9<sup>th</sup> International Congres of Food Technologists, Biotechnologists and Nutritionists Zagreb, Croatia, October 03 – 05, 2018



# OPTIMIZATION OF EXTRACTION METHODS FOR ISOLATION OF VOLATILE COMPOUNDS AND HESPERIDIN FROM ORANGE PEEL

Stela Jokić<sup>1</sup>, Maja Molnar<sup>1</sup>, Ana-Marija Cikoš<sup>1</sup>, Martina Jakovljević<sup>1</sup>, Igor Jerković<sup>2</sup>

<sup>1</sup>Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhaca 20, 31000 Osijek, Croatia <sup>2</sup>Faculty of Chemistry and Technology, University of Split, Ruđera Boškovića 35, 21000 Split, Croatia



**ByPrOExtract** 

# **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 such as limonoids, dietary fibres and phenolic compounds (flavonoids and phenolic acids).

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 flavonoids, obtaining essential oil, because of non-polar characteristics of  $CO_2$ . Ultrasound- assisted extraction (UAE) is extraction technique for obtaining polar compounds such as phenolics. The aim of this work was to obtain extracts from orange peel rich in volatile compounds and hesperidin. Because of different polarity of this class of compounds, SC- $CO_2$  extraction was used to obtain essential oil from orange peel which was analysed using GC/MS and UAE of the reused sample was used to obtain extracts rich in flavonoid hesperidin which were analysed using HPLC. Due to different composition depending on applied process parameters, optimal extraction conditions were defined for the two the most abundant compounds (limonene in SC- $CO_2$  extract and hesperidin in extract obtain by UAE) by using response surface methodology (RSM).

## **PLANT MATERIAL**

The orange peels (*Citrus sinensis* L.) variety "Washington Navel" were obtained in November 2017 from small family farm Dalibor Ujević (Opuzen, Croatia). Before extraction, the orange 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.



#### SUPERCRITICAL CO<sub>2</sub> (SC-CO<sub>2</sub>) 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 40.0 g of grounded orange peel 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) determined by central composite rotatable design (CCRD). The applied extraction time was set to 90 minutes and  $CO_2$  mass flow rate was kept at 2 kg/h.



### **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  $\mu$ L 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.

# **ULTRASOUND-ASSISTED EXTRACTION (UAE)**

An ultrasound bath with temperature control and working at 37 kHz of frequency and the ultrasonic power of 50 W (Elma, Elmasonic P 70 H, Elma Schmidbauer GmbH, Gottlieb-Daimler, Singen, Germany) was used for the UAE experiments. In all experiments, 1.0 g of orange peel remained after SFE was mixed with different aqueous ethanol solutions in a 50 mL flask. At each power level the temperature was varied at 30, 50 and 70 °C, the time varied at 15, 30 and 45 min, solvent-solid ratio 10, 30 and 50 mL/g, and ethanol : water ratio (20%, 50%, 80% v/v). 29 treatments in all were conducted according to Box-Behnken Design (BBD). Ultrasonic power, temperature and extraction time were controlled from the instrument panel. Flasks were always positioned at the same distance from the transducer, and no additional agitation was applied. After extraction, crude extracts were immediately filtered through the filter paper. Extracts were collected in glass flasks and stored at 4°C until the analysis.

### HPLC ANALYSIS

Hesperidin was determined using a RP-HPLC method with UV detection on a Cosmosil 5C18-MS-II column (Nacalai Tesque, Inc., Kyoto, Japan), 150 mm long with an internal diameter of 4.6 mm, with gradient elution of acetonitrile as phase A and water as phase B, at room temperature. A gradient program was used as follows: 22–22% A at 0–10 min, 22–61% A at 10–30 min, reequilibration duration between two individual runs was 5 min. Detection was carried out at 280 nm.The flow rate was 1.0 mL/min, an injection volume of 20 µL and a UV detection wavelength of 280 nm. Hesperidin standard stock solutions were prepared in a solvent and calibration was obtained at eight concentrations (5.0-250.0 mg/L). Linearity of the hesperidin calibration curve was confirmed by  $R^2=0.9991$  with a limit of detection (LOD) of 0.008 mg/L, a quantification limit (LOQ) of 0.026 mg/L and hesperidin retention time of 16.03 minute.

