

Original Research

Recovery of Phenolic Compounds from Orange Peel Waste by Conventional and Assisted Extraction Techniques Using Sustainable Solvents

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Academic Editor: Vincent Courdavault

Submitted: 22 September 2023 Revised: 8 November 2023 Accepted: 16 November 2023 Published: 12 December 2023

Abstract

Background: The valorization of orange peel waste (OPW) through the extraction of bioactive compounds is a clear example of the circular economy. OPW contains many value-added compounds, among which bioactive phenolic compounds (flavonoids and phenolic acids) could be extracted and used for industrial applications, such as pharmaceuticals or cosmetics. **Methods:** In this work, the extraction of phenolic compounds from orange peel was carried out by conventional (orbital shaker) and assisted (ultrasound and microwave) extraction techniques using deionized water, 80% (v/v) ethanol in water, and ethyl acetate as solvents. The effect of temperature, extraction time, and type of technique was evaluated and discussed following spectrophotometric (total phenolic content and total flavonoid content) and high-performance liquid chromatography (HPLC) analyses of the extracts. **Results:** The most effective extraction in terms of efficiency was achieved by microwave-assisted extraction using 80% (v/v) ethanol in water as the extraction solvent, at 373 K for 6 min, which obtained 7.2 ± 0.1 mg gallic acid equivalent (GAE)/g OPW and 13.3 ± 0.1 mg quercetin equivalent (QE)/g OPW, with the main bioactive compound extracted being hesperidin (58.2 ± 0.2 mg/g OPW). The most effective extraction in terms of energy consumption was achieved using ultrasound-probe-assisted extraction, yielding 8.8 ± 0.0 mg GAE/g OPW; 17.1 ± 0.1 mg QE/g OPW; 40.0 ± 0.2 mg hesperidin/g OPW, with an energy consumption of 18 kJ. **Conclusions:** Ultrasound and microwave-assisted extractions can be considered efficient extraction technologies for the valorization of OPW as they reduce extraction time and energy consumption and increase extraction yield.

Keywords: natural phenolic compounds; assisted extraction; sustainable solvents; orange waste valorization; hesperidin

1. Introduction

Annually, more than 1.6 billion tons of food waste are produced worldwide [1]. The valorization of food waste through sustainable technologies to produce value-added bioproducts is considered a potential alternative, which can be implemented to address the problems related to waste management within the framework of the circular bioeconomy [2]. In particular, the citrus industry greatly contributes to food waste, with more than 140 million tons of citrus fruits produced annually (FAO-Citrus Fruit Statistics, 2020), generating large amounts of by-products during their processing. The most abundant citrus fruit is orange (*Citrus Sinensis*), with an annual production of 76 million tons, of which approximately 30% is used for juice processing, leading to up to 20 million tons of solid residue [3]. Traditionally, incineration, landfill, ensiling, or animal feeding have been used for citrus waste management, yet these conventional techniques require high costs and energy and can cause serious health problems and environmental impacts [2,4,5]. Therefore, the production of bioproducts from such renewable waste is being explored as a potential valoriza-

tion strategy to reduce the environmental impact and cost of citrus waste management. Moreover, using the citrus waste generated during processing could help to increase profits in the orange industry [5].

Orange peel waste (OPW) contains a wide variety of value-added compounds, including fermentable sugars, organic acids, pectin, flavonoids, polyphenols, some pigments, and essential oils, such as D-limonene, among others [6,7]. In this regard, OPW can be used to produce pectin, flavonoids, natural colorants, nutraceutical products, and antioxidant phenolic compounds. The latter compounds (more specifically, flavonoids and phenolic acids) could be used to prevent food oxidation; in addition, their consumption has been associated with multiple health benefits due to their nutraceutical and medical properties [2,8–10].

In particular, flavonoids are natural compounds with a stable phenolic structure, which provide strong antioxidant and radical scavenging activities [11] as well as significant biological properties, such as protection against ultraviolet (UV) radiation [12]. Flavonoids are among the main antioxidant molecules and possess a wide range of prop-



erties that make them interesting in fighting various diseases, acting as chemotherapeutic, chemo-preventive, and antiangiogenic agents [13]. According to their structure, flavonoids can be classified as flavones (e.g., apigenin, luteolin, rutin), flavanones (e.g., hesperidin, naringin, naringenin), flavonols (e.g., quercetin, kaempferol), isoflavones, and catechins [11,12,14,15]. The main flavonoids present in orange peels are hesperidin, naringin, neohesperidin, narirutin, rutin, and quercetin [16,17]. Among them, hesperidin is the most abundant flavonoid [12,18]. Hesperidin is of great interest as it is effectively used as a supplement with pharmaceutical effects because of its significant potential as a therapeutic agent for a wide range of diseases and disorders [19]. In addition, several phenolic acids, such as caffeic acid, chlorogenic acid, *trans*-ferulic acid, cinnamic acid, and *p*-coumaric acid, are widely reported as orange peel constituents [9,20]. Phenolic acids are substances that contain a phenolic ring together with a single carboxylic acid functional group and have several biological functions, such as nutrient uptake, enzyme activity of protein synthesis, or photosynthesis [13]. The growing interest in phenolic acids is attributed to their protective role against oxidative damage diseases, such as coronary heart disease, stroke, and cancers [13]. The levels of these phenolic acids in fruit are higher in the peel than in the pulp, particularly for the hydroxycinnamic acids [8].

Conventional solvent extraction has been widely used to recover the aforementioned bioproducts from natural matrices [21]. However, this traditional technique has several drawbacks, such as the need for long extraction times, which leads to low extraction yields, high energy consumption, safety hazards, and environmental risk, in addition to low-quality extracts due to oxidative degradation [21,22]. In the development of more environmentally friendly and efficient extraction methods, new technologies such as supercritical fluid extraction, pressurized liquid extraction, ultrasound-assisted extraction, and microwave-assisted extraction have been explored as suitable alternatives to conventional technologies [23]. On the one hand, ultrasound-assisted extraction (UAE) has been shown to decrease the extraction time and increase the efficiency of the process [24]; this improvement is attributed to cavitation bubbles that cause a rupture in the cell membrane, thereby allowing a higher diffusion rate of the solvent into the matrix, improving mass transfer, and releasing bioactive compounds [25–27]. In addition, previous studies have proven that the use of ultrasound as an assisted extraction technology enhances the antioxidant activity of the extract [28,29]. Alternatively, microwave-assisted extraction (MAE) has been shown to reduce the required solvent volume and extraction time, while obtaining a high extraction yield [30]. During MAE, the substances are heated up in a targeted and selective way with practically no heat lost [31]. This heat is based on the direct effect of microwaves on the molecules by ionic conduction and dipole moment [32]. In a plant ma-

trix, this phenomenon causes a high pressure inside the cell wall, causing the cell to rupture, which results in the release of solutes from within the matrix [33]. A higher extraction yield is also achieved by the increase in temperature, which favors a faster penetration of the solvent into the cell wall of the plant matrix [34].

Traditionally, bioproducts have been extracted with organic solvents. In this work, the solvents selected were deionized water (H₂O), 80% (v/v) ethanol in water (80% EtOH), and ethyl acetate (EA). Water was selected for use as a reference. Ethanol is a polar food-grade solvent, sustainably produced by the fermentation of the sugar present in the biomass [35]. The composition of the mixture of water and ethanol was selected by considering the previous results obtained by Li *et al.* [36], who analyzed the effects of ethanol concentrations, using pure water to 95% ethanol, on the phenolic compounds extraction from citrus peel. It was confirmed that extraction efficiency improved as the ethanol concentration increased up to 80%. Furthermore, ethyl acetate was chosen as a nonpolar organic solvent to study the polarity effect on the extraction efficiency. Ethyl acetate possesses low toxicity, and it was widely used as a solvent in the food, cosmetic, and pharmaceutical industries. Ethyl acetate is produced directly from biomass through the esterification of ethanol with acetic acid [35].

This work aimed to evaluate and compare conventional and assisted extraction techniques (orbital shaker, ultrasound bath, ultrasound probe, and microwaves) in the extraction of phenolic compounds from OPW, especially flavonoids and phenolic acids. For this purpose, different extraction conditions were tested as a function of the extraction solvent, the extraction time, or the working temperature. Water, 80% EtOH, and ethyl acetate were used as solvents to evaluate their role in the phenolic extraction yields and selectivity. The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were measured by spectrophotometric methods. Afterward, the phenolic profiles of the extracts were quantified by high-performance liquid chromatography with diode array detection (HPLC-DAD). In addition to the experimental screening, the energy consumption in the best conditions of each extraction technique was evaluated. The systematic evaluation of the different extraction techniques contributes to the efficient design of the methodology used for the valorization of the naturally occurring phenolic compounds found in OPW, not only in terms of quantification but also for energy efficiency.

2. Materials and Methods

2.1 Orange Peel Waste Sample Preparation

About 3 kg of fresh oranges, *Citrus Sinensis*, of Navel Lane Late variation were purchased from a local store in Madrid (Spain). The oranges were squeezed, and the peels were dried in an oven at 313 K for 24 h, then, ground in a mill until a homogenous powder was obtained. The re-

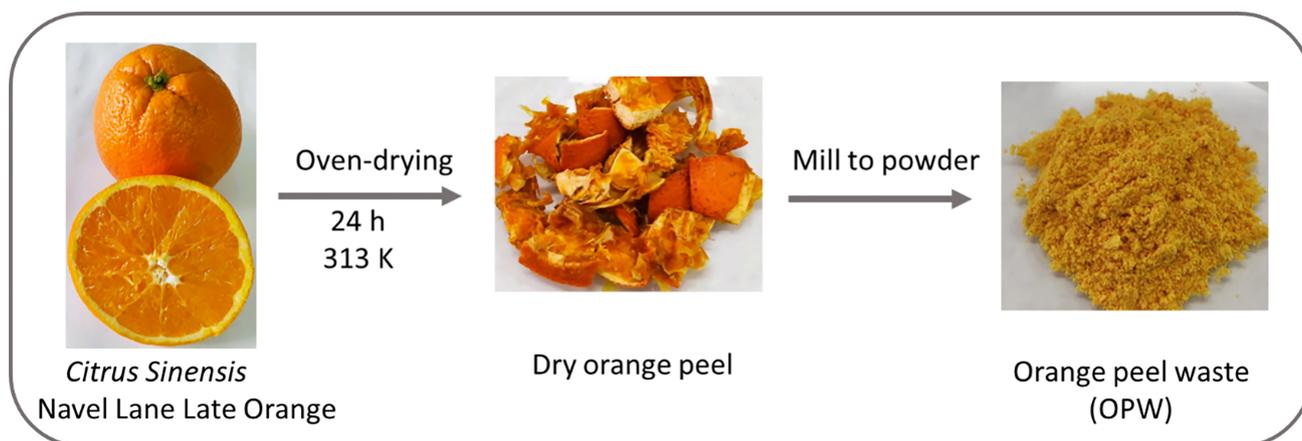


Fig. 1. Representation of the orange peel waste (OPW) preparation.

sultant powder, labeled as orange peel waste (OPW), was stored in a freezer, and maintained at 269 K until experiments were carried out. A schematic diagram of the preparation of the OPW can be observed in Fig. 1.

2.2 Chemicals and Reagents

The solvents used in this work were deionized water, ethanol absolute (CAS Number: 64-17-5; purity ≥ 99.5 wt.%), supplied by J. T. Baker (Gliwice, Upper Silesia, Poland), and ethyl acetate (CAS Number: 141-78-6; purity ≥ 99.5 wt.%), supplied by HoneyWell (Seelze, Lower Saxony, Germany). In all cases, solvents were used as received without any additional purification.

The Folin–Ciocalteu phenol reagent (FC) (2N) (CAS number: 12111-13-6) and sodium carbonate anhydrous (CAS Number: 497-19-8; purity $\geq 99.5\%$) were used to perform the Folin–Ciocalteu method following purchase from Sigma-Aldrich (St. Louis, MO, USA). To determine the flavonoid content in each essay, sodium nitrite (CAS Number: 7632-00-0; analysis purity) from Sigma-Aldrich, aluminum chloride (CAS Number: 7446-70-0; purity $>98\%$) from EMD Millipore Corporation (Darmstadt, Hessen, Germany), and sodium hydroxide (CAS Number: 1310-73-2; for certificate analysis) from J.T. Baker were used to conduct the corresponding aluminum complexation reactions.

The chemicals used as standards for HPLC analysis were hesperidin (CAS Number: 520-26-3; purity ≥ 80 wt.%), naringin (CAS Number: 10236-47-2; purity ≥ 95 wt.%), (+)-catechin hydrate (CAS Number: 225937-10-0; purity ≥ 98 wt.%), rutin hydrate (CAS Number: 207671-50-9; purity ≥ 94 wt.%), quercetin (CAS Number: 117-39-5; purity ≥ 95 wt.%), gallic acid (CAS Number: 149-91-7; purity ≥ 97 wt.%), caffeic acid (CAS Number: 331-39-5; purity ≥ 98 wt.%), *p*-coumaric acid (CAS Number: 501-98-4; purity ≥ 98 wt.%), and *trans*-ferulic acid (CAS Number: 537-98-4; purity 99 wt.%), all of which were purchased from Sigma-Aldrich. For the HPLC mobile phase,

acetonitrile (CAS Number: 75-05-08), and water (HPLC grade purity) were purchased from Honeywell, and acetic acid (CAS Number: 64-19-7; purity ≥ 99 wt.%) was bought from Labkem (Vilassar de Dalt, Barcelona, Spain).

2.3 Extraction Methods

All the extractions were carried out by adding 0.5 g of OPW to 5 mL of the solvent (i.e., biomass:solvent ratio = 1:10, in g/mL). In this work, deionized water (H_2O), a solution of 80% (v/v) ethanol in water (80% EtOH), and ethyl acetate (EA) were used as solvents.

Extraction conditions for each technique (orbital shaker, ultrasound, and microwave) were evaluated in terms of extraction time and temperature. The selected values for each variable were based on preliminary assays and the operative limits of each instrument.

For orbital shaker extraction (OSE), an orbital shaker (Labnet VORTEMP 1550 orbital shaker) with controlled temperature and an accuracy of ± 0.5 K was used. The extractions were performed by varying the extraction temperature between 303 and 333 K, and the extraction time at 30, 60, and 90 min, under a fixed shaker rate of 900 rpm.

For ultrasound bath-assisted extraction (UBAE), a bath (Elmasonic S 15 H, Elma) was used with an ultrasound frequency of 37 kHz, power of 35 W, and a controlled temperature. In this case, the extractions were carried out at 333 K for 30 min.

Ultrasound probe-assisted extraction (UPAE) was evaluated using a portable ultrasonic homogenizer UP200Ht (200 W, 26 kHz) (Hielscher Ultrasound technology). The extraction process was carried out using a power of 10 W (based on preliminary tests) and various extraction times of 1.5, 3, and 6 min. Although temperature could not be controlled in this case, it was measured at the end of each experiment.

A microwave reactor (Monowave 400, Anton Paar) equipped with an infrared temperature sensor for temperature control was used in microwave-assisted extractions

(MAEs). For the microwave extraction, a hermetically closed vessel was used, with a fixed stirring rate of 1000 rpm. Samples were heated at 333 and 373 K for 1.5, 3, or 6 min.

After all extraction assays were performed, the obtained samples were centrifuged with Unicen 21 Centrifuge (Ortoalresa, Spain) for 10 min at 4200 rpm, to promote phase separation. The extracts were analyzed using different analytical techniques, which are described in the following section.

2.4 Analytical Methods

2.4.1 Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined after extraction by the Folin–Ciocalteu spectrophotometric quantification method, adapted from Cañadas *et al.* [37]. Briefly, 0.1 mL of the extract and 0.1 mL of the Folin–Ciocalteu reagent were added to a glass vial and thoroughly mixed. After incubating for 3 min in the absence of light, 2 mL of 2% (w/v) sodium carbonate aqueous solution was added to the mix and incubated in the dark for another 30 min. Then, the absorbance of the resulting solution was measured at 765 nm by a JASCO V-730 UV–Vis spectrophotometer (JASCO, Spain). Spectrophotometric analyses were carried out in triplicate using the corresponding tested solvent as a blank.

The TPC values, expressed as mg of gallic acid equivalent (GAE) per g of OPW, were obtained from Eqn. 1:

$$TPC (mg GAE/g OPW) = \frac{[GAE]}{m OPW} \cdot V solvent \quad (1)$$

where [GAE] is the gallic acid equivalent present in the extract expressed as mg of gallic acid per L, calculated from the calibration curve previously obtained with standard solutions of gallic acid (in mg/L); V solvent is the amount of solvent used during the extraction, expressed in L; m OPW is the mass of the sample used, expressed in g. The results were expressed as TPC (mg GAE/g OPW) ± relative standard deviation (RSD, %). The RSD, expressed as a percentage, was calculated as the typical deviation of the three results obtained divided by the average value.

2.4.2 Determination of Total Flavonoids Content (TFC)

Total flavonoid contents were determined following the procedure reported by Peçkal *et al.* [38], with slight modifications. In a glass vial, 1 mL of deionized water was mixed with 0.45 mL of the solvent extract and 0.15 mL of 5% (w/v) of NaNO₂. After 5 min, 0.15 mL of 10% (w/v) of AlCl₃ was added and thoroughly mixed for 5 min. Then, the solution was neutralized with 1 mL of 1 N NaOH aqueous solution. The mixture was left for 15 min at room temperature and then measured at 510 nm by a JASCO V-730 UV–Vis spectrophotometer (JASCO, Spain). Spectrophotometric analyses were carried out in triplicate using the corresponding tested solvent as a blank.

The results, expressed as mg of quercetin equivalent per g of OPW, were calculated using Eqn. 2:

$$TFC (mg QE/g OPW) = \frac{[QE]}{m OPW} \cdot V solvent \quad (2)$$

where [QE] is the quercetin equivalent present in the extract expressed as mg of quercetin/L, calculated with the calibration curve measured using standard solutions of quercetin (representative flavonoid); V solvent is the amount of solvent used during the extraction expressed in L; m OPW is the mass of the sample used, expressed in g. The results were expressed as TFC (mg QE/g OPW) ± relative standard deviation (RSD, %). The RSD was calculated as the typical deviation of the three results obtained divided by the average value.

2.4.3 HPLC Analysis

Liquid chromatographical analysis of the most common phenolic compounds found in OPW (hesperidin, naringin, catechin, rutin, quercetin, gallic acid, caffeic acid, *p*-coumaric acid, and *trans*-ferulic acid [8,9,16–18]) was performed in a JASCO 4000 Series HPLC system (JASCO, Spain) equipped with a MD-4015 photo diode array (PDA) detector, using a Fortis C18 column (250 mm × 4.6 mm, 5 μm) at room temperature. The compounds were separated by gradient elution using different ratios of 1.25% aqueous acetic acid solution (v/v) (A) and acetonitrile (B). The gradient method conditions were as follows: 21% of phase B for the first 12 min; linear increase from 21% to 35% of B from 12 to 15 min; maintain the conditions until 22 min; linear increase of B to 50% from 22 to 28 min; linear decrease to 21% of B between 28 and 32 min at a flow rate 1 mL/min. Re-equilibration of the column was carried out using the starting conditions for 4 min before the next assay. Total analysis per sample was performed in 36 min.

Detection and identification of phenolic compounds (PC) were performed using a PDA detector at the following wavelengths: 271 nm for the detection of gallic acid; 282 nm for the detection of catechin, naringin, and hesperidin; 323 nm for the detection of caffeic acid, *p*-coumaric acid, and *trans*-ferulic acid; 370 nm for the detection of rutin and quercetin. The HPLC chromatogram of the target phenolic compounds is presented in **Supplementary Fig. 1** (available as Supporting Information).

Quantifying the extraction of the target compounds was carried out from the integration of the peak area and using the calibration curves prepared with standard solutions for each compound. The results, expressed as mg of target compound per g of OPW, were obtained from Eqn. 3:

$$PC (mg/g OPW) = \frac{[PC]}{m OPW} \cdot V solvent \quad (3)$$

where [PC] is the concentration of the phenolic compound (in mg PC/mL), measured by HPLC, and calculated by the

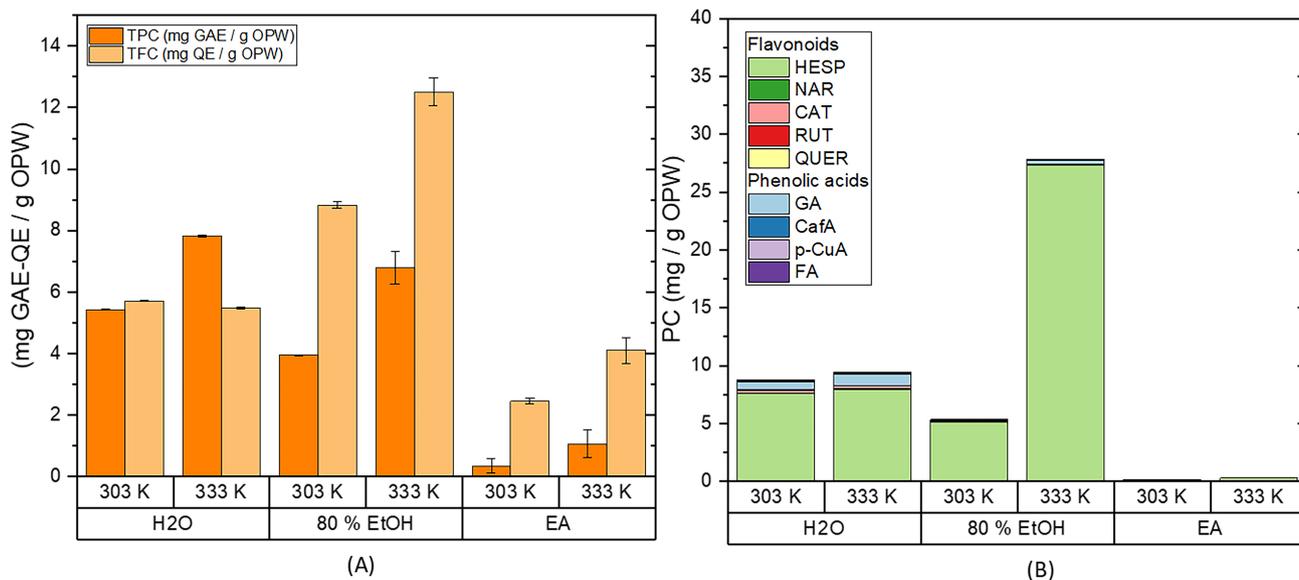


Fig. 2. Effect of temperature on the extraction from OPW using the orbital shaker. (A) Total phenolic content (TPC), total flavonoid content (TFC), and (B) high-performance liquid chromatography (HPLC) quantification of the flavonoids and phenolic acids present in the extract. Extraction conditions: biomass:solvent ratio = 1:10; $t_{\text{extraction}} = 60$ min; $T = 303$ and 333 K; stirring rate: 900 rpm.

calibration curve; V of the solvent is the amount of solvent used during the extraction, expressed in L; m OPW is the amount of sample used expressed in g. In all cases, HPLC analyses were performed in triplicate, and the results were expressed as mg PC/g OPW \pm relative standard deviation (RSD, %). The RSD was calculated as the typical deviation of the three results obtained divided by the average value.

3. Results and Discussion

3.1 Orbital Shaker Extraction (OSE)

First, the compounds of interest were extracted from OPW and evaluated using the orbital shaker to study the effect of temperature on the process. The experiments were performed at 303 and 333 K, and the extraction time was maintained at 60 min. In this case, temperatures higher than 333 K were not tested to avoid possible thermal degradation of phenolic compounds since the extraction time used is quite prolonged. The results obtained from the TPC, TFC, and HPLC analyses of the extracts, for the three solvents evaluated, are shown in Fig. 2, and reported in **Supplementary Table 1**, available as Supporting Information.

As can be seen in Fig. 2A, in general, TPC and TFC increase alongside temperature, except for water, for which the TFC values are quite similar at both temperatures. These results suggest that a higher temperature enhances the extraction of phenolic compounds from OPW using the orbital shaker. The positive effect of the temperature on the extraction, specifically using 80% EtOH and ethyl acetate as solvents, could be explained due to the reduction in their surface tension and viscosity with temperature, which favors solubility and increases the diffusion coefficients of phenolic compounds [39].

Comparing Fig. 2A,B, it is also possible to observe that the TFC and HPLC analyses show a similar trend, which can be related to the fact that hesperidin (a flavonoid) was the major compound identified in the extracts by HPLC, as can also be seen in **Supplementary Table 1**, available as Supporting Information. Nevertheless, it is important to remark that only eight phenolic compounds were identified by HPLC analysis (quercetin was not found in any analysis), and therefore, any discrepancies between TFC and HPLC may be due to the extraction of other flavonoids not identified by HPLC.

The increase in TPC values by temperature for water, which does not agree with the values obtained by the TFC and HPLC analyses, could be attributed to the low selectivity of this solvent; in particular, water could be promoting the extraction of other compounds from OPW, such as sugar, short-chain carbohydrates, and/or ascorbic acid [40], which are known to interfere with the Folin–Ciocalteu method due to their reducing capacities [41,42]. The low selectivity of water was confirmed by HPLC analysis since the presence of gallic acid, (+)-catechin hydrate, and *trans*-ferulic acid in water extract is remarkable, which does not happen for the other two solvents. The percentage of hesperidin in the aqueous extract represents about 84% of the phenolic compounds identified, which is lower than with 80% EtOH (about 99%) or in ethyl acetate, where only hesperidin was identified (see **Supplementary Table 1**).

Fig. 2 illustrates that the best extraction efficiency was achieved when using 80% EtOH at 333 K. Specifically, TPC increased from 3.9 to 6.8 mg GAE/g OPW, TFC increased from 8.8 to 12.5 mg QE/g OPW, and the total phenolic compounds quantified by HPLC increased from 5 to

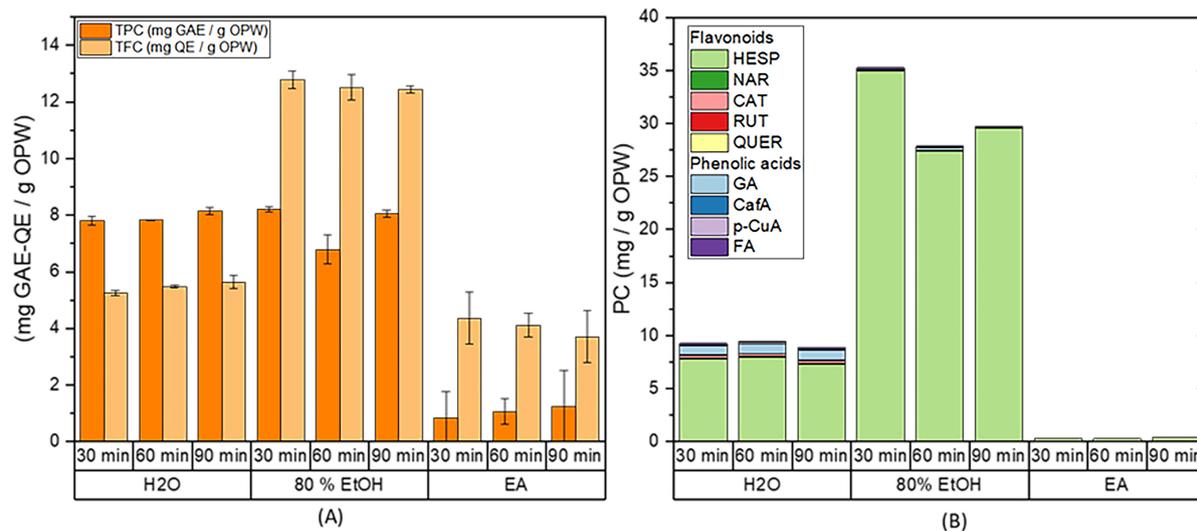


Fig. 3. Effect of time on the extraction from OPW using the orbital shaker. (A) Total phenolic content (TPC) and total flavonoids content (TFC), and (B) HPLC quantification of the flavonoids and phenolic acids present in the extract. Extraction conditions: biomass:solvent ratio = 1:10; $T = 333\text{ K}$; $t_{\text{extraction}} = 30, 60, \text{ and } 90\text{ min}$; stirring rate: 900 rpm.

29 mg PC/g OPW following the increases in temperature. The effectiveness of the ethanolic mixture agrees with the results published by Li *et al.* [36], who compared the ability of pure H₂O and a solution of 80% EtOH to extract phenolic compounds from citrus wastes. The use of ethanol enhances the extraction capacity because the solubility of the phenolic compounds in this alcohol is higher than in water, while the addition of water to pure ethanol favors the desorption of the solute from the matrix [43].

In contrast, lower extraction values were obtained when using ethyl acetate, which also increased as the temperature increased (TPC increased from 0.4 to 1.1 mg GAE/g OPW, TFC increased from 2.5 to 4.1 mg QE/g OPW, and total phenolic compounds quantified with HPLC increased from 0.1 to 0.3 mg PC/g OPW). For this solvent, it is important to remark that only hesperidin was identified in the HPLC chromatograms, thereby revealing that the presence of flavonoids and phenolic acids in this extract is limited in comparison with the other studied solvents (8.7–9.4 and 5.3–27.8 mg PC/g OPW for water and 80% EtOH, respectively). These results agree with those published by Safdar *et al.* [43], who compared 80% EtOH and pure ethyl acetate in the extraction of phenolic compounds from *Citrus reticulata L.* (25 mg GAE/g with 80% EtOH and 9 mg GAE/g with ethyl acetate). This could be explained due to the hydrophobic character of ethyl acetate, whose capacity to be introduced into the cell wall is lower than the hydrophilic solvents.

Taking all these results into account, the best temperature for stabilization was 333 K and then the effect of extraction time at this temperature was also evaluated using three different times (30, 60, and 90 min). The results for TPC, TFC, and HPLC analysis are shown in Fig. 3 and **Supplementary Table 1** (available as Supporting Information).

The obtained results show little or no influence of the stirring time on the extraction of phenolic compounds, suggesting that 30 min should be sufficient to achieve equilibrium when these solvents are used. A similar conclusion was also obtained by Vergara-Salinas *et al.* [44], who found no influence by extraction time, between 5 and 30 min when extracting phenolic compounds from thyme samples using pressured hot water as the solvent. Thus, the choice of a longer time can lead to no significant effect on the extraction efficiency, as suggested by Silva *et al.* [45]. The decreasing extraction efficiency for phenolic compounds at 90 min using water and 80% EtOH could be attributed to the oxidation of some phenolic compounds by extended exposure to oxidizing factors, such as oxygen and light [46–48].

The results obtained in this part of the study agree with those trends plotted in Fig. 2, confirming that 80% EtOH is the solvent with the highest extraction capacity (27.8–35.2 mg PC/g OPW) followed by deionized water (8.8–9.5 mg PC/g OPW), and ethyl acetate (0.3–0.4 mg PC/g OPW) from the HPLC results. Then, since longer extraction times do not promote further recovery of phenolic compounds from OPW by OSE, it could be concluded that the best operating conditions for this technique are 333 K and 30 min, thereby avoiding the possible degradation of the phenolic compounds and saving costs by reducing extraction time. Since the operational power of the orbital shaker is 515 W, the energy consumption for the optimal conditions (333 K and 30 min) is 927 kJ.

3.2 Ultrasound-Assisted Extraction (UAE)

Extractions by an ultrasonic bath (USBE) were performed using the best conditions established for OSE (333 K and 30 min). The obtained results are reported in **Supplementary Table 2**, available as Supporting Information, and

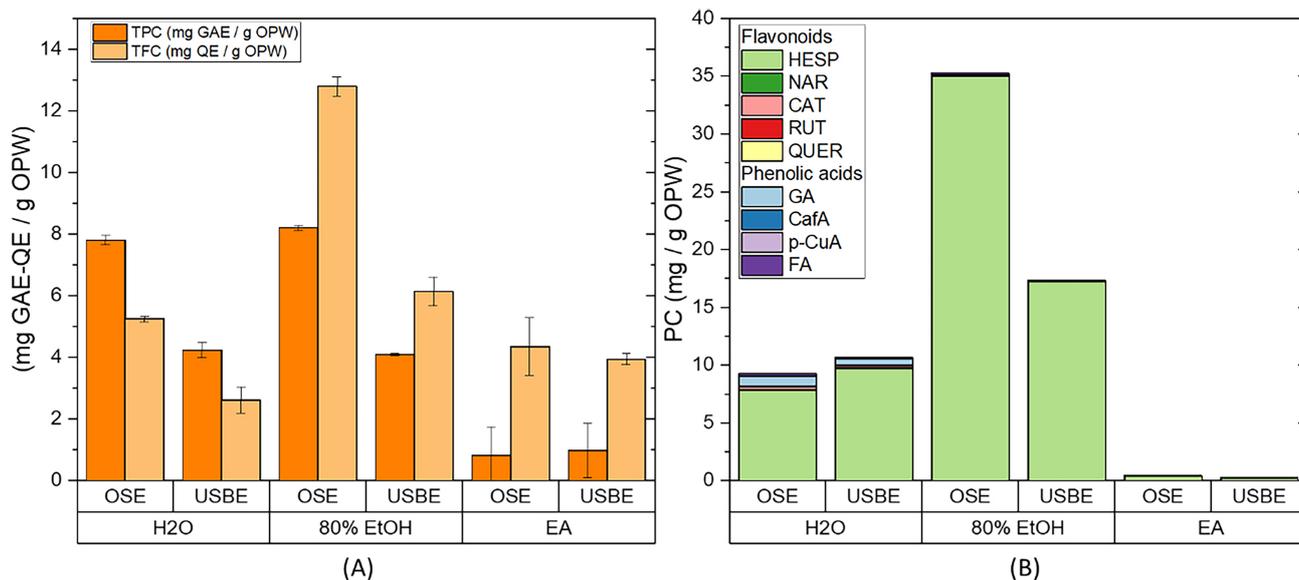


Fig. 4. Extraction from OPW by orbital shaker (OSE) and an ultrasonic bath (USBE). (A) Total phenolic content (TPC), total flavonoid content (TFC), and (B) HPLC quantification of the flavonoids and phenolic acids present in the extract. Extraction conditions: biomass:solvent ratio = 1:10; $t_{\text{extraction}} = 30$ min; $T = 333$ K.

a comparison between the USBE and OSE results is also plotted in Fig. 4. The best extraction efficiency was also achieved using the 80% EtOH mixture as the solvent but the use of this ultrasonic bath did not improve the extraction process. In fact, the TPC and TFC values were lower for USBE than OSE, while HPLC analysis revealed a small increase in phenolic compounds in water (9.3 to 10.7 mg PC/g OPW) and ethyl acetate (3 to 4 mg PC/g OPW) extracts, although also a significant decrease in phenolic compounds in the 80% EtOH extract (35.2 to 17.4 mg PC/g OPW). In all cases, a higher percentage of hesperidin compared to the other seven phenolic compounds was detected.

Although ultrasound energy is commonly applied to intensify extraction by increasing the number of cavitation bubbles, which causes the cell walls to rupture, thereby reducing particle size and improving mass transfer [7,49], the ultrasonic bath used in this work did not improve the extraction results. This could be due to the fact that this bath only allowed the temperature to be controlled, and no other parameters, such as frequency, power, or distance of the sample to the irradiation surface, known to affect the intensification process [24]. Moreover, the performance of the equipment could also be influenced by the orientation of the sample, sonication volume, and purity of the water used in the bath [26,50]. It is worth noting that, although there was no significant improvement in the extraction efficiency using USBE, there was a reduction in energy consumption from 927 kJ, when using OSE, to 63 kJ with UBAE.

Since extraction using indirect ultrasound (ultrasonic bath) was not as effective as expected, an ultrasound probe was tested as an alternative for performing the ultrasound-assisted extraction (USPE). After some preliminary assays,

the power was set at 10 W since higher power values resulted in solvent evaporation due to higher final temperatures, while the recipient was open. In this case, the extraction time effect was evaluated at 1.5, 3, and 6 min, reaching a maximum final temperature of 333 K. The results of the TPC, TFC, and HPLC analysis are shown in Fig. 5 and **Supplementary Table 3** available as Supporting Information.

Comparing the Figs. 3,5, it is possible to observe that the TPC, TFC, and HPLC results follow a similar trend, yet those obtained by the ultrasonic probe are clearly better. Furthermore, they were obtained at shorter times (30–90 min using an orbital shaker vs. 1.5–6 min for the ultrasound probe). These facts confirm that ultrasound-assisted extraction using a probe instead of a bath improves the extraction of phenolic compounds from OPW since the probe transmits a higher energy to the medium and the mass transfer is improved due to the lack of barriers in the propagation path of ultrasound waves, hence, enabling shorter extraction times [26,51]. Once again, the best extraction efficiency was achieved using 80% EtOH as solvent.

The HPLC analysis clearly shows a decrease in the extraction of phenolic compounds as time increases, which can be due to the heat generated during the sonication process or the sample oxidation since the vials are opened. This can lead to a change in the characteristics of the medium, resulting in the degradation of the phenolic compounds [51]. Such decreases were not observed in the TPC and TFC values because sonication could also be facilitating the extraction of other compounds that can interfere with the spectrophotometric measurements. Therefore, a time of 1.5 min was considered enough to ensure a high extraction yield and avoid possible degradation of the phenolic compounds. In

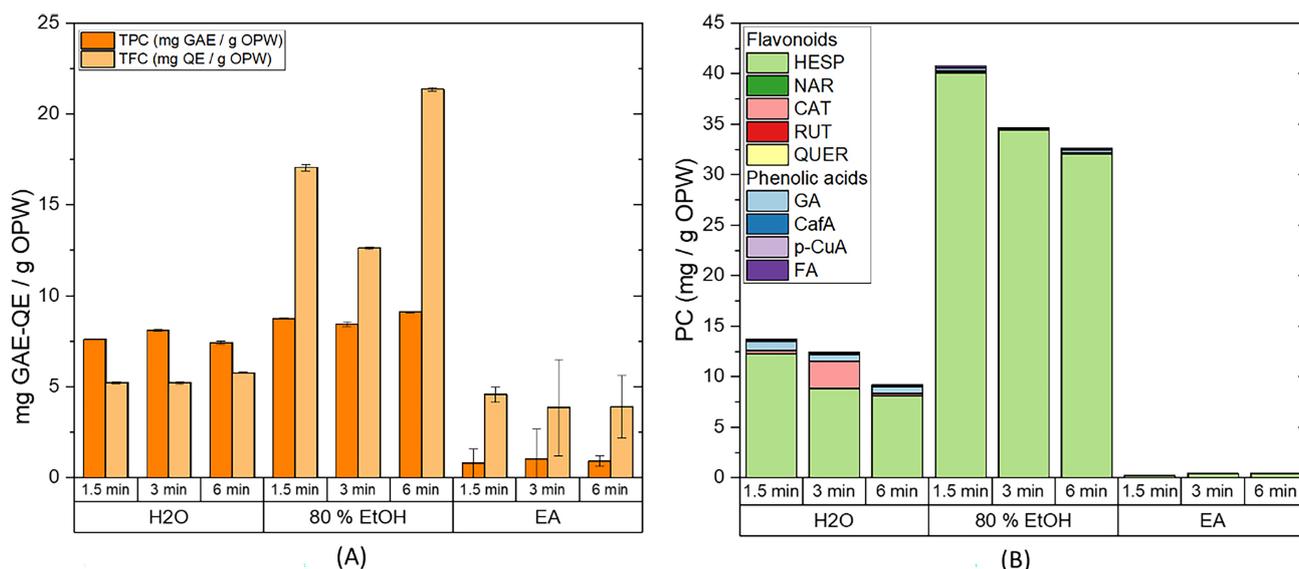


Fig. 5. Extraction from OPW by ultrasound probe (USPE). (A) Total phenolic content (TPC), total flavonoid content (TFC), and (B) HPLC quantification of the flavonoids and phenolic acids present in the extract. Extraction conditions: biomass:solvent ratio = 1:10; $t_{\text{extraction}} = 1.5, 3$ and 6 min; $p = 10$ W; $T_{\text{max}} \approx 333$ K.

addition, the energy consumed during USPE (18 kJ) is significantly lower than during the extraction using the bath (63 kJ). Therefore, the direct use of ultrasound as an assisted extraction technique is a better option in terms of extraction and energy efficiency. In brief, ultrasound-assisted extraction using the probe is more efficient than the orbital shaker and ultrasound bath-assisted extraction, as the extraction time can be shortened from 30 min to 1.5 min, achieving better extraction yields.

3.3 Microwave-Assisted Extraction (MAE)

Subsequently, the best conditions established for USPE (1.5 min and 333 K) were used to test whether microwave-assisted extraction (MAE) could improve the obtained results. Moreover, the study temperature was extended to 373 K to evaluate the effect of temperature and time alongside this type of extraction technology. The obtained results are plotted in Figs. 6,7, and reported in **Supplementary Table 4**, available as Supporting Information.

As can be seen in Fig. 6, microwave-assisted extraction at 333 K failed to improve the results obtained by USPE. A possible explanation is that the energy required to increase the temperature up to 333 K is very low; therefore, the effect of the microwaves is also very small. Nevertheless, a significant improvement in extraction efficiency, especially for hesperidin, can be observed from the HPLC analysis when the temperature was set at 373 K (11.4 to 22.8 mg PC/g OPW in water, 22.3 to 43 mg PC/g OPW in 80% EtOH, and 0.1 to 0.4 mg PC/g OPW in ethyl acetate). At 373 K, the required energy is higher and the effect of microwaves on the sample is more relevant, which facilitates the desorption of the compounds from the matrix and the solvent penetration on the matrix by reducing the sur-

face tension and surface viscosity [31]. On the other hand, microwaves accelerated extraction by increasing the temperature and internal pressure in the plant cell walls, which leads to an enhancement in porosity, thereby allowing the solvent to better penetrate [10].

Considering the good results obtained at 373 K, this work was completed by evaluating the effect of time on the MAE (Fig. 7), concluding that MAE efficiency increases with time while achieving the best results using 80% EtOH at 6 min (58.6 mg PC/g OPW). This can be explained by the fact that ethanol is known to be a good microwave absorber, which increases the extraction efficiency [52].

The energy required when using MAE for 6 min with a maximum operating power of 850 W was 306 kJ. The extraction using microwave and 80% EtOH as a solvent, enhanced the efficiency from 35.2 mg PC/g OPW using OSE to 58.6 mg PC/g OPW with MAE; moreover, MAE was able to reduce the energy consumption from 927 kJ to 306 kJ. This demonstrates that MAE is a good alternative to conventional extraction techniques.

4. Conclusions

This work evaluates the improvement in the extraction of phenolic compounds from orange peel waste (OPW) by ultrasound and microwave-assisted extraction methods compared to traditional extraction by an orbital shaker. Extraction temperature, extraction time, and the solvent used have been studied as operational parameters to optimize the recovery of natural antioxidants from OPW.

After optimizing the orbital shaker extraction, it was concluded that increasing temperatures up to 333 K improved the extraction efficiency, while times longer than

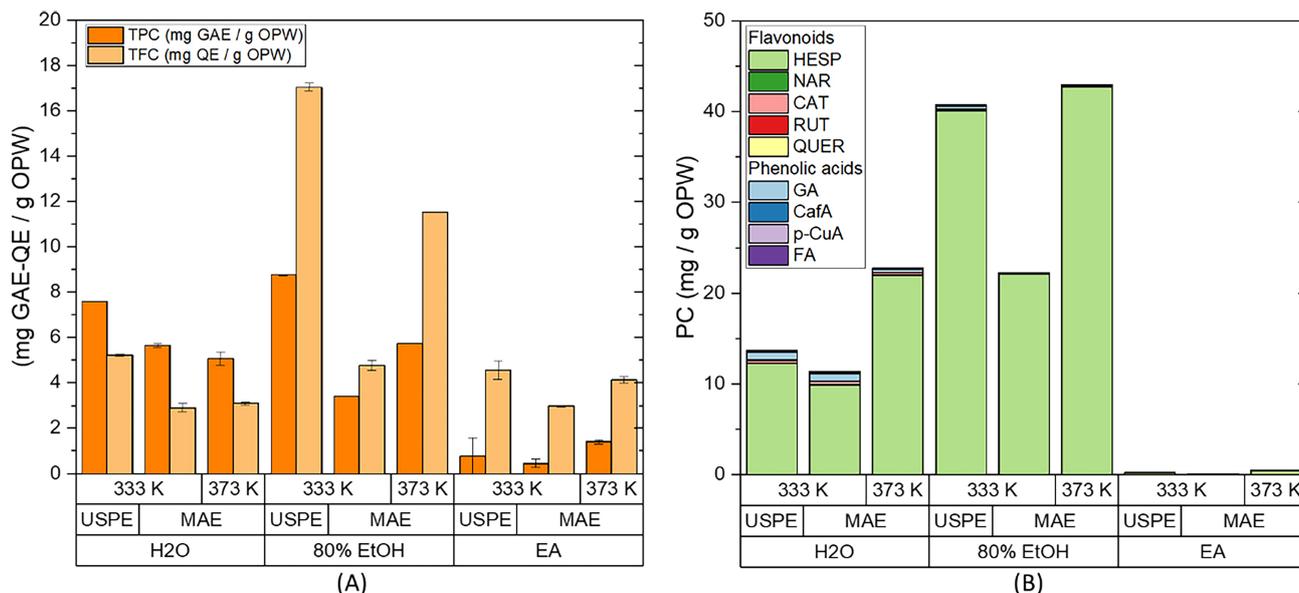


Fig. 6. Extraction from OPW by ultrasound probe (USPE) and microwaves. (A) Total phenolic content (TPC), total flavonoid content (TFC), and (B) HPLC quantification of the flavonoids and phenolic acids present in the extract. Extraction conditions: biomass:solvent ratio = 1:10; $t_{\text{extraction}} = 1.5$ min.

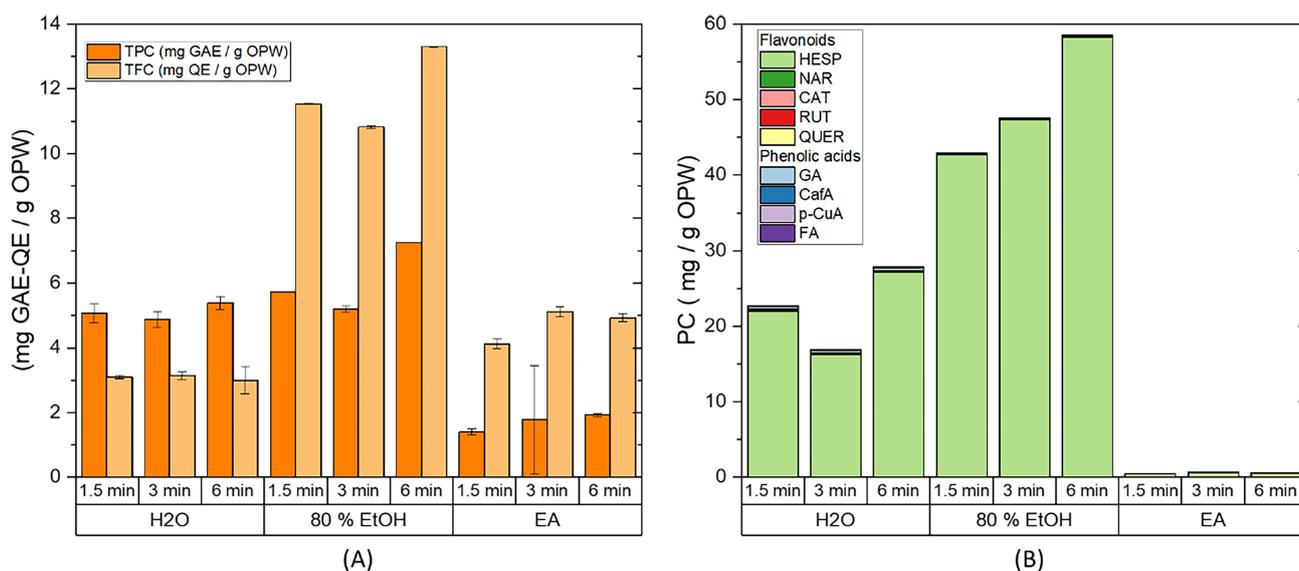


Fig. 7. Effect of time on the extraction from OPW by microwave-assisted extraction (MAE). (A) Total phenolic content and total flavonoid content, and (B) HPLC quantification of the flavonoids and phenolic acids present in the extract. Extraction conditions: biomass:solvent ratio = 1:10; $t_{\text{extraction}} = 1.5, 3, 6$ min; $T = 373$ K; stirring rate: 1000 rpm.

30 min decreased the extraction yields probably due to the degradation of the target compounds. Regarding ultrasound-assisted extraction (UAE), it was found that the use of an ultrasound bath did not improve the extraction yield, while the use of an ultrasound probe enhanced the extraction efficiency. The ultrasound probe allows higher energy to be directly transmitted to the matrix, reducing extraction time from 60 min to 1.5 min. As for the use of microwave-assisted extraction (MAE), it was concluded to be the most effective technique, as the extraction time

can be reduced from 60 min to 6 min, while significantly enhancing the recovery of polyphenolic compounds at increasing temperatures, up to 373 K.

Concerning the extraction solvent (i.e., H₂O, 80% EtOH, and EA) it was observed that hydrophobic solvents, such as EA are not effective for solid-liquid extraction from OPW, as the penetration capacity into the matrix is lower. As for the polar solvents tested, extraction with H₂O was found to be less selective, while extraction with 80% EtOH was demonstrated to be the most efficient since the solu-

bility of the phenolic compounds is higher in ethanol, and the addition of water promotes the desorption of the target compounds from the matrix. The HPLC analysis indicates that hesperidin was the main extracted compound.

Regarding energy consumption, after the optimization of the extraction with each technique, it was concluded that the assisted extraction techniques provide a significant decrease in the energy required to carry out the extraction. In particular, USPE consumed 18 kJ compared to the 927 kJ normally consumed for traditional extraction using the orbital shaker under optimum extraction conditions.

In summary, UAE with a probe and MAE can be considered an efficient extraction technology for the valorization of OPW when 80% EtOH is used as the solvent, whereby it reduces the extraction time by at least 10 times, the energy consumption by 50 times, and increased the extraction efficiency by almost two times.

Availability of Data and Materials

All data points generated or analyzed during this study are included in this article. Any additional information will be provided upon request to the corresponding author.

Author Contributions

BSM, RC, MGM and EJG designed the research study. RC contributed to the conception of the present work. BSM performed the research, analyzed the data and wrote the manuscript. MGM and EJG provided oversight and critical revision of the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors thank Paula Sánchez Morilla for her help with the analysis of the samples.

Funding

This work was financially supported by the Comunidad Autónoma de Madrid (Spain): SUSTEC P2018/EMT- 4348 project, PEJ-2020-AI/AMB-18391 and PEJ-2021-TL/AMB-21722 grants. This work has been also supported by the Madrid Government (Comunidad de Madrid - Spain) under the Multiannual Agreement with Universidad Politécnica de Madrid in the line Excellence Programme for University Professors, in the context of the V PRICIT (Regional Plan of Research and Technological Innovation).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbe1504030>.

References

- [1] Senit JJ, Velasco D, Manrique AG, Sanchez-Barba M, Toledo JM, Santos VE, *et al.* Orange peel waste upstream integrated processing to terpenes, phenolics, pectin and monosaccharides: Optimization approaches. *Industrial Crops and Products*. 2019; 134: 370–381.
- [2] Mohsin A, Hussain MH, Zaman WQ, Mohsin MZ, Zhang J, Liu Z, *et al.* Advances in sustainable approaches utilizing orange peel waste to produce highly value-added bioproducts. *Critical Reviews in Biotechnology*. 2022; 42: 1284–1303.
- [3] González-Miquel M, Diaz I. Valorization of citrus waste through sustainable extraction processes. *Food industry wastes* (pp. 113–133). Elsevier: New York. 2020.
- [4] Panwar D, Panesar PS, Chopra HK. Recent Trends on the Valorization Strategies for the Management of Citrus By-products. *Food Reviews International*. 2021; 37: 91–120.
- [5] Lin CSK, Pfaltzgraff LA, Herrero-Davila L, Mubofu EB, Abderahim S, Clark JH, *et al.* Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. *Energy & Environmental Science*. 2013; 6: 426–464.
- [6] Panić M, Andlar M, Tišma M, Rezić T, Šibalić D, Cvjetko Bubalo M, *et al.* Natural deep eutectic solvent as a unique solvent for valorisation of orange peel waste by the integrated biorefinery approach. *Waste Management (New York, N.Y.)*. 2021; 120: 340–350.
- [7] Boukroufa M, Boutekedjiret C, Petigny L, Rakotomanomana N, Chemat F. Bio-refinery of orange peels waste: a new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*. 2015; 24: 72–79.
- [8] Wang YC, Chuang YC, Hsu HW. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. *Food Chemistry*. 2008; 106: 277–284.
- [9] Özcan MM, Ghafoor K, Al Juhaimi F, Uslu N, Babiker EE, Mohamed Ahmed IA, *et al.* Influence of drying techniques on bioactive properties, phenolic compounds and fatty acid compositions of dried lemon and orange peel powders. *Journal of Food Science and Technology*. 2021; 58: 147–158.
- [10] M'hiri N, Ioannou I, Boudhrioua NM, Ghoul M. Effect of different operating conditions on the extraction of phenolic compounds in orange peel. *Food and Bioproducts Processing*. 2015; 96: 161–170.
- [11] Gattuso G, Barreca D, Gargiulli C, Leuzzi U, Caristi C. Flavonoid composition of Citrus juices. *Molecules (Basel, Switzerland)*. 2007; 12: 1641–1673.
- [12] Sharma K, Mahato N, Lee YR. Extraction, characterization and biological activity of citrus flavonoids. *Reviews in Chemical Engineering*. 2019; 35: 265–284.
- [13] Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR. Extraction and analysis of polyphenols: recent trends. *Critical Reviews in Biotechnology*. 2011; 31: 227–249.
- [14] Omoba OS, Obafaye RO, Salawu SO, Boligon AA, Athayde ML. HPLC-DAD Phenolic Characterization and Antioxidant

- Activities of Ripe and Unripe Sweet Orange Peels. *Antioxidants* (Basel, Switzerland). 2015; 4: 498–512.
- [15] Hegde P, Agrawal P, Gupta PK. Isolation and optimization of polyphenols from the peels of orange fruit. *Journal of Chemical and Pharmaceutical Sciences*. 2015; 8: 463–468.
- [16] Kadhom IM, Ibrahim NS, Al-zubaidy NA. Estimation of phenolic compounds and evaluation of their antioxidant activity of some parts of the orange plant (*Citrus Sinensis* L.). *European Journal of Molecular & Clinical Medicine*. 2020; 7: 4811–4822.
- [17] Sawalha SMS, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A. Quantification of main phenolic compounds in sweet and bitter orange peel using CE-MS/MS. *Food Chemistry*. 2009; 116: 567–574.
- [18] Manthey J A, Grohmann K. Concentrations of hesperidin and other orange peel flavonoids in citrus processing byproducts. *Journal of Agricultural and Food Chemistry*. 1996; 44: 811–814.
- [19] Garg A, Garg S, Zaneveld LJ, Singla AK. Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. *Phytotherapy Research: PTR*. 2001; 15: 655–669.
- [20] Petrotos K, Giavasis I, Gerasopoulos K, Mitsagga C, Papaioannou C, Gkoutosid P. Optimization of the Vacuum Microwave Assisted Extraction of the Natural Polyphenols and Flavonoids from the Raw Solid Waste of the Pomegranate Juice Producing Industry at Industrial Scale. *Molecules* (Basel, Switzerland). 2021; 26: 1033.
- [21] Rodsamran P, Sothornvit R. Extraction of phenolic compounds from lime peel waste using ultrasonic-assisted and microwave-assisted extractions. *Food Bioscience*. 2019; 28: 66–73.
- [22] Sharmila G, Nikitha VS, Ilaiyarasi S, Dhivya K, Rajasekar V, Kumar NM, *et al.* Ultrasound assisted extraction of total phenolics from *Cassia auriculata* leaves and evaluation of its antioxidant activities. *Industrial Crops and Products*. 2016; 84: 13–21.
- [23] Gomez L, Tiwari B, Garcia-Vaquero M. Emerging extraction techniques: Microwave-assisted extraction. *Sustainable Seaweed Technologies* (pp. 207–224). Elsevier: Amsterdam. 2020.
- [24] Ma Y, Ye X, Hao Y, Xu G, Xu G, Liu D. Ultrasound-assisted extraction of hesperidin from Penggan (*Citrus reticulata*) peel. *Ultrasonics Sonochemistry*. 2008; 15: 227–232.
- [25] Ma YQ, Chen JC, Liu DH, Ye XQ. Effect of ultrasonic treatment on the total phenolic and antioxidant activity of extracts from citrus peel. *Journal of Food Science*. 2008; 73: T115–T120.
- [26] Dash DR, Pathak SS, Pradhan RC. Improvement in novel ultrasound-assisted extraction technology of high value-added components from fruit and vegetable peels. *Journal of Food Process Engineering*. 2021; 44: e13658.
- [27] Montero-Calderon A, Cortes C, Zulueta A, Frigola A, Esteve MJ. Green solvents and Ultrasound-Assisted Extraction of bioactive orange (*Citrus sinensis*) peel compounds. *Scientific Reports*. 2019; 9: 16120.
- [28] Savic IM, Savic Gajic IM, Milovanovic MG, Zerajic S, Gajic DG. Optimization of Ultrasound-Assisted Extraction and Encapsulation of Antioxidants from Orange Peels in Alginate-Chitosan Microparticles. *Antioxidants* (Basel, Switzerland). 2022; 11: 297.
- [29] Selahvarzi A, Ramezan Y, Sanjabi MR, Namdar B, Akbarmivehie M, Mirsaeedghazi H, *et al.* Optimization of ultrasonic-assisted extraction of phenolic compounds from pomegranate and orange peels and their antioxidant activity in a functional drink: Antioxidant effect of optimized pomegranate and orange peel extracts in the functional drink. *Food Bioscience*. 2022; 49: 101918.
- [30] Esquivel-Hernández DA, Ibarra-Garza IP, Rodríguez-Rodríguez J, Cuéllar-Bermúdez SP, Rostro-Alanis MDJ, Alemán-Nava GS, *et al.* Green extraction technologies for high-value metabolites from algae: a review. *Biofuels, Bioproducts and Biorefining*. 2012; 6: 246–256.
- [31] Mandal V, Mohan Y, Hemalatha S. Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Pharmacognosy Reviews*. 2007; 1: 7–18.
- [32] Xing S, He Y, Zhu H, Wang X. An approach to sample selection from big data for classification. 2016 IEEE International Conference on Systems, Man, and Cybernetics (SMC) (pp. 2928–2935). IEEE: Budapest, Hungary, 2016.
- [33] Bagade SB, Patil M. Recent Advances in Microwave Assisted Extraction of Bioactive Compounds from Complex Herbal Samples: A Review. *Critical Reviews in Analytical Chemistry*. 2021; 51: 138–149.
- [34] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, *et al.* Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*. 2013; 117: 426–436.
- [35] Zirahi A, Sadeghi Yamchi H, Haddadnia A, Zirrahi M, Hassanzadeh H, Abedi J. Ethyl acetate as a bio-based solvent to reduce energy intensity and CO₂ emissions of in situ bitumen recovery. *AIChE Journal*. 2020; 66: e16828.
- [36] Li BB, Smith B, Hossain MM. Extraction of phenolics from citrus peels: I. Solvent extraction method. *Separation and Purification Technology*. 2006; 48: 182–188.
- [37] Cañadas R, González-Miquel M, González EJ, Díaz I, Rodríguez M. Hydrophobic eutectic solvents for extraction of natural phenolic antioxidants from winery wastewater. *Separation and Purification Technology*. 2021; 254: 117590.
- [38] Pękal A, Pyrzynska K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Analytical Methods*. 2014; 7: 1776–1782.
- [39] Ozturk B, Parkinson C, Gonzalez-Miquel M. Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. *Separation and Purification Technology*. 2018; 206: 1–13.
- [40] Barrales FM, Silveira P, Barbosa PDP, Ruviano AR, Paulino BN, Pastore GM, *et al.* Recovery of phenolic compounds from citrus by-products using pressurized liquids — An application to orange peel. *Food and Bioproducts Processing*. 2018; 112: 9–21.
- [41] Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science*. 2007; 30: 3268–3295.
- [42] Georgé S, Brat P, Alter P, Amiot MJ. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and Food Chemistry*. 2005; 53: 1370–1373.
- [43] Safdar MN, Kausar T, Jabbar S, Mumtaz A, Ahad K, Saddozai AA. Extraction and quantification of polyphenols from kinnow (*Citrus reticulata* L.) peel using ultrasound and maceration techniques. *Journal of Food and Drug Analysis*. 2017; 25: 488–500.
- [44] Vergara-Salinas JR, Pérez-Jiménez J, Torres JL, Agosin E, Pérez-Correa JR. Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (*Thymus vulgaris*). *Journal of Agricultural and Food Chemistry*. 2012; 60: 10920–10929.
- [45] Silva EM, Rogez H, Larondelle Y. Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. *Separation and Purification Technology*. 2007; 55: 381–387.
- [46] Benmeziane F, Djamaï R, Cadot Y, Seridi R. Optimization of extraction parameters of phenolic compounds from Algerian fresh table grapes, (*Vitis Vinifera*). *International Food Research Journal*. 2014; 21: 1061–1065.
- [47] Uma DB, Ho CW, Wan Aida WM. Optimization of extraction parameters of total phenolic compounds from henna (*Lawsonia inermis*) leaves. *Sains Malaysiana*. 2010; 39: 119–128.
- [48] Hismath I, Wan Aida WM, Ho CW. Optimization of extraction conditions for phenolic compounds from neem (*Azadirachta*

- indica) leaves. *International Food Research Journal*. 2011; 18: 931–939.
- [49] Feng H, Yang W, Hielscher T. Power ultrasound. *Food Science and Technology International*. 2008; 14: 433–436.
- [50] Sivakumar M, Pandit AB. Ultrasound enhanced degradation of Rhodamine B: optimization with power density. *Ultrasonics Sonochemistry*. 2001; 8: 233–240.
- [51] Lavilla I, Bendicho C. *Fundamentals of Ultrasound-Assisted Extraction. Water extraction of bioactive compounds* (pp. 391–316). Elsevier: Amsterdam. 2017.
- [52] Inoue T, Tsubaki S, Ogawa K, Onishi K, Azuma JI. Isolation of hesperidin from peels of thinned Citrus unshiu fruits by microwave-assisted extraction. *Food Chemistry*. 2010; 123: 542–547.