTRACTION OF VOLATILE COMPOUNDS FROM MANDARIN PEELS

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INTRODUCTION

Citrus peels are considered as primary waste, but they can have beneficial effect on our health due to the presence of bioactive natural compounds. During the industrial processing of mandarin, large amounts of waste, including peels, are produced. Since it was shown that the peels contain higher concentration of bioactive compounds compared to pulps and thus have more potential for being used as source of bioactive compounds. Mandarin peels, due to its compounds, have been found to have health-related properties including antioxidant, anticancer and anti-inflammatory. Mandarin peel and its essential oil have been studied particularly due to their unique and specific aroma. Characteristic aroma is formed by the presence of terpene hydrocarbons, esters, ketones, aldehydes and alcohols.

By discovering supercritical solvents, toxic and ecologically unacceptable organic solvents are gradually replaced with supercritical CO_2 (SC-CO_2). SC-CO_2 extraction gave extracts with less polar compounds, obtaining essential oil, because of non-polar characteristics of CO_2. The aim of this work was to obtain extracts from mandarin peels, of different variety (*Zorica rana, Chahara, Okitsu, Kuno*), rich in volatile compounds. SC-CO_2 extraction was used to obtain essential oil from mandarin peels which was analysed using GC/MS.

PLANT MATERIAL

The mandarin peels of *Citrus reticulata* Blanco cultivars of different variety (*Zorica rana, Chahara, Okitsu, Kuno*) were obtained in November 2017 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.

Okitsu

Kuno

Chahara

Zorica rana

SUPERCRITICAL CO_2 (SC-CO_2) EXTRACTION

The experiment was carried out in a supercritical fluid extraction (SFE) system which shematic diagram is given in Figure 1. For each extraction a 100.0 g of grounded mandarin peels 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 two main parameters (pressure, temperature). The applied extraction time was set to 60 minutes and CO_2 mass flow rate was kept at 2 kg/h.

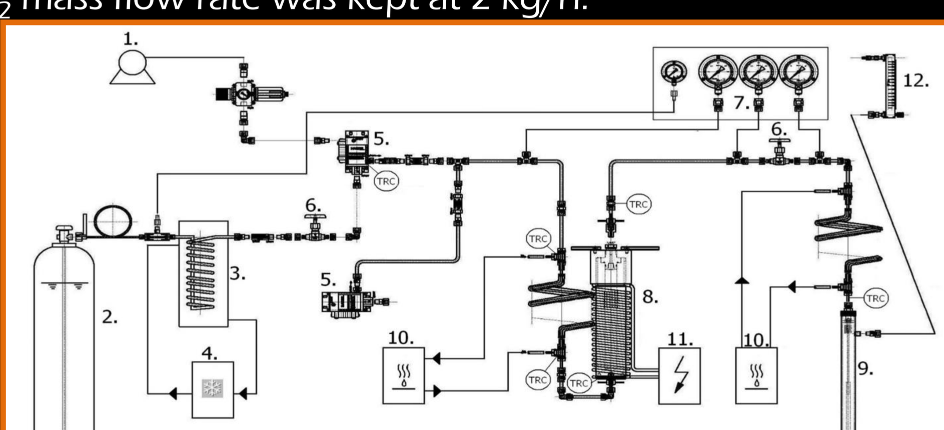

Figure 1. Supercritical fluid extraction system sheme

1.Compressor; 2. CO_2 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

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.

