NATURAL RESOURCES, GREEN TECHNOLOGY AND SUSTAINABLE DEVELOPMENT/4-GREEN2022

14th - 16th September 2022, Zagreb, Croatia

SEPARATION OF BIOACTIVE COMPOUNDS FROM MANDARIN PEEL *Citrus unshiu* USING SUBCRITICAL WATER EXTRACTION

Silvija Šafranko*, Marija Banožić, Ina Ćorković, Martina Jakovljević, Krunoslav Aladić, Stela Jokić

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, 31000 Osijek, Croatia

*** silvija.safranko@ptfos.hr**

INTRODUCTION

Mandarin peel contains significant amounts of bioactive and high-value components, among which phenolic acids and flavonoids are the most persistent groups of plant phenolics exhibiting health-related properties including antioxidant, anticancer and anti-inflammatory. Subcritical water extraction (SWE) technique is considered as an promising green alternative to conventional extraction methods, being also efficient for extraction of variety types of bioactive compounds from plant material. The efficiency of supercritical CO_2 (SC-CO_2) extraction for obtaining high-quality essential oil from citrus peel is well-known, however the utilization of generated remain free of non-polar components is not commonly studied for obtaining extracts rich in phenolic compounds. The primary aim of this study is to investigate the possibility of using mandarin peel of the *Citrus unshiu* variety using subcritical water extraction (SWE) technique, and to evaluate phytochemicals, total phenolic content and antioxidant activity of the prepared extracts. After SC-CO_2 extraction, the exhausted citrus waste was subjected to SWE in a wide temperature range (130 – 220 °C) using solvent-solid ratio (10 – 30 mL/g) in periods from 5 to 15 min. Identification and quantification of individual bioflavonoids, of which hesperidin (0.16 – 15.07 mg/g of plant) was determined as the most abundant flavanon in mandarin peel, and also other polyphenolic compounds as possible products of thermal degradation, was performed using high performance liquid chromatography with a diode array detector (HPLC-DAD). At higher temperatures the presence of 5-HMF and chlorogenic acid was detected. Antioxidant activity and total phenolic content in extracts were determined using spectrophotometric methods. Process optimization was performed by response surface methodology (RSM) using Design Expert® software.

Citrus Unshiu fruit

MATERIALS AND METHODS

The mandarin peels (*Citrus unshiu*) of the variety "Kuno" were obtained in November 2019 from a small family farm Dalibor Ujević (Opuzen, Croatia).

All chemicals and solvents were of analytical grade.