Table	le 1. The most significant volatile compounds of SC-CO <sub>2</sub> orange peel extracts (according to CCRD)															Table 2. Hesperidin content detected in U						
No.	Compound	Rt	RI	Run 1	Run 2	Run 3	Run 4	Run 5	A Run 6	rea perce Run 7	entage (% Run 8	) Run 9	Run 10	Run 11	Run 12	Run 13			Run	Temperature (°C)	Time (min)	Solve ratio
				21,00	21,00	12,00	12,00	30,00	21,00	21,00	21,00	30,00	21,00	21,00	33,73	8,27 50.00	MPa °C		1.	70	45	
1.	Limonene	6,602	1034	25.8	3.3	73.7	89.0	15.9	58.8	16.4	4.6	52.4	5.4	27.9	9.3	87.0	<u> </u>		2.	70	30	
2.	Linalool	8,855	1100	2.5	0.8	1.7	1.3	1.2	2.3	1.5	0.3	2.8	0.9	2.7	2.8	0.7			3.	30	30	
3.	a-Terpineol	12,286	1191	1.1	1.6	0.7	_	1.9	0.8	2.2	0.9	1.0	1.3	1.4	1.7	0.2			4.	30	45	
4	Decanal	12,864	1204	1.5	0.5	1.0	1.4	0.7	1.5	1.0	0.2	1.7	0.7	1.7	1.6	0.9			<u> </u>	50	15	
-	trans-	21 572	1415	1.5	1.0	0.4	0.7	1.5	0.4	1.0	0.2	0.7		1.0	1.0	0.7			7.	50	30	
5.	Caryophyllene	21,572	1415	1.5	1.0	0.4	0.2	1.5	0.6	1.8	0.6	0.7	1.1	1.0	1.0	0.1			8.	50	45	
6.	Valencene	24,564	1489	2.5	4.3	1.5	1.3	5.2	1.9	7.1	3.5	2.3	4.6	3.2	3.6	0.8			9.	50	30	
7.	δ-Cadinene	25,808	1520	1.1	2.4	0.6	0.2	3.2	0.8	3.8	2.0	1.0	2.3	1.3	1.7	0.1			10.	70	15	
8.	β-Sinensal	32,345	1692	-	2.7	0.7	-	2.3	0.9	3.1	2.9	1.2	2.9	1.7	2.1	-			11.	30	30	-
9.	α-Sinensal	34,369	1749	-	2.3	0.3	-	1.5	0.6	1.8	1.5	0.4	1.1	0.3	1.2	-			12.	70	30	-
10.	Hexadecanoic acid	41,697	1966	11.0	29.8	2.7	-	19.2	4.5	14.7	13.6	4.8	13.5	8.8	15.3	-			13. 14.	50 30	30 15	
11.	Manool	44,189	2045	17.6	0.8	0.5	-	1.0	2.2	0.7	0.3	1.3	0.8	0.8	0.7	_			15.	50	30	
12.	(Z)-Octadec-9-en- 1-ol	44,407	2052	-	3.4	0.3	-	1.7	0.6	2.4	2.4	1.0	2.1	0.7	0.9	-			16. 17.	50 70	30 30	
13.	Octadecan-1-ol	45,184	2077	-	1.9	0.1	-	0.9	0.4	0.8	0.8	0.4	0.7	0.1	0.3	-			18.	50	30	
14.	Linoleic acid	47,065	2132	6.8	23.5	5.6	-	27.6	7.7	25.4	51.1	9.2	36.4	28.3	36.0	-			19.	50	45	
	Extraction yield (g	g/100 g)		2.1	1.05	1.40	0.15	0.93	1.63	0.98	1.15	3.23	0.98	1.95	3.10	0.25			20.	50	30	
mAU	25-					011													21.	50	15	
																	22.	70	30			
20—											25							23.	50	30		
																			24.	50	30	
	15		Ŭн Ŭ														26.	50	45	1		









**Figure 2** Chromatographic separation of hesperidin, as the most abundant flavonoid, by RP-HPLC

Figure 3 Three-dimensional plots for the hesperidin as a function of different UAE parameters



25.00

30.00

35.00

10.00 15.00 20.00

5,00

#### CONCLUSION

The results showed that the concetrations of dominant compounds (limonene and hesperidin) were different applying different extraction conditions. The limonene predominance of 89 % was found when the pressure of 12.00 MPa and the temperature of 40 °C (Run 4) were applied for the SC-CO<sub>2</sub> extraction. The highest concentration of hesperidin (23.0 µg/mg) was obtained with the temperature of 70°C, the time of 30 min, ethanol:water ratio 50% v/v and the solvent-solid ratio of 50 mL/g (Run 22) for the UAE. It is necessary to find the most efficient method of extraction of selected bioactive components and then optimize the extraction procedure to enable their application. Therefore, SC-CO<sub>2</sub> extraction and UAE applied to food by-products such as orange peel exhibited a strong potential for the industrial development in the production of the extracts rich in bioactive compounds.

#### ACKNOWLEDGMENT

This work has been supported in part by Croatian Science Foundation under the project "Application of the extraction of bioactive components from by products of plant origin" (UIP-2017-05-9909)

27.

28.

29.