RESULTS

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

No.	Compound	Rt	RI	*Zorica rana*		*Kuno*		*Okitsu*		*Chahara*	
				100 bar	300 bar	100 bar	300 bar	100 bar	300 bar	100 bar	300 bar
1.	α-Thujene	4,082	932	-	-	0.1	0.1	-	-	-	-
2.	α-Pinene	4,237	940	-	-	0.6	0.3	-	0.1	-	-
3.	Sabinene	5,091	978	-	-	0.2	0.1	-	0.1	-	-
4.	β-Pinene	5,191	982	0.1	-	0.4	0.3	0.1	0.1	-	-
5.	β-Myrcene	5,478	992	0.6	0.4	2.3	1.9	0.5	1.0	0.1	0.1
6.	Octanal	5,783	1003	-	-	0.1	0.1	0.1	0.1	-	-
7.	Phellandrene	5,876	1007	-	-	0.1	0.1	-	-	-	-
8.	p-Cymene	6,440	1028	1.2	1.1	0.1	0.1	0.5	0.9	0.3	0.1
9.	**Limonene**	6,602	1034	37.2	35.1	66.8	66.6	35.4	52.8	11.7	3.5
10.	trans-β-Ocymene	7,110	1051	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
11.	**γ-Terpinene**	7,467	1062	3.9	4.4	8.4	8.5	5.0	6.6	1.3	1.0
12.	cis-Sabinene hydrate	7,755	1071	0.1	0.1	0.1	0.1	0.1	0.1	-	-
13.	α-Terpinolene	8,437	1089	0.4	0.4	0.6	0.6	0.4	0.6	0.2	0.1
14.	**Linalool**	8,855	1100	3.4	4.3	1.6	1.7	3.0	2.6	4.3	2.2
15.	Nonanal	8,891	1101	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
16.	Citronellal	10,793	1155	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2
17.	Terpinen-4-ol	11,744	1179	0.2	0.3	0.1	0.1	0.2	0.2	0.3	0.2
18.	α-Terpineol	12,286	1191	1.4	1.6	0.6	0.6	1.2	0.9	2.0	2.2
19.	Decanal	12,864	1204	0.6	0.7	0.4	0.4	0.6	0.4	0.8	0.6
20.	trans-Carveol	13,405	1219	-	-	-	-	0.1	-	-	-
21.	Citronellol	13,809	1230	0.3	0.3	0.1	0.1	0.2	0.1	0.2	0.1
22.	(Z)-Citral	14,283	1242	-	-	0.1	0.1	-	-	-	-
23.	(E)-Citral	15,511	1271	-	-	0.1	0.1	-	-	-	-
24.	4-(1-Methyl-ethenyl)-cyclohex-1-ene-1-carbox-aldehyde (Perilla alhehyde)	15,631	1274	0.3	0.3	0.1	0.2	0.2	0.2	0.5	0.4
25.	Thymol	16,609	1295	0.2	0.4	-	-	-	0.3	-	-
26.	Carvacrol	16,992	1304	0.7	0.9	0.1	0.1	0.1	0.1	0.4	0.6
27.	Undecanal	17,023	1305	-	-	0.1	-	0.1	-	-	-
28.	δ-Elemene	18,253	1337	0.5	0.5	0.2	0.2	0.5	0.3	0.8	0.6
29.	Citronellyl acetate	18,982	1354	-	-	-	-	0.2	0.2	0.3	0.1
30.	Neryl acetate	19,452	1365	0.4	0.4	0.1	0.1	0.2	0.2	0.4	0.4
31.	α-Copaene	19,881	1375	1.3	1.4	0.5	0.6	1.2	0.9	1.7	1.1
32.	Geranyl acetate	20,255	1384	0.6	0.6	0.2	0.2	0.9	0.6	1.1	1.1
33.	β-Cubebene	20,419	1387	1.1	1.1	0.5	0.5	1.1	0.8	1.3	0.9
34.	β-Elemene	20,518	1389	2.9	2.9	0.5	0.5	2.9	1.9	3.8	3.2
35.	Dodecanal	21,254	1407	0.2	0.2	0.1	0.1	0.1	0.1	0.3	0.2
36.	Limonen-10-yl acetate	21,349	1409	0.3	0.3	0.1	0.1	0.5	0.3	0.7	0.3
37.	trans-Caryophyllene	21,572	1415	1.2	1.2	0.3	0.3	1.1	0.8	1.6	1.5
38.	α-Guaiene	22,373	1436	0.2	-	0.1	-	0.2	0.1	0.3	0.1
39.	α-Humulene	22,953	1450	2.1	2.0	0.5	0.5	2.4	1.4	2.8	2.6
40.	**Germacrene D**	24,085	1477	5.0	4.9	1.9	1.9	5.5	3.2	7.2	7.6
41.	Valencene	24,564	1489	0.3	0.3	0.1	0.1	0.6	0.3	0.4	0.9
42.	Bicyclogermacrene	24,698	1492	1.1	1.1	0.3	0.3	1.6	0.9	1.9	2.8
43.	α-Muurolene	24,873	1496	-	-	0.1	0.2	-	-	0.7	0.9
44.	**Eremophilene**	25,064	1500	10.4	10.4	2.6	2.8	11.1	7.2	15.2	18.6
45.	**(E,E)-α-Farnesene**	25,294	1506	7.6	7.5	3.5	3.5	8.8	5.3	12.8	15.8
46.	δ-Cadinene	25,808	1520	1.9	1.9	0.7	0.7	1.7	1.1	2.6	3.2
47.	Elemol	26,833	1547	0.2	0.2	-	-	0.1	0.1	0.2	0.1
48.	Germacrene B	27,026	1552	0.9	0.9	0.3	0.3	0.9	0.5	1.4	1.7
49.	Dodecanoic acid	27,640	1568	0.2	0.2	-	-	0.1	-	0.3	0.9
50.	Spathulenol	27,873	1574	0.1	-	-	-	-	-	-	-
51.	Tetradecanoic acid	34,903	1764	0.6	0.7	0.2	0.3	0.4	0.3	0.8	2.6
52.	Hexadecanoic acid	41,697	1966	1.8	2.4	0.6	1.1	2.0	1.4	5.4	4.8
53.	Linoleic acid	47,065	2132	0.5	0.5	0.6	1.0	2.1	0.9	4.2	11.3

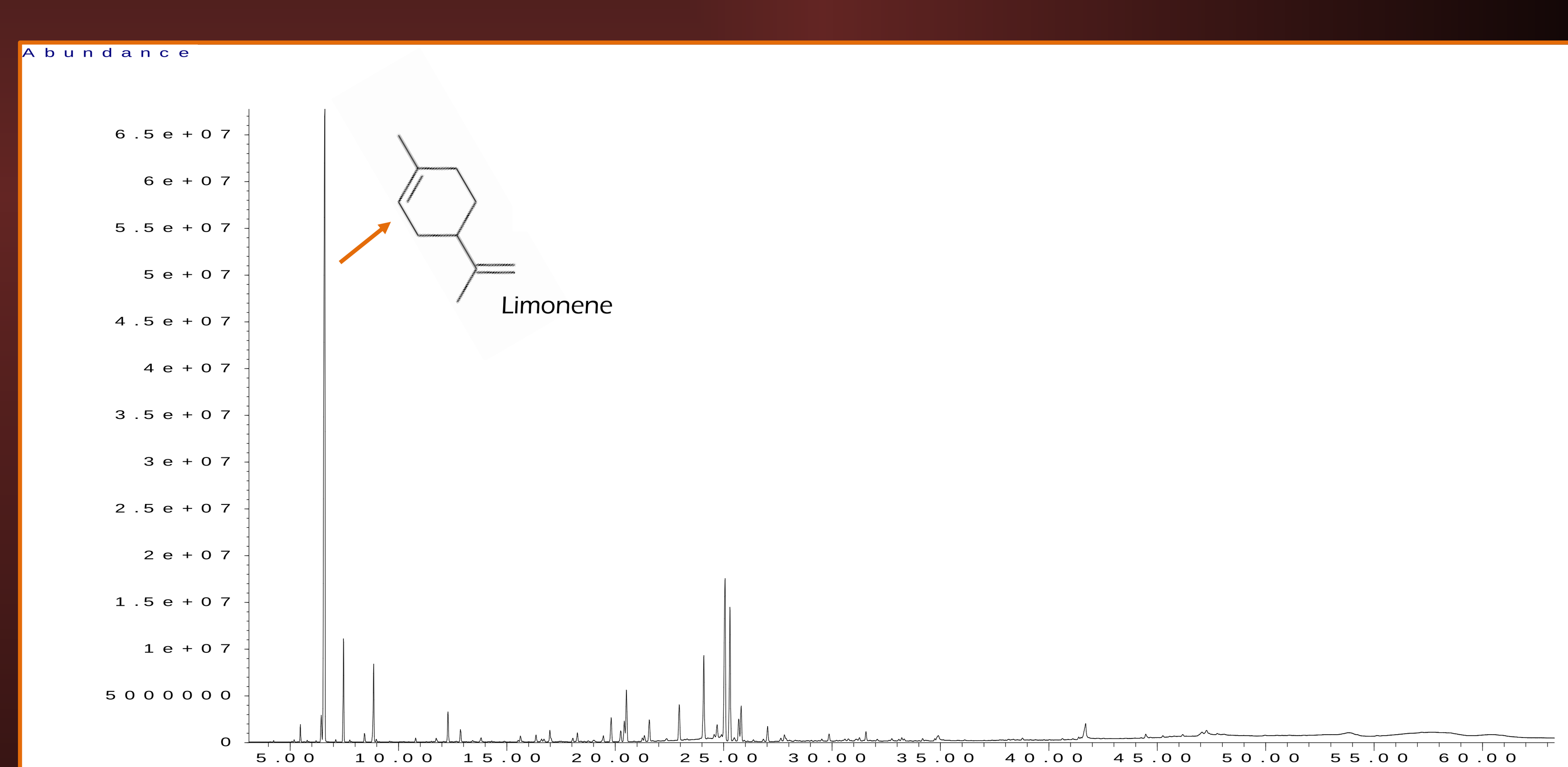

Figure 2 Chromatographic separation of volatile compounds from mandarin peel variety *Zorica rana* (100 bar; 40°C) by GC-MS with limonene (RT 6.602) as the most abundant compound

CONCLUSION

The limonene predominance was found in samples variety *Zorica rana, Okitsu* and *Kuno*, followed by eremophylene in variety *Zorica rana, Chahara* and *Okitsu*, and γ-terpinene in variety *Kuno*. The results showed that volatile composition were completely different according to used mandarin variety. Furthermore, SC-CO_2 extraction applied to food by-products such as mandarin peel exhibited a strong potential for the industrial development in the production of the extracts rich in bioactive compounds.

ACKNOWLEDGMENT

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