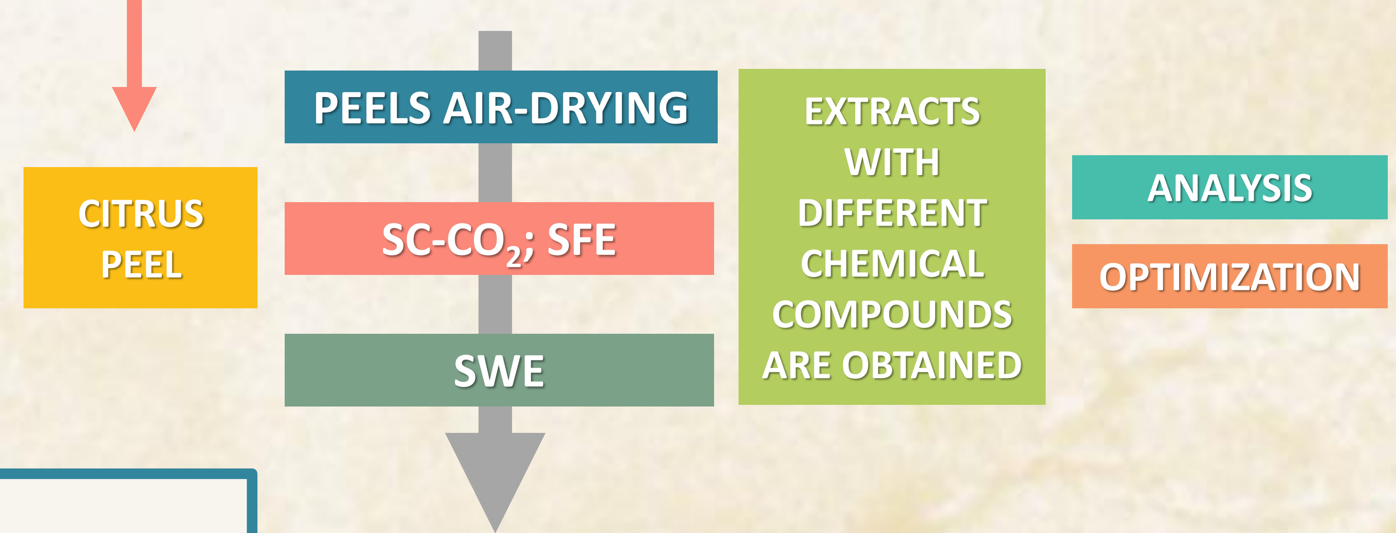
SC-CO_2; SFE | SWE | ANALYSIS

STEP-BY-STEP PROCEDURE

QUANTIFICATION → GC-MS, HPLC

BIOACTIVITY → TPC, DPPH

PROCESS OPTIMIZATION → RSM



GC-MS ANALYSIS

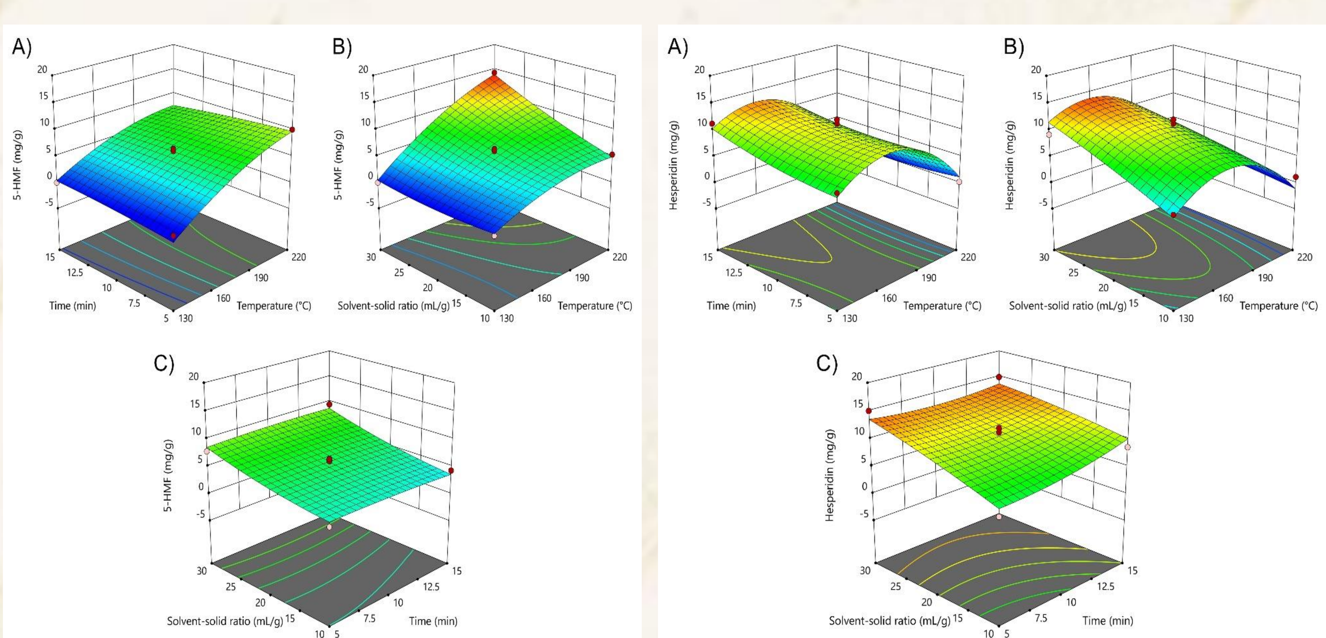
The volatiles profile of SC-CO_2 extracts of mandarin peel var. *Kuno* determined by GC-MS analysis.

No.	Compound	100 bar (%)	300 bar (%)	No.	Compound	100 bar (%)	300 bar (%)
1.	α-Pinene	0.03	0.09	27.	Undecanal	0.09	0.06
2.	Sabinene	-	0.04	28.	δ-Elemene	0.31	0.4
3.	Hexanoic acid	0.05	0.03	29.	α-Cubebene	0.11	0.08
4.	β-Myrcene	0.13	0.54	30.	Citronellyl acetate	0.24	0.15
5.	α-Terpinene	-	0.04	31.	Neryl acetate	0.43	0.28
6.	p-Cymene	0.04	-	32.	α-Copaene	1.46	0.89
7.	Limonene	13.16	30.65	33.	Geranyl acetate	0.72	0.45
8.	(Z)-β-ocymene	0.02	0.05	34.	β-Elemene	0.99	0.86
9.	γ-Terpinene	1.75	3.69	35.	Dodecanal	0.23	0.13
10.	cis-Sabinene hydrate	0.11	0.16	36.	trans-Caryophyllene	0.9	0.54
11.	α-Terpinolene	0.17	0.32	37.	Valencene	0.51	0.3
12.	Linalool	2.18	1.58	38.	α-Muurolene	0.63	-
13.	Nonanal	0.07	0.06	39.	Eremophilene	6.7	3.99
14.	trans-p-mentha-2,8-dien-1-ol	-	0.02	40.	γ-Cadinene	2.21	1.38
15.	trans-Limonene oxide	0.02	0.02	41.	Germacrene B	1.11	0.69
16.	Citronellal	0.11	0.1	42.	Dodecanoic acid	0.34	0.32
17.	Terpinen-4-ol	0.22	0.16	43.	t-Muurulol	0.07	0.06
18.	Octanoic acid	0.08	0.05	44.	3-Oxo-α-ionol	-	-
19.	α-Terpineol	2.1	1.31	45.	α-Sinensal	0.08	0.05
20.	Decanal	0.53	0.37	46.	Tetradecanoic acid	2.42	1.56
21.	β-Citronelol	0.19	0.13	47.	Nootkatone	0.14	0.13
22.	Perilla aldehyde	0.43	0.3	48.	Octadecan-1-ol	0.18	0.17
23.	Nonanoic acid	0.08	0.06	49.	Heptadecanoic acid	0.19	0.4
24.	p-Mentha-1,8-dien-9-ol	0.23	0.15	50.	Linoleic acid	15.44	19.04
25.	Thymol	0.09	0.08	51.	Oleic acid	2.87	-
26.	Carvacrol	0.19	0.12				

HPLC ANALYSIS

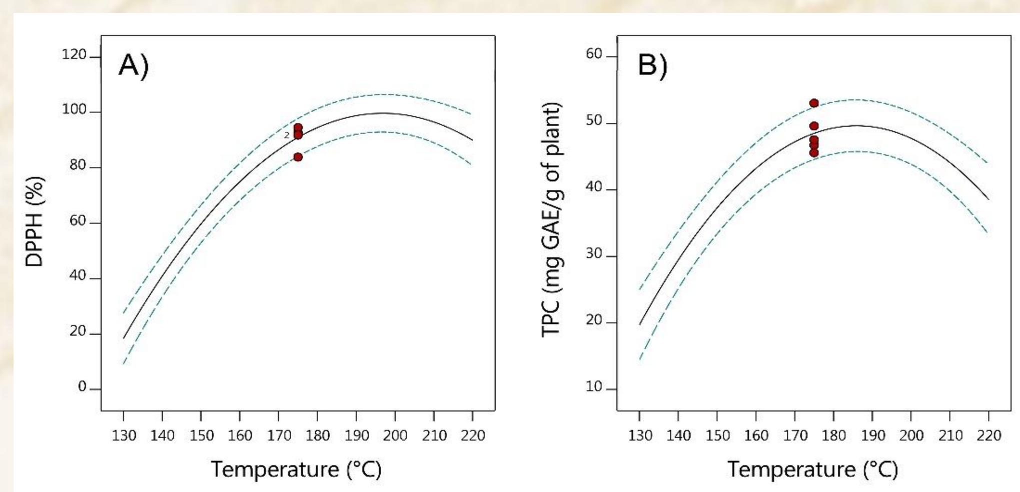
Experimental design for SWE procedure and obtained contents of polyphenolic compounds analyzed by HPLC.

Experimental design (BBD)				Compound (mg/g of peels)				
Run	Temperature (°C)	Time (min)	Solvent-solid ratio (mL/g)	5-HMF	Hesperidin	Narirutin	Rutin	Chlorogenic acid
1.	175	10	20	4.68	9.28	3.65	3.14	0.27
2.	175	15	10	4.32	8.56	1.05	0.89	1.90
3.	130	10	30	0.01	9.19	3.83	1.31	0.28
4.	220	5	20	10.08	0.19	0.09	0.19	58.93
5.	175	15	30	9.48	14.89	4.27	3.91	8.69
6.	175	10	20	6.48	10.52	3.63	3.03	3.62
7.	220	10	10	5.38	1.15	0.03	0.80	21.26
8.	130	15	20	0.00	11.25	4.87	1.04	0.00
9.	130	10	10	0.00	3.66	1.99	0.52	0.08
10.	175	5	30	7.83	15.07	4.72	4.21	0.28
11.	175	5	10	3.67	5.28	1.91	1.68	0.79
12.	175	10	20	6.18	9.61	3.44	2.94	1.98
13.	175	10	20	6.02	11.23	3.38	2.81	1.84
14.	220	15	20	6.39	0.16	0.08	0.18	54.48
15.	130	5	20	0.03	7.34	3.76	1.04	0.29
16.	175	10	20	6.41	11.99	5.11	4.27	9.10
17.	220	10	30	14.33	0.35	0.11	0.26	68.58



5-HMF HESPERIDIN

TPC AND DPPH



2D diagram of the effect of the applied extraction temperature on A) antiradical activity and B) total phenolic content

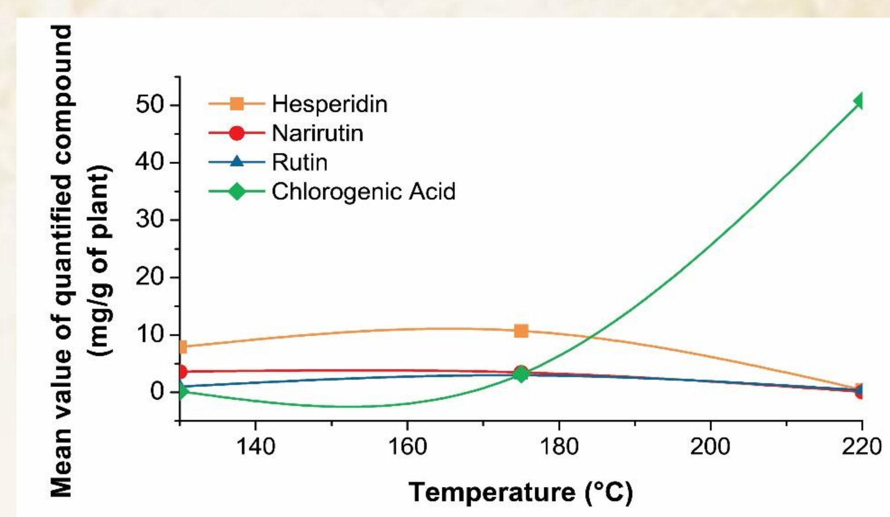


Figure 3. The effect of temperature on the recovery of the three most abundant phenolic compounds and chlorogenic acid in SWE extracts.

Figure 1. 3D diagrams of the effects of A) time and temperature; B) solvent-solid ratio and temperature, and C) solvent-solid ratio and time on the 5-HMF and hesperidin extraction from mandarin peel (*Citrus unshiu* var. *Kuno*) using SWE technique.

PROCESS OPTIMIZATION

Table 1. The uncoded and coded levels of independent variables used in the RSM design for SWE technique from citrus material pretreated by SC-CO_2 extraction.

Independent variable	Symbol	Level		
		Low (-1)	Center (0)	High (+1)
Temperature (°C)	X_1	130	175	220
Extraction time (min)	X_2	5	10	15
Solvent-solid ratio (mL/g)	X_3	10	20	30

CONCLUSIONS

This study focuses on the innovative green extraction techniques of the mandarin peel (*Citrus unshiu* variety *Kuno*) to obtain the highly valuable components.

1. Firstly, SC-CO_2 extractions at operating pressures of 100 and 300 bar were performed to obtain aromatic components of different chemical complexities, among which limonene was detected as the most dominant volatile compound at both applied pressures, followed by α-farnesene, linoleic and hexadecanoic acids;
2. The exhausted residue of mandarin peel remained after SC-CO2 extraction was further used for extraction of polyphenolic compounds using SWE.
3. Based on the obtained results, the most abundant phenolic components in mandarin peel extracts obtained by SWE were hesperidin, narirutin, and rutin.
4. The concentration of these components was dependent on the applied temperature and solvent-solid ratio, with significant decrease observed in extracted content above 160 °C. At these conditions, the higher contents of chlorogenic acid and 5-HMF were detected, which are suggested as by-products of thermal degradation.
5. The extracts obtained at higher temperatures ≤175 °C exhibited good antiradical activity, however the formation of 5-HMF was also observed.
6. Potential limitation of SWE could be attributed to the formation of undesirable compo-nents by applying the higher extraction temperatures. Hence, efficient optimization pro-cess for obtaining highly valuable and at the same time reducing the content of undesira-ble components is undoubtedly a study of interest and essential toward possible large-scale applications.

ACKNOWLEDGEMENT

HrZZ Croatian Science Foundation

ByProExtract

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