beverages

Article

Hydroxypropyl-β-Cyclodextrin as a Green Co-Solvent in the Aqueous Extraction of Polyphenols from Waste Orange Peels

Achillia Lakka, Stavros Lalas and Dimitris P. Makris *

Green Processes & Biorefinery Group, Department of Food Science & Nutrition, School of Agricultural Sciences, University of Thessaly, N. Temponera Str., 43100 Karditsa, Greece; ACHLAKKA@uth.gr (A.L.); slalas@uth.gr (S.L.)
* Correspondence: dimitrismakris@uth.gr; Tel.: +30-24410-64792

Received: 4 June 2020; Accepted: 3 August 2020; Published: 10 August 2020

Abstract: There is, to-date, an expanding interest concerning the use of cyclodextrins as green food-grade co-solvents in the aqueous extraction of polyphenols, however, data regarding polyphenol extraction from waste orange peels (WOP) are lacking. On this ground, hydroxypropyl β-cyclodextrin (HP-β-CD), a highly water-soluble cyclodextrin, was used to develop a simple and straightforward methodology for the effective recovery of WOP polyphenols. Process optimization by response surface showed that maximum total polyphenol recovery (26.30 ± 1.49 mg gallic acid equivalents g^{-1} dry mass) could be accomplished with 15 mM HP-β-CD at 40 °C. On the other hand, integration of ultrasonication pretreatment was found unsuitable, as it resulted in reduced polyphenol yield. Examination of solvent acidity indicated that polyphenol extraction may be enhanced at pH 4, but the difference was non-significant ($p > 0.05$) compared to yields attained at pH 2, 3, and 5. Extraction of WOP polyphenols with HP-β-CD was shown to provide significantly higher hesperidin yield compared to 60% (*v*/*v*) aqueous ethanol, which suggested selectivity of HP-β-CD toward this polyphenolic metabolite.

Keywords: antioxidants; extraction; hydroxypropyl β-cyclodextrin; orange peels; polyphenols

1. Introduction

Currently, agri-food industry waste represents a highly significant environmental issue and valorization of fruit and vegetable side streams is paramount to establishing sustainable routes of waste management. To-date, waste biomass originating from food industry processing residues is rather undervalorized, being used as a source of fertilizers, fuel, and livestock feeds. However, plant-food processing by-products and wastes may now be regarded as a prominent potential source for value-added natural products and chemicals [1,2]. At present, both emerging and conventional technologies must be combined in a coordinated manner to allow for an integral management of food processing wastes, thereby minimizing impacts on the environment, and reducing wastage of natural resources.

Globally, the production of citrus fruits for the year 2016/17 amounted to more than 8200 million metric tons, approximately 7209 of which was represented by oranges [3]. Citrus peel and pulp are the major waste streams of citrus juice processing, accounting for nearly 55–60% of the fresh fruit weight, and it has been estimated that the volume of such wastes may be more than 15×10^6 tons worldwide [4]. There is currently an expanding interest concerning the green valorization of citrus processing wastes for the recovery of a variety of value-added compounds such as flavonoids, carotenoids, pectins, and essential oil, which could be used in the pharmaceutical, food, and cosmetics industry as functional ingredients, additives, colorants, flavorings, etc. [5]. Flavonoids are a class of biopotent constituents largely occurring in orange peels and possess a spectrum of activities including anti-inflammatory, antioxidant, anticancer, and cardioprotective [6–8].

By virtue of these biological properties, orange peel flavonoids have been a subject of extensive research, and numerous techniques for efficient solid–liquid extraction have been developed [5,9]. However, in the framework of green chemistry principles, a key concern for the establishment of sustainable extraction processes is the use of bio-based solvents, instead of conventional volatile, petroleum-based ones, for minimal environmental impact, but also increased efficiency and higher product quality [10,11]. Cyclodextrins (CDs) are cyclic supermolecules, composed of at least six D-(+)-glucopyranoside units, which are linked to each other with α-1,4-glycosidic bonds [12]. The principal attribute of CDs is their relatively hydrophobic cavity and their hydrophilic external surface, which enables CDs to form inclusion complexes with structurally versatile compounds. The guest molecule attachment into the CD cavity is stabilized through van der Waals forces, hydrophobic interactions, and hydrogen bonds [13]. CDs have been gaining acceptance as an eco-friendly means of recovering polyphenolic substances, and several investigations have studied parameters critical to solid–liquid extraction such as CD concentration, CD type, extraction technique, time, and temperature [14]. The advantages offered using CDs for polyphenol extraction are related to the improvement of extraction performance and extraction time minimization, improved antioxidant activity of the extracts generated, higher extract stability, and higher bioavailability of the encapsulated molecules.

On this basis, the current examination was aimed at developing a simple and straightforward process to effectively recover WOP polyphenols in a fully green manner. To this end, hydroxypropyl β-CD was employed because of its high water solubility, and process modeling was established through deployment of response surface methodology. The role of ultrasonication pretreatment and pH was also examined to assess their effect on extraction performance.

2. Materials and Methods

2.1. Chemicals

Hesperidin, narirutin, Folin-Ciocalteu reagent, hydroxypropyl β-cyclodextrin, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), and 2,2-diphenylpicrylhydrazyl (DPPH) were from Sigma-Aldrich (Darmstadt, Germany). Iron chloride hexahydrate and citric acid anhydrous were from Merck (Darmstadt, Germany). Aluminum chloride anhydrous (98%) and sodium carbonate anhydrous (99%) were from Penta (Praha, Czechia). Solvents used for chromatographic purposes were HPLC grade.

2.2. Waste Orange Peels

Orange (*Citrus sinensis* cv Valencia) peels were collected from an orange-processing plant (Prefecture of Thessaly, central Greece) and transported within 30 min to the laboratory, where they were processed with a sharp cutter to remove remaining flesh from peels (flavedo and albedo). This material, assigned as waste orange peels (WOP), was spread over stainless steel trays to layers of 0.5-cm thickness, and then dried in a laboratory oven (Binder BD56, Bohemia, NY, USA) for 480 min at 70 °C. After pulverization in a ball mill, the powder was sieved to obtain a uniform feed with an average particle diameter of 0.379 mm and stored at −17 °C.

2.3. Extraction Procedure

A precise amount of 0.667 g of WOP was transferred into a glass flask with 20 mL of deionized water (solvent-to-solid ratio 30 mL g^{-1}) containing HP-β-CD. The mixture was subjected to ultrasonication and then extracted for 150 min at 500 rpm on a magnetic stirrer (Witeg, Wertheim, Germany). The concentration of HP-β-CD (C_{CD}), the extraction temperature (T), and the time of ultrasonication (t_{US}) were dictated by the experimental design. Ultrasonication was carried out in an Elma D-78224 Singen HTW heated ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) and set at a power of 550 W, a frequency of 50 Hz, and acoustic energy density of 78.6 W L^{-1}. Ultrasound-assisted pretreatment of all samples was accomplished at ambient temperature (26 ± 2 °C). In the experiments carried out to assess the effect of pH, citric acid was added to the extraction medium at a standard concentration of 1 g L^{-1}. Then, the pH of the medium was adjusted by using 5 M NaOH. The extracts generated were centrifuged for 10 min at 10,000× *g*.

2.4. Experimental Design and Response Surface Optimization

The three process (independent) variables selected to set up the design of experiment were the concentration of HP-β-CD (C_{CD}), the time of ultrasonication pretreatment (t_{US}), and the temperature (T). Construction of the predictive model was performed by deploying a response surface methodology via a Box–Behnken design with three central points. The basis for choosing the process variables was literature data [15,16] illustrating their profound influence on the extraction yield in total polyphenols (Y_{TP}), which was the response. The actual and codified levels of the variables are analytically presented in Table 1. Codification of variable levels was accomplished as described elsewhere in detail [17]. Assessment of the model derived was based on the lack-of-fit test and analysis of variance (ANOVA). These tests enabled the determination of the significance of the model as well as of each polynomial coefficient and the model coefficient R^2. The model (mathematical equations) was composed of only significant terms and insignificant ones ($p > 0.05$) were not considered.

Table 1. Process variable levels and corresponding codification.

Independent Variables	Code Units	Coded Variable Level		
		−1	0	1
C_{CD} (mM)	X_1	0	7.5	15
t_{US} (min)	X_2	0	15	30
T (°C)	X_3	40	55	70

2.5. Total Polyphenol Determination and Antioxidant Activity

The concentration of total polyphenols in the extracts produced was determined with a Folin-Ciocalteu methodology reported elsewhere [17] using gallic acid as calibration standard. Yield in total polyphenols (Y_{TP}), expressed as mg gallic acid equivalents g^{-1} dry mass (dm), was computed as previously described [18]. The antioxidant activity was estimated by performing antiradical activity (A_{AR}) and a ferric-reducing power (P_R) assays, as described earlier in detail [17]. Expression of the results was in μmol DPPH g^{-1} dm and μmol ascorbic acid equivalents (AAE) g^{-1} dm for A_{AR} and P_R, respectively.

2.6. Chromatographic Analyses

A Shimadzu CBM-20A liquid chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) was used that was equipped with a Shimadzu SPD-M20A detector and Shimadzu LC solution software. A Phenomenex Luna C18(2) (100 Å, 5 μm, 4.6 × 250 mm) (Phenomenex, Inc., Torrance, CA, USA) was employed for all analyses and maintained at 40 °C. Analyses were carried out with (A) 0.5% aqueous formic acid and (B) 0.5% formic acid in MeCN/water (6:4) at a flow rate of 1 mL min^{-1} and 20 μL injection volume. The elution program implemented was: 100% A to 60% A in 40 min; 60% A to 50% A in 10 min; 50% A to 30% A in 10 min, and then constant for another 10 min. Hesperidin and narirutin (for chromatograms see Figure S1) were detected at 280 nm, and quantified using calibration curves with concentrations ranging from 0 to 50 μg mL^{-1} ($R^2 > 0.998$).

2.7. Statistical Analysis

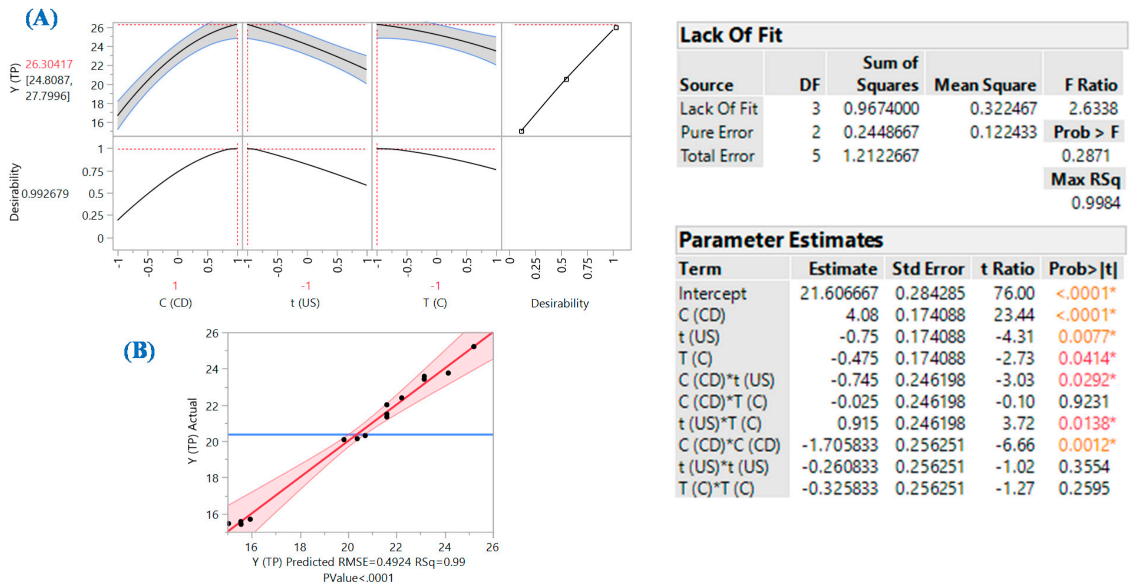
At least two extractions were performed for each experiment and analyses were carried out in triplicate. All values reported are means ± standard deviation (sd). SigmaPlot™ 12.5 (Systat Software Inc., San Jose, CA, USA) was employed to perform linear correlations (95% significance level). JMP™

Pro 13 (SAS, Cary, NC, USA) was employed to do distribution analysis, set up the experimental design, and run response surface statistics (ANOVA, lack-of-fit).

3. Results and Discussion

3.1. Design of Experiment and Extraction Process Optimization

The evidence emerged by recent studies on HP-β-CD-aided polyphenol extraction, revealed the importance of its concentration on the extraction performance [19–21]. Likewise, the time of ultrasonication pretreatment was also shown to be crucial in this regard [16,17]. Thus, these two parameters, along with temperature, whose effect on the extraction performance is undisputed, were selected to be the independent variables of the experimental design, and Y_{TP} was chosen as the response. The influence exerted by each of the process (independent) variables was assessed by performing ANOVA, and the overall fitting of the predictive model to the experimental measurements was assessed by lack-of-fit and ANOVA (Figure 1).



Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	0.9674000	0.322467	2.6338
Pure Error	2	0.2448667	0.122433	**Prob > F**
Total Error	5	1.2122667		0.2871
				Max RSq
				0.9984

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	21.606667	0.284285	76.00	<.0001*
C (CD)	4.08	0.174088	23.44	<.0001*
t (US)	-0.75	0.174088	-4.31	0.0077*
T (C)	-0.475	0.174088	-2.73	0.0414*
C (CD)*t (US)	-0.745	0.246198	-3.03	0.0292*
C (CD)*T (C)	-0.025	0.246198	-0.10	0.9231
t (US)*T (C)	0.915	0.246198	3.72	0.0138*
C (CD)*C (CD)	-1.705833	0.256251	-6.66	0.0012*
t (US)*t (US)	-0.260833	0.256251	-1.02	0.3554
T (C)*T (C)	-0.325833	0.256251	-1.27	0.2595

Figure 1. Analytical information associated with model fitting and assessment, constructed by implementing response surface methodology. (**A**,**B**) correspond to the desirability function and actual-to-predicted plot. Asterisk (*) in the inset table "Parameter Estimates" indicates statistically significant terms, at least at a 95% significance level.

The non-significant terms (Figure 1, "Parameter estimates") were excluded from the model, hence its final form was:

$$Y_{TP} = 21.61 + 4.08X_1 - 0.75X_2 - 0.48X_3 - 0.75X_1X_2 + 0.92X_2X_3 - 1.71X_1^2 \quad (1)$$

The square correlation coefficient (R^2), which is an indicator of the total variability around the mean provided by the model [22], was 0.99 ($p = 0.0001$), and was determined by taking into consideration the proximity of the predicted and measured response values (Figure 1B). These values are given in full detail in Table 2. This fact suggests that the model was very well adjusted to the experimental data.

The visualization of the effect of independent variables on the response was based on the model derived, and it was presented in the form of 3D plots (Figure 2). The desirability function (Figure 1A) enabled the estimation of the maximum Y_{TP}, which was 26.30 ± 1.49 mg GAE g^{-1} dm. The optimal settings predicted by the model to achieve this value were $C_{CD} = 15$ mM, $t_{US} = 0$ min, and $T = 40$ °C. To confirm the validity of this prediction, three individual extractions were carried out under these conditions, and the Y_{TP} determined was 25.10 ± 0.06 mg GAE g^{-1} dm. This finding indicated that the predictions provided by the model were credible.

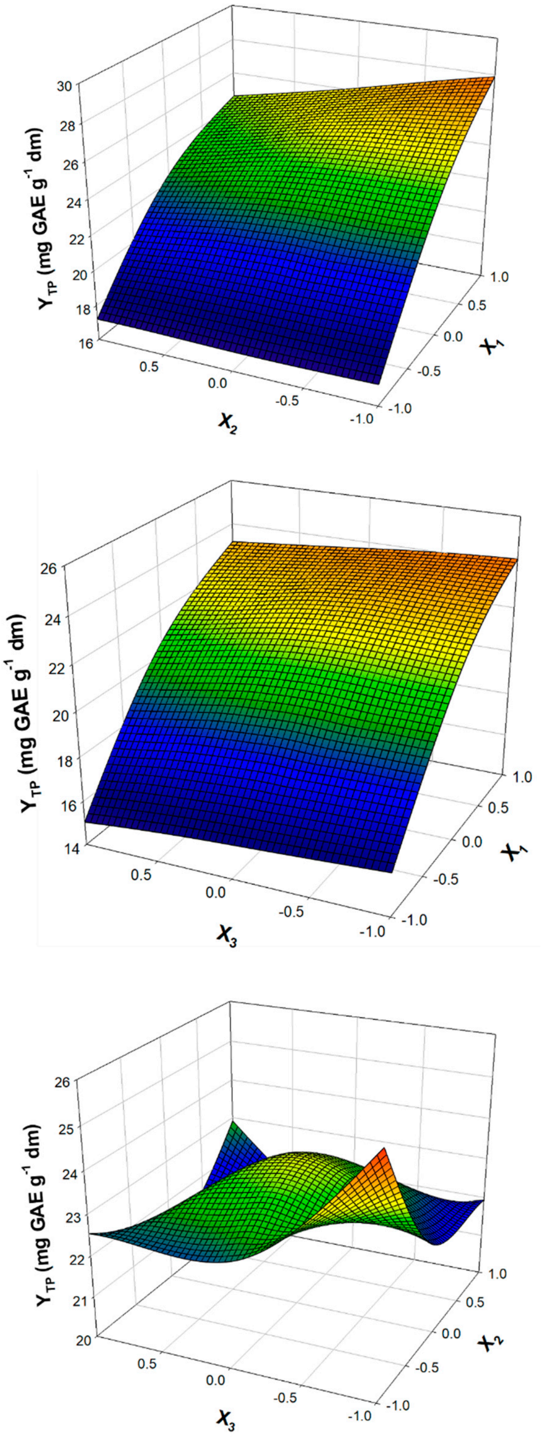


Figure 2. Visualization of the effect of independent variables on Y_{TP} in the form of 3D plots.

The maximum Y_{TP} attained (26.30 ± 1.49 mg GAE g^{-1} dm) was comparable to 26.88 mg GAE g^{-1} [23] and 23.63 mg GAE g^{-1} dm [24], both achieved with microwave-assisted extraction, but far higher than the 7 mg GAE g^{-1} dm achieved with high voltage electrical discharges combined with enzymic hydrolysis [25], 7.29 mg GAE g^{-1} dm with aqueous extraction [26], 13.89 mg GAE g^{-1} dm with ultrasound-assisted extraction [27], and approximately 16 mg GAE g^{-1} dm achieved with infrared heating pretreatment and ultrasonication extraction [28]. Despite differences in the polyphenol peel content, which may arise from the influence of factors such as sun exposure, soil type, cultivation practices, maturation stage, etc. [24], critical comparison of the above data might indicate that the HP-β-CD-aided extraction was a high-performance process.

Table 2. Analytical presentation of the design points of the response surface methodology and the corresponding measured and predicted response values.

Design Point	Independent Variables			Response (Y_{TP}, mg GAE g^{-1} dw)	
	X_1 (C_{CD}, mM)	X_2 (t_{US}, min)	X_3 (T, °C)	Measured	Predicted
1	−1	−1	0	15.41	15.56
2	−1	1	0	15.56	15.55
3	1	−1	0	25.21	25.21
4	1	1	0	22.38	22.22
5	0	−1	−1	23.57	23.16
6	0	−1	1	20.13	20.38
7	0	1	−1	20.08	19.83
8	0	1	1	20.30	20.71
9	−1	0	−1	15.69	15.94
10	1	0	−1	23.75	24.15
11	−1	0	1	15.45	15.04
12	1	0	1	23.41	23.15
13	0	0	0	21.49	21.61
14	0	0	0	21.33	21.61
15	0	0	0	22.00	21.61

The optimum C_{CD} value of 15 mM was the highest level used in the experimental design, close to HP-β-CD solubility [29]. This finding suggests that increased Y_{TP} may be achieved simply by raising C_{CD}. Such a trend was also reported for the extraction of red grape polyphenols [30] and pomegranate fruit [19]. On the other hand, other investigations stressed that after a certain point, increases in C_{CD} resulted in non-significant increases in Y_{TP} [21]. This could enable the use of amounts lower of HP-β-CD, resulting in a lower-cost process. Therefore, a thorough examination of the effect of C_{CD} on Y_{TP} should be an indispensable element of an extraction process development.

The time of ultrasonication pretreatment (t_{US}) had a negative effect on WOP polyphenol extraction (Figure 1, "Parameter estimates"), and according to the model (Equation (1)), maximization of Y_{TP} required no ultrasonication pretreatment (t_{US} = 0 min). This phenomenon might appear to be a paradox, considering the undisputed contribution of ultrasonication on the polyphenol extraction from peels of several citrus species [31–35]. Furthermore, the integration of ultrasonication as a pretreatment stage has been demonstrated to significantly enhance subsequent stirred-tank extraction [16]. However, data on the effect of ultrasonication on the cyclodextrin-aided polyphenol extraction are extremely limited, and therefore the actual effect is largely unknown. The combination of β-cyclodextrin with ultrasound-assisted extraction (UAE) for polyphenol recovery from some vinification wastes has been shown to provide exceptional boosting in extraction yield [36]. Contrary to that, extraction of polyphenols with various deep eutectic solvents (DES) in the presence of β-cyclodextrin was either enhanced or suppressed [37]. Although these discrepancies would not allow for argument on the effect of HP-β-CD/ultrasonication combination on WOP polyphenol extraction, it could be hypothesized that incorporation of HP-β-CD in the extraction medium during ultrasonication pretreatment might obstruct polyphenol extraction because of the increased viscosity of the liquid phase, which reduces solute (polyphenol) diffusivity [18].

Subsequent stirred-tank extraction is most probably favored by the presence of HP-β-CD, since stirring creates turbulence that fosters diffusivity [38] and enables the formation of polyphenol/HP-β-CD complexes, which enhance polyphenol solubility [29]. Based on the latter argument, it could also be supported that during ultrasonication pretreatment, irradiation with ultrasounds might destabilize polyphenol/HP-β-CD complexes and does not contribute to polyphenol solubilization in the liquid phase. This could be another reason for the negative effect of the ultrasonication pretreatment observed.

The effect of temperature (T) was also negative, and the optimum level determined was 40 °C, which represented the lowest limit tested (Table 1). This outcome was in line with a recent study on HP-β-CD-aided polyphenol extraction from potato peels [21], where it was found that $T > 30$ °C did not favor increased polyphenol yield. Increases in T have been explicitly demonstrated to increase the aqueous solubility of sparingly soluble polyphenols in the presence of various cyclodextrins [39]. On the other hand, it was found that the binding constants of certain polyphenol complexes with α-, β-, and γ-cyclodextrin decreased as T increased [40]. Results on the interactions of the stilbene (E)-piceatannol with HP-β-CD were in concurrence, illustrating a decrease in the complexation constant as a response to increasing T [41]. Such a phenomenon was attributed to weakening of hydrogen bonds between the guest and the host molecule due to heating. In addition, it was observed that at around 50–60 °C, there may be decomposition of polyphenol-cyclodextrin inclusion complexes, and therefore the reduced Y_{TP} found for $T > 40$ °C can be justified.

3.2. The Effect of pH Regulation

The influence of the pH of the extraction medium on Y_{TP} was assessed through regulation of the pH in deionized water and in 15 mM HP-β-CD solution by adding 1 g L^{-1} citric acid. Extraction was then carried out at 40 °C, and the results are depicted in Figure 3. Over the pH range of 2 to 5, the difference in Y_{TP} between deionized water and HP-β-CD was from 33.6% (pH 3) to 54% (pH 4), highlighting the effect of HP-β-CD on WOP polyphenol recovery. For the extraction with HP-β-CD, the highest Y_{TP} was found at pH 4 (29.13 ± 1.10 mg GAE g^{-1} dm). However, no statistical difference was seen compared to the Y_{TP} achieved at the other pH tested. This is in contrast to recent studies on the importance of the pH in the HP-β-CD-aided extraction of polyphenols [15,16], where pH effects on polyphenol extraction performance was found to be significant. Optimum pH value for HP-β-CD-aided extraction of *S. fruticosa* polyphenols was shown to be 3.90 [16], very close to pH 4 determined in this study.

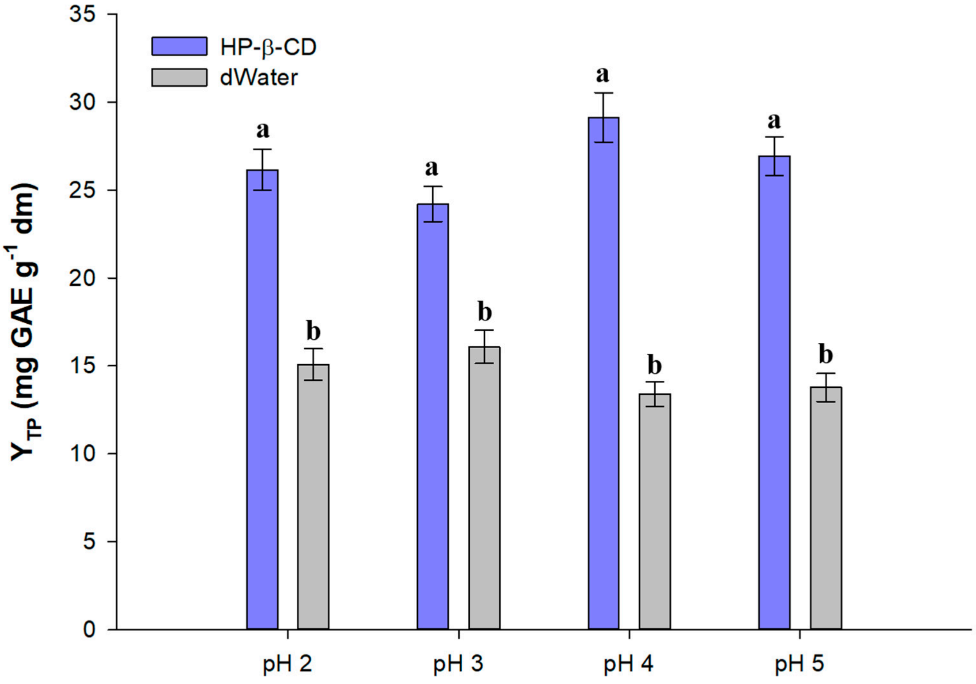


Figure 3. Effect of pH on the total polyphenol yield obtained with deionized water (dWater) and 15 mM hydroxypropyl-β-cyclodextrin (HP-β-CD) at 40 °C. Bars designated with different letters correspond to statistically different values ($p < 0.05$).

Since pH affects the dissociation of phenolic hydroxyls and carboxyl functions, its role in polyphenol–cyclodextrin interactions has been implicated. Investigations on caffeic acid (CA)/HP-β-CD interactions illustrated that complex formation constants were sensitive to pH, manifesting a decline with pH increases [42]. Such a phenomenon was attributed to CA hydrophobicity, which is related to its ionization state. Considering that hydrophobic interactions between the guest molecule and the HP-β-CD cavity contribute to the formation of inclusion complexes, acidic pH would maintain CA in a non-ionized and therefore more hydrophobic form. This would entail greater stability constant. Data on rosmarinic acid/β-CD interactions were along the same line [43]. A similar conclusion was reached for catechin and epicatechin interactions with HP-β-CD [44].

3.3. Comparative Assessment and Polyphenolic Composition

To further illuminate the potential of HP-β-CD as a co-solvent in the aqueous extraction of WOP polyphenols, a comparative appraisal was performed, considering not only Y_{TP}, but also the yield in total flavonoids (Y_{TFn}), the antiradical activity (A_{AR}), and the ferric-reducing power (P_R) (Table 3).

Table 3. Appraisal of hydroxypropyl β-cyclodextrin (HP-β-CD) as a co-solvent in the production of polyphenol-containing aqueous extracts from waste orange peels (WOP). Extractions were performed at 40 °C, and values given represent average ± standard deviation.

Solvent	Y_{TP} (mg GAE g^{-1} dm)	Y_{TFn} (mg RtE g^{-1} dm)	A_{AR} (μmol DPPH g^{-1} dm)	P_R (μmol AAE g^{-1} dm)
HP-β-CD [a]	29.13 ± 1.10	4.06 ± 0.06	119.09 ± 3.57	29.50 ± 0.44
Water [a]	13.39 ± 0.75	2.47 ± 0.04	85.32 ± 2.56	20.04 ± 0.30
60% EtOH	20.40 ± 0.98	4.02 ± 0.06	128.70 ± 3.86	33.11 ± 0.50
60% MeOH	18.03 ± 0.89	3.42 ± 0.05	119.86 ± 3.60	36.52 ± 0.55

[a] Adjusted at pH 4.0.

Compared to 60% ethanol, which was the most effective conventional solvent, extraction with HP-β-CD gave by 30% higher Y_{TP}, virtually equal Y_{TFn}, 7.5% lower A_{AR} ($p > 0.05$), and 10.9% lower P_R ($p > 0.05$). This outcome demonstrated that HP-β-CD was highly effective in assisting aqueous extraction of WOP polyphenols at 40 °C.

The extracts obtained with HP-β-CD and 60% EtOH were analyzed by HPLC to identify the principal polyphenolic constituents and determine quantitative differences. The chromatogram recorded at 280 nm revealed that both extracts were dominated by two polyphenols, narirutin and hesperetin (Figure 4). This was in accordance with earlier examinations, which found these two phytochemicals to be the major WOP metabolites [45–47]. The difference in narirutin recovery between HP-β-CD and 60% EtOH extraction was 10.4%, but the difference for hesperidin was 62% (Table 4). This fact indicates that there might be selectivity displayed by HP-β-CD toward hesperidin. Such a phenomenon would appear reasonable, since as pointed out above, less polar compounds interact more strongly with the HP-β-CD cavity. Thus, hesperidin, which is less polar that narirutin, may form more stable complexes with HP-β-CD that facilitate its solubilization and recovery.

Table 4. Yields of the major WOP polyphenols, achieved with hydroxypropyl-β-cyclodextrin (HP-β-CD) and 60% aqueous ethanol (EtOH), at 40 °C.

Extract	Yield (mg g dm^{-1}) ± sd		
	Narirutin	Hesperidin	Sum
HP-β-CD	5.11 ± 0.20	30.55 ± 1.22	35.66
60% EtOH	4.58 ± 0.18	11.62 ± 0.46	16.20

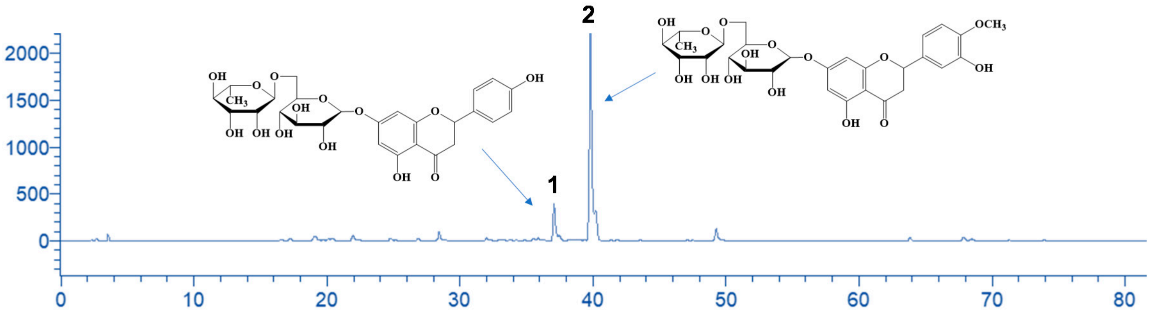


Figure 4. HPLC trace of a waste orange peels (WOP) extract, produced with hydroxypropyl β-cyclodextrin (HP-β-CD), at pH 4 and 40 °C. The trace was recorded at 280 nm. Peak assignment: 1, narirutin; 2, hesperetin.

Overall, HP-β-CD-aided extraction afforded a 54.6% higher yield than 60% aqueous EtOH. This finding demonstrates the high extraction efficiency of HP-β-CD.

The level of narirutin determined (5.11 ± 0.20 mg g^{-1} dm) was in accordance with previous studies that reported values of 0.26–4.52 mg g^{-1} dm [46]. However, other authors reported narirutin levels varying from 0.78 to 1.00 mg g^{-1} dm [45] and 0.73 to 2.85 mg g^{-1} dm [47]. Likewise, recoveries of 7.02–26.81 mg g^{-1} dm [46] and 1.56–20.00 mg g^{-1} dm [47] were determined for hesperidin, although much lower levels of 0.18–4.00 have also been found [45].

4. Conclusions

This study illustrated the efficiency of WOP polyphenol extraction at low temperature by employing HP-β-CD. Such an extraction technique for WOP polyphenol recovery, to the best of the authors' knowledge, has not been previously reported. The results indicated that ultrasonication pretreatment provided no benefit to increasing extraction yield, and that extraction was favored at 40 °C. Under optimal settings, HP-β-CD-aided extraction produced extracts with total polyphenol yield and antioxidant characteristics comparable to those obtained with common conventional solvents such as aqueous methanol and ethanol. The principal polyphenolic phytochemicals identified in the extracts were narirutin and hesperidin. Future examinations should demonstrate the applicability of such extracts in food and cosmetics to pave the way for large-scale valorization of WOP as cost-effective, abundant, and bio-potent natural constituents.

Supplementary Materials: The following are available online at http://www.mdpi.com/2306-5710/6/3/50/s1, Figure S1: Chromatograms recorded as 280 nm, of narirutin (upper figure) and hesperidin (lower figure) standards. Both compounds were dissolved in HPLC-grade methanol at 50 µg mL^{-1}.

Author Contributions: Conceptualization, S.L. and D.P.M.; Formal analysis, A.L., S.L., and D.P.M.; Investigation, A.L., S.L., and D.P.M.; Writing—original draft preparation, S.L. and D.P.M.; Writing—review and editing, S.L. and D.P.M.; Supervision, D.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Torres, A.F.; Xu, X.; Nikiforidis, C.V.; Bitter, J.H.; Trindade, L.M. Exploring the treasure of plant molecules with integrated biorefineries. *Front. Plant Sci.* **2019**, *10*, 478. [CrossRef] [PubMed]
2. Ben-Othman, S.; Jõudu, I.; Bhat, R. Bioactives from agri-food wastes: Present insights and future challenges. *Molecules* **2020**, *25*, 510. [CrossRef] [PubMed]
3. USDA. *Citrus: World Markets and Trade*; United States Department of Agriculture Foreign Agricultural Service, July 2017. Available online: https://www.fas.usda.gov/data/citrus-world-markets-and-trade (accessed on 7 August 2020).

4. Mahato, N.; Sharma, K.; Sinha, M.; Cho, M.H. Citrus waste derived nutra-/pharmaceuticals for health benefits: Current trends and future perspectives. *J. Funct. Foods* **2018**, *40*, 307–316. [CrossRef]
5. Putnik, P.; Bursać Kovačević, D.; Režek Jambrak, A.; Barba, F.J.; Cravotto, G.; Binello, A.; Lorenzo, J.M.; Shpigelman, A. Innovative "green" and novel strategies for the extraction of bioactive added value compounds from citrus wastes—A review. *Molecules* **2017**, *22*, 680. [CrossRef] [PubMed]
6. Khan, M.K.; Dangles, O. A comprehensive review on flavanones, the major citