Turkish Journal of Agriculture - Food Science and Technology, 11(12): 2417-2425, 2023
DOI: https://doi.org/10.24925/turjaf.v11i12.2417-2425.6519

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Comparison of Different Extraction Methods on the Recovery Efficiencies of Valuable Components from Orange Peels

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ARTICLE INFO

Research Article

Received : 02.11.2023
Accepted : 07.12.2023

Keywords:
Antioxidant activity
Soxhlet extraction
Supercritical carbon dioxide
Ultrasound-assisted extraction
Valuable content

ABSTRACT

Supercritical-CO_2 extraction, Soxhlet extraction, and ultrasound-assisted extraction methods were conducted in this study to recover valuable components, specifically phenolic antioxidant compounds, from orange peels. Basic operating parameters such as temperature and pressure, which affect the extraction efficiency of phenolic substances in orange peel with supercritical-CO_2, were designed using the central composite design methodology. In the Soxhlet and ultrasound-assisted extraction methods, 2-hour extraction processes were carried out using ethanol at different concentrations (50%, 80% and 100%) as a solvent. Yield comparison was made by performing total phenolic content, antioxidant activity and total flavonoid content analyses in the extracts. The total phenolic content (TPC) in the extracts was determined to be 5034 mg GAE/L for supercritical-CO_2 extraction at 61.5°C and 20 MPa. In comparison, Soxhlet extraction yielded a TPC of 1728 mg GAE/L, while the ultrasound-assisted extraction method resulted in a TPC of 4056 mg GAE/L. It was determined that the optimum operating parameters of supercritical-CO_2 extraction were 60°C and 26.4 MPa in case all the responses were maximized. The best phenolic recovery was obtained at 100% ethanol in Soxhlet extraction and 80% ethanol in ultrasound-assisted extraction. Although supercritical-CO_2 extraction is an environmentally friendly application, the recovery rate of valuable components from raw materials is lower than in Soxhlet extraction and ultrasound-assisted extraction. However, since the volume of the extracts obtained from the supercritical-CO_2 extraction is small, the ratio of phenolic compounds is higher.

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Introduction

Citrus fruits are a natural source of many critical bioactive compounds for humans, such as ascorbic acid, flavonoids, phenolic compounds, pectins, and antioxidant substances (Fernández-López et al., 2005). Studies on flavonoids found in citrus fruits show that they minimize the risk of developing heart disease (Zayed et al., 2021). It has also been reported to produce antibodies that fight carcinogenic cells by strengthening the immune system due to its antioxidant properties (Elangovan et al., 1994; Javanmardi et al., 2003).

Valuable components are present in the edible parts of citrus fruits and the inedible peel, which make up almost half of the fruit mass. The peel is part of citrus fruits, containing nearly the highest flavonoid concentrations (Anagnostopoulou et al., 2006). These components have many biological effects such as antibacterial, antiviral, anti-inflammatory, anti-allergic and antithrombotic (Cook and Samman, 1996).

Although citrus fruits are used in many sectors (such as fruit juice, puree, frozen pulp (fruit pulp), fermented beverages, gels, candies, and ice cream), the most industrial use is fruit juice processing. Compared to other uses, it is estimated that 50-60% is used in the fruit juice processing industry (Satari and Karimi, 2018; Zema et al., 2018). The fruit and vegetable processing industry generates significant by-product waste, accounting for approximately 25 to 30% of the commodity group (Sagar et al., 2018). These wastes can be used in the production of dietary fiber (Pathania and Kaur, 2022), enzymes, ethanol, biocolors (Sharma et al., 2016) and adsorbents (Shrivastava and Singh, 2022), or they can be utilized direct land spreading and composting (Zema et al., 2018). It is possible to use these wastes as animal feed (Panwar et al., 2021) and as raw material in biofuel production through biorefinery (Yadav et al., 2022). The fruit juice processing plant wastes are segment membranes, peels (albedo,

flavedo), pulp, and seeds (Zema et al., 2018; Suri et al., 2022). The pulp of orange fruit consists of 60-65% peel, 30-35% slices, 0-10% seeds, dice, juice sacs, and axis pieces on a dry basis. In the production of orange juice, waste is generated in the amount of 0.5 kg/kg of raw oranges. Citrus wastes have a high organic matter content and a low pH value, including valuable components that can be recycled (Alvarez et al., 2018; Zema et al., 2018; Bozkir et al., 2021).

Recycling methods of valuable compounds from plant wastes can be classified into two main categories: conventional and novel techniques. The conventional methods of steam distillation, hydro distillation, or Soxhlet extraction are often used to recover valuable components from waste. However, these techniques may not be suitable for sensitive compounds that may be lost or degraded at high temperatures (Sagar et al., 2018; Phong et al., 2022). Due to the increasing energy prices and the need to reduce environmental impacts, the infrastructure for the necessary experiments to recover valuable components from citrus wastes can be established using extraction techniques that require lower energy needs and minimize environmental problems. Examples of these extraction methods are supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), microwave accelerated distillation (MAD), microwave steam distillation (MSD), microwave hydro diffusion and gravity (MHY), and sonication-assisted extraction (SAE) (Negro et al., 2016). Supercritical-CO_2 (SC-CO_2) extraction is one of the most suitable methods for extracting valuable oils and some organic compounds from plants. Because considering the operating conditions of the process, the critical temperature and pressure of the solvent used must not affect the structure of the extract. CO_2 used as a solvent is non-toxic, inexpensive, non-flammable, and chemically stable. Thanks to its operating conditions, SC-CO_2 exhibits high diffusivity (similar to gases) and high solvent power (similar to liquids), allowing a higher mass transfer and extraction rate. In addition to these advantages, it can be said that low solvent consumption and no residue are left (Mira et al., 1999; Atti-Santos et al., 2005). Compared to other extraction methods, the Soxhlet extraction (SE) method has the most significant disadvantage: the amount of solvent used is high and causes health/environmental problems. The ultrasonic extraction method is based on increasing the interaction between solvent and solute through sound waves. Ultrasonic waves create compression and expansion cycles in the medium due to their movement. During the expansion cycle, the molecules separate and form bubbles that absorb energy and begin to increase in size, whereas during the compression cycle, the molecules come together due to the increase in pressure and temperature at the microscale collapse (Rao and Rathod, 2015). Compared to the Soxhlet extraction method, the ultrasound-assisted extraction (UAE) method can increase extraction efficiency using less solvent (Rathod et al., 2017).

In this study, SC-CO_2 extraction was optimized to recover valuable components from orange peel. Additionally, the efficiencies of SC-CO_2 extraction, SE and UAE methods were compared.

Material and Methods

Materials

Orange peel (albedo and flavedo) was obtained from Mersin/Türkiye and was used in extraction studies. SC-CO_2 extraction, 99.9% pure CO_2 was used as the supercritical fluid (Ar-Oksijen, Konya, Türkiye), and ethanol (Merc, Germany) was used as the solvent in the experiments carried out by Soxhlet extraction and ultrasonic extraction method.

Methods

Preparation of the samples

The citrus peels used in the experiments were dried in an oven at 75 °C for 12 hours. Argun et al. (2023) found that the decomposition of phenolics was not significantly affected at 70 °C unless exposed to sunlight. The dried samples were ground into powder in the GRT-10BL laboratory grinding mill (Akyol, Türkiye). The prepared samples were stored in the refrigerator at +4 °C until use.

Extraction methods

Supercritical CO_2 extraction

SC-CO_2 extractor has a 500 mL column in which temperature and pressure are controlled (Superex F-500; Figure 1). The pressure can be adjusted up to 35 MPa and the temperature up to 70 °C. The desired temperature and pressure values were adjusted, and then the sample (50 g) was placed in the extractor with the help of a cloth. After reaching the set operating conditions, the device was kept in a static state for 20 minutes, and then it was brought to a dynamic state with a carbon dioxide flow rate of 2 ± 0.3 mL/min for 100 minutes.

CO_2 was separated from the extracts spontaneously by reducing the pressure at the extractor outlet and the extracts were collected in a 50 mL falcon tube. The orange peel extracts were coded as PKE (Figure 2).

Figure 1. Photograph of the supercritical carbon dioxide extraction system

Figure 2. Photos of the orange peel extracts (PKE) obtained by SC-CO_2 extraction.

Table 1. Independent variables and working ranges used in SC-CO_2 extraction.

Variables	Working conditions		
	Minimum	Average	Maximum
Pressure (MPa)	8.5	20	31.5
Temperature (°C)	38.5	50	61.5

Table 2. Experimental conditions used in the extraction of orange peel samples with SC-CO_2.

Experiment Code	Working conditions			Extraction time and mode
	Temperature (°C)	Pressure (MPa)	CO_2 consumed (kg)	
PKE-1	38.5	20	0.5	20 min static, 100 min dynamic
PKE-2	40	10	0.4	
PKE-3	40	30	0.4	
PKE-4	50	20	0.5	
PKE-5	50	20	0.5	
PKE-6	50	31.5	0.6	
PKE-7	50	8.5	0.3	
PKE-8	50	20	0.5	
PKE-9	60	30	0.6	
PKE-10	60	10	0.4	
PKE-11	61.5	20	0.5	

Response surface methodology (RSM) was used in the experimental planning. RSM consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to experimental data obtained in relation to experimental design. In applying RSM as an optimization technique, linear or square polynomial functions are used (Bezerra et al., 2008). The central composite design method was used to evaluate the effect of independent variables (pressure and temperature) on dependent variables: volumetric recovery, mass recovery, TPC, TPC recovery, DPPH, and ABTS. Experimental working ranges of the independent variables and specific conditions are summarized in Table 1 and Table 2.

Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction was performed in a high-frequency ultrasonic bath (Kudos, China). In the study, 1 g of dried and ground orange peel samples was taken, and 10 mL of a solvent mixture with ethanol: water ratio of 50%, 80%, and 100%, respectively, was added. The extraction process was carried out at a frequency of 53 kHz, a power of 100 W, temperatures between 20-26 °C and for 120 minutes.

Soxhlet extraction (SE)

Ethanol was used as an organic solvent for Soxhlet extraction. 10 g of ground orange peel sample was placed in extraction cartridges. Ethanol/water ratios were determined as 50%, 80% and 100%. 100 ml of ethanol solution was taken into 200 ml flat-bottomed flasks, of which the empty weight was taken and placed in the apparatus. The Soxhlet extraction apparatus was operated on the heater for 2 hours under a fume hood. At the end of the extraction period, the solution collected in the balloon was evaporated at 65 °C under a fume hood using a Buchi R 100 brand rotary evaporator (Buchi, Germany). The amount of extract obtained at the end of this process was calculated gravimetrically.

Determination of total phenolic content (TPC)

TPC analysis of the extracts was performed using the Folin-Ciocalteu reagent method (Singleton et al., 1999). 20 µL of the sample or diluted sample was taken into 15 mL flasks, and 1580 µL of methanol/water mixture was added to it, and 1600 µL of methanol/water solution was taken for the blank. 100 µL of 2N Folin–Ciocalteu reagent (FCR) was placed on them, and they waited for 5 minutes. Then, 300 µL of sodium carbonate (20%, w/v) was added and mixed. The mixtures were waited for 30 minutes at 45 °C in darkness for color change. At the end of the incubation, the samples were transferred to 2 mL falcon tubes and were centrifuged at 4000 rpm for 5 minutes. Finally, absorbance values were read at 765 nm wavelength in a spectrophotometer (Hach Lange DR-5000). The calibration curve was prepared using standard gallic acid to calculate TPC ($y = 0.0441x$, R^2: 0.994). TPC was expressed as mg gallic acid equivalent (GAE)/L extract.

Determination of total flavonoid content (TFC)

TFC analysis of the extracts was performed according to Zhishen et al. (1999). 250 µL of the samples (250 µL of ethanol for the blank) was taken and placed in 15 mL falcon tubes. 1250 µL of pure water was added to it. 75 µL of 5% $NaNO_2$ solution was added to the mixture, mixed, and waited 6 minutes. At the end of the waiting period, 150 µL of 10% $AlCl_3$ solution was added, mixed, and waited 5 minutes. Finally, 500 µL of 1 M NaOH solution was added, and the total volume was completed to 2500 µL with 275 µL of pure water. A light orange color was observed in the prepared samples. The absorbance values of the prepared samples were read at 510 nm wavelength in a spectrophotometer (Hach Lange DR-5000). The calibration curve was prepared by using standard quercetin to TFC calculation ($y = 0.0009x$, R^2: 0.9868). TFC was expressed as mg quercetin equivalent (QE)/g extract.

Determination of DPPH$^{\bullet}$ scavenging capacity

The free radical scavenging capacity of the samples was measured according to Rai et al. (2006). 1900 µL of DPPH solution was taken into falcon tubes with a volume of 2000 µL, and 100 µL of the sample was added and shaken. Prepared samples were kept in the dark for 30 min. At the end of the time, the absorbance values of the samples were read at 517 nm wavelength. The calibration curve was prepared using the Trolox standard and expressed as µM TE.

Determination of ABTS$^{\bullet+}$ scavenging capacity

The radical cation scavenging capacity of the samples was determined according to Re et al. (1999). At room temperature, a seven mM ABTS$^{\bullet+}$ stock solution containing 2.45 mM potassium persulfate was prepared and kept in the dark for 12-16 hours. The ABTS$^{\bullet+}$ working solution was prepared by diluting the stock ABTS$^{\bullet+}$ solution with a 1:1 water:ethanol (v/v) mixture such that the absorbance of the total mixture was 0.70 ± 0.02 at 734 nm. 1000 µL of ABTS$^{\bullet+}$ solution was taken into 2000 µL falcon tubes, and 10 µL of the sample was added to it. The lids of the falcon tubes were closed, mixed with vortex, and waited for 6 minutes. At the end of the waiting period, the absorbance values of the samples were read by adjusting the spectrophotometer to a wavelength of 734 nm. The calibration curve was prepared using the Trolox standard and expressed as µM TE.

Calculation of recovery yields

The extraction yields were calculated concerning extract volume (%, v/w) and extracted mass (%, w/w) according to Equation (1):

$$Yield\ (\%) = \frac{X_{ext} \times 100}{M_{OP}} \quad (1)$$

where X is the volume (mL) or mass (g) of the extract and M_{OP} is the mass of the orange peel (OP) (g).

The TPC and TFC recoveries (%, w/w) were calculated considering the mass balance according to Equation (2):

$$\%R_{TPC,TFC} = \frac{C_{ext} \times M_{ext}}{C_{OP} \times M_{OP}} \times 100 \quad (2)$$

Where C is the concentration of the individual TPC and TFC in a particular matrix expressed in mg/g DW, and M is the mass of the extract and OP (g).

Statistical analysis

The significance of the statistical relationship between the independent variables and the results was evaluated according to the ANOVA results. The design of the experimental conditions and the ANOVA tests of the results were carried out with the Minitab 18 software. The obtained statistical model was analyzed at a 95% confidence interval (P <0.05).

Results and Discussion

Recovery studies with SC-CO_2 extraction

The appearance of the extracts obtained at higher pressures was dark orange, indicating more TFC in the extracts (Figure 2). These differences in color tones are also confirmed by TFC recovery efficiencies (Figure 3). It was observed that TFC is more enriched than TPC in the extracts. This richness indicates that SC-CO_2 dissolves more apolar flavonoids than other phenolics. The extraction yields for volumetric recovery and mass recovery were increased at lower temperatures and pressures, while TPC values were increased at higher pressures (Figure 3). It was determined that the volumetric recovery values of the extraction process varied between 4-8% and the mass recovery values between 2-5%. The highest recovery efficiencies in volume and mass were obtained for 38.5°C, 20 MPa, and 40°C, 10 MPa conditions.

It was determined that the TPC values of the extracts ranged between 1678-5034 mg GAE/L extract (R_{TPC}: 0.8-2.5%), and the TFC values ranged between 25-43 mg QE/g extract (R_{TFC}: 9-20%) (Figure 3 and Table 3). The ABTS$^{\bullet+}$ scavenging activity of the extracts was found to be 1708-16107 µM TE, and the DPPH$^{\bullet}$ scavenging activity was seen as 2423-7602 µM TE (Table 3).

ANOVA data showing the effects of experimental conditions on the responses are given in Table 4. It was observed that the effects of temperature on the extract yield and the effects of pressure on the TPC, TPC recovery, DPPH• scavenging activity, and ABTS•+ scavenging activity recovery values were significant (P<0.05). Argun et al. (2022) determined that increasing the temperature and pressure increased the extract yield and phenolic substance recovery from orange processing wastewater. Espinosa-Pardo et al. (2017) reported that the extract yield and TPC content increased in SC-CO_2 extraction of phenolic compounds from processed pulp from orange juice with increasing pressure.

Optimization of SC-CO_2 extraction according to the response surface methodology (RSM) is presented in Table 5. According to the central composite design, the optimum extraction conditions to maximize volumetric recovery, mass recovery, TPC, TPC recovery, DPPH$^{\bullet}$ scavenging activity, and ABTS$^{\bullet+}$ scavenging activity values of the extracts were determined as 22 MPa and 40°C.

Table 3. Valuable components variation at the different experimental conditions for SC-CO_2 extraction.

	TPC (mg GAE/L extract)	TPC recovery (%)	TFC (mg QE/g extract)	TFC recovery (%)	ABTS$^{\bullet+}$ scavenging activity (µM TE)	DPPH$^{\bullet}$ scavenging activity (µM TE)
PKE-1	4717	2.46	34.6	19.8	4791	5448
PKE-2	2880	1.72	29.9	18.6	1708	4926
PKE-3	3696	1.65	42.9	18.5	11245	6130
PKE-4	2827	1.78	38.2	19.6	16107	6550
PKE-5	2268	1.38	34.4	18.8	9066	7254
PKE-6	4263	1.27	36.1	13.1	13006	6947
PKE-7	1678	0.75	38.0	17.0	2947	4845
PKE-8	2222	1.16	33.6	17.7	9122	7602
PKE-9	4558	2.04	38.0	17.3	12168	6237
PKE-10	3356	1.00	25.3	9.1	1760	2423
PKE-11	5034	2.25	39.7	17.9	8144	6492

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalents

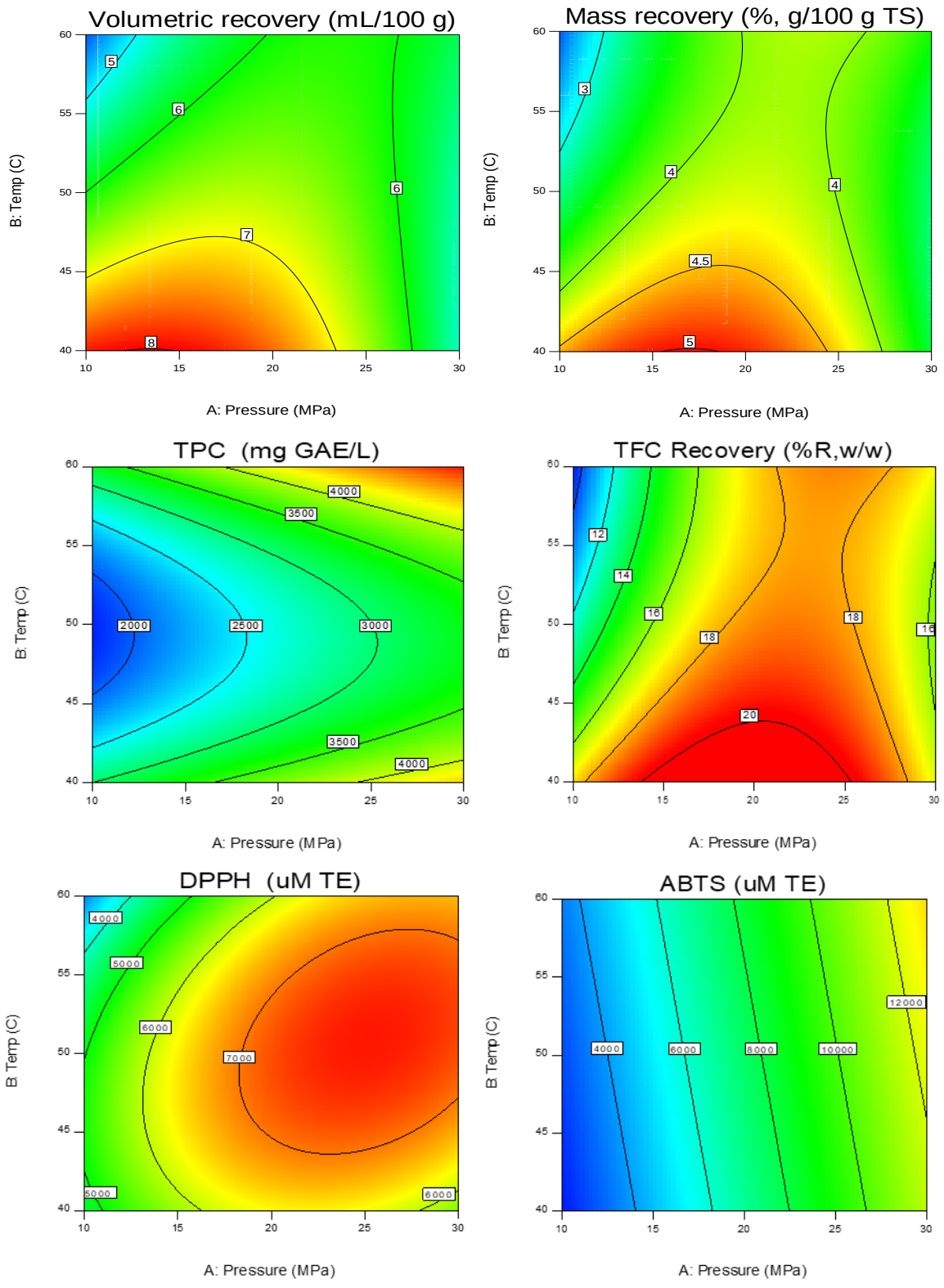

Figure 3. Dual effects of pressure and temperatures on the extraction yields and quality parameters of the extracts

However, it was determined that some values were higher at the 26 MPa and 60°C, and there was no significant difference between the two optimization solutions. Espinosa-Pardo et al. (2017) emphasized that the highest TPC content obtained using SC-CO_2 extraction from orange peel was at 40 °C and 350 bar. Still, there was no significant difference between the study performed at 60 °C and 250 bar. Based on these results, it can be concluded that temperature effects solvent density and selectivity in obtaining total phenolics. Argun et al. (2022) determined the optimum extraction conditions to maximize the mass recovery, TPC, TPC Recovery, and antioxidant capacity values of the extracts obtained from orange processing wastewater as 28.7 MPa and 60°C. Santos et al. (2019) obtained a TPC value of 23 mg GAE/g under 55°C and 30 MPa pressure (optimized parameters) in their extraction using SC-CO_2 extraction from feijoa peel.

Table 4. Statistical relevance between experimental conditions and obtained results for SC-CO_2 extraction.

Source	Volumetric recovery (mL/100 g)		Mass recovery (%, g/100 g)		TPC mg GAE/L		TPC recovery (%, w/w)	
	F	P	F	P	F	P	F	P
Model	Quadratic		Quadratic		Quadratic		Quadratic	
	4.8	0.06	4.1	0.07	4.9	0.05	8.4	0.018*
Pressure	1.5	0.27	5.10^{-3}	0.95	8.7	0.03*	5.9	0.059
Temperature	7.8	0.04*	4.7	0.08	1.0	0.36	0.8	0.42
Lack of Fit	9.7	0.09	31.9	0.03	5.7	0.15	0.2	0.91
Std. Dev.	0.72		0.55		656		0.25	
Mean	6.14		3.80		3409		1.57	
C.V. %	11.75		14.44		19.25		15.99	
R-Squared	0.83		0.81		0.83		0.89	
Adeq Precision	6.67		6.49		6.92		9.26	
Source	TFC mg QE/g		TFC recovery (%, w/w)		DPPH$^{\bullet}$ scavenging activity µM TE		$ABTS^{\bullet+}$ scavenging activity µM TE	
	F	P	F	P	F	P	F	P
Model	Linear		Quadratic		Quadratic		Linear	
	1.3	0.32	8.8	0.02*	6.9	0.03*	5.4	0.03*
Pressure	2.6	0.15	6.5	0.05	15.5	0.01*	10.6	0.01*
Temperature	0.1	0.81	8.2	0.04*	0.4	0.55	0.3	0.63
Lack of Fit	0.5	0.79	4.4	0.19	2.4	2.44	0.1	0.99
Std. Dev.	5.69		1.74		732		3755	
Mean	36.29		16.45		5896		7558	
C.V. %	15.69		10.56		12.41		49.68	
R-Squared	0.25		0.90		0.87		0.58	
Adeq Precision	2.75		9.89		7.64		5.58	

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalents; *: P <0.05 level of significance.

Table 5. Optimum supercritical condition and results for orange peel extraction by using for pre-determined goals.

Variables		Goal	Range of experimental value		I	Solution 1 (Desirability: 0.74)	Solution 2 (Desirability: 0.73)
			Lower limit	Upper limit			
Experimental variables	A: Pressure (MPa)	in range	10	30	3	22.1	26.4
	B: Temperature (°C)	in range	40	60	3	40.0	60.0
Results	Volumetric recovery (mL/100 g)	maximize	4.00	8.00	3	7.25	6.03
	Mass recovery (%, g/100 g TS)	maximize	2.17	5.05	3	4.78	4.01
	TPC (mg GAE/L)	maximize	1678	5034	3	3876	4761
	TPC recovery (%, w/w)	maximize	0.75	2.50	3	2.12	2.14
	DPPH (µM TE)	maximize	2423	7603	3	6377	6643
	ABTS (µM TE)	maximize	1708	16107	3	7814	11300
	TFC recovery (%, w/w)	maximize	9.1	19.82	3	21.2	18.28

I: Importance; TS: Total solids; TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; TE: Trolox equivalents.

Table 6. Comparison of the extraction yield, TPC and antioxidant activity values of the studied extraction methods.

Extraction type	Extraction conditions	Solvent /sample	Yield, %	TPC, mg GAE/g*	$\%R_{TPC}$	TFC, mg QE/g	DPPH, µmol TE/g	ABTS, µmol TE/g
Soxhlet extraction	50% ethanol	20	23	22.5^{abc}	28.5^{a}	0.6^{a}	12.9^{e}	33.8^{e}
	80% ethanol		12	33.9^{bc}	42.9^{ab}	5.8^{a}	15.1^{f}	41.5^{f}
	100% ethanol		49	34.6^{c}	45.7^{ab}	10.5^{ab}	13.3^{e}	33.9^{e}
Ultrasound-assisted extraction	50% ethanol	10	-	36.8^{c}	46.7^{ab}	1.2^{a}	6.6^{a}	20.8^{d}
	80% ethanol		-	42.3^{c}	98.2^{b}	13.0^{ab}	7.6^{b}	21.3^{d}
	100% ethanol		-	30.3^{abc}	55.2^{ab}	14.2^{abc}	7.7^{b}	21.5^{d}
SC-CO_2 extraction	38.5°C, 20 MPa	~10	4.6	7.1^{a}	2.5^{a}	34.6^{bcd}	8.2^{c}	7.2^{a}
	50°C, 20 MPa		4.4	4.7^{a}	1.4^{a}	38.2^{cd}	10.7^{d}	15.2^{c}
	61.5°C, 20 MPa		3.7	8.3^{ab}	2.3^{a}	39.7^{d}	10.6^{d}	13.4^{b}

TPC: Total phenolic content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Trolox equivalents; *: mg GAE/g extract for SC-CO_2 extraction, mg GAE/g dry peel for SE and UAE.; Mean values expressed with different letters in the same column are significantly different (P< 0.05).

Recovery studies with Soxhlet extraction (SE)

Extraction yield, TPC, DPPH$^{\bullet}$ scavenging activity, and ABTS$^{\bullet+}$ scavenging activity values of the extracts obtained by SE using 50% (v/v), 80% (v/v), and 100% (v/v) ethanol solutions from ground orange peel are given in Table 6. It was observed that the obtained extract yields changed significantly depending on the change in ethanol ratios, and the highest value was reached for 100% ethanol. Likewise, it can be said that TPC values get the best values in 100% ethanol. DPPH$^{\bullet}$ scavenging activity and ABTS$^{\bullet+}$ scavenging activity values were found to be higher at 80% ethanol. It is seen that the amount of antioxidant substance recovery from orange peels is higher than the antioxidant activity values of the extracts obtained by the SC-CO_2 method. The high extraction efficiency obtained by the Soxhlet method may be because ethanol dissolves the polar phenolic compounds better than SC-CO_2 (Azwanida, 2015).

Soxhlet extraction is a continuous process compared to percolation and maceration methods and is advantageous because it is easy, requires less time, and is less solvent (Azwanida, 2015; Alara et al., 2021). Alias and Abbas (2017) found the TPC values as 28.78 mg GAE/mg DW and 207.72 mg GAE/mg DW in the extracts they obtained from pineapple peels using SE and Microwave Assisted Extraction (MAE) methods, respectively.

Recovery studies with ultrasound-assisted extraction (UAE)

TPC and antioxidant activity values of extracts obtained from orange peels with UAE (53 kHz) at different alcohol ratios are summarized in Table 6. While the TPC value was higher at 80% ethanol, the antioxidant activity values were a little higher at 100% ethanol, which could be partly due to higher TFC concentration. The amount of flavonoids with higher antioxidant activity increases in the extracts because of high ethanol concentration. Rodrigues et al. (2015) observed the positive linear effect of ethanol concentration on the extraction of monomeric anthocyanin and cyanidin-3-O-glucoside from jabuticaba peel. It was concluded that UAE obtained maximum extraction efficiencies of TPC with the range of 47%-98%. Odabaş and Koca (2016) reported that higher extraction time (45 min) and medium ethanol concentration (approximately 67%) for UAE application resulted in increased extraction of total phenolic compounds. Some researchers reported that optimum TPC concentration could be obtained by using ethanol concentration near 70% in maceration, like our findings (Nepote et al., 2005; Vongsak et al., 2013). In their study on the extraction of bioactive components from lemon peels, Jagannath and Biradar (2019) found the TPC and TFC values to be 7.17 mg GAE/100 g and 4.52 mg CE/100 g, respectively, with the UAE method under optimum conditions. Their study stated that the UAE method was better than Soxhlet in the extraction of total phenolics and flavonoids, retention of vitamin C, and antioxidant activity.

Comparison of different extraction methods

TPC concentration of the SC-CO_2 extracts was a maximum of 8.3 mg GAE/g which is lower than Soxhlet and ultrasound-assisted extraction. However, the concentration value of the extracts (if calculated as mg GAE/L extract) obtained by SC-CO_2 extraction reached up to 5034 mg GAE/L while the TPC concentrations in the Soxhlet and UAE extracts were a maximum of 1728 mg GAE/L and 4056 mg GAE/L, respectively. This situation may be due to the dilution of phenolics in the increasing extract volume because of the higher yield of methods other than SC-CO_2 extraction. The DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging activity values of the extracts obtained by the SE method were the highest. The highest %R_{TPC} value (98.2%) was reached in UAE using 80% ethanol (Table 6). It is reported that UAE is a more economical extraction method than Soxhlet, provides higher extraction efficiency and requires less extraction time (Ciğeroğlu et al., 2018). By using a co-solvent, SFE can be made an effective technique for the extraction of essential oils and polar compounds, although the performance of UAE is better. Extraction performance can be improved by combining or integrating two different extraction techniques. Combining ultrasound with SFE increases extraction efficiency (Osorio-Tobón, 2020). Although SC-CO_2 extraction is an environmentally friendly and least damaging method to bioactive components, the extract yield and recovery rate of valuable components were found to be lower.

Conclusion

In this study, valuable components found in orange peels, generated as waste in various sectors, were tried to be recovered using SC-CO_2, SE, and UAE methods. In SC-CO_2 extraction, optimum extraction conditions were found to be 22 MPa and 40 °C (or 26.4 MPa and 60 °C) to maximize the volumetric recovery, mass recovery, TPC, TPC recovery, DPPH$^{\bullet}$ scavenging activity, and ABTS$^{\bullet+}$ scavenging activity values of the extracts according to the central composite design. While SE and UAE methods give higher values in terms of extract efficiency and recovery of valuable components, it is a fact that SC-CO_2 extraction is an environmentally and product-friendly method. The use of co-solvent can increase the efficiency of SC-CO_2 extraction. Increasing sensitivity to environmental protection and the spread of zero waste policies bring environmentally friendly applications such as SC-CO_2 extraction to the fore rather than applications that use chemicals such as SE and UAE methods. However, the effectiveness of this application needs to be increased. In addition, in SC-CO_2 extraction, since the extract is obtained in pure form without solvent removal, the products obtained may be more practical and economical.

Acknowledgments

The authors thank the Scientific and Technological Research Council of Turkey (TUBITAK grant No: 120Y351) and Konya Technical University Research Fund (Grant No: 211001019) for their financial support.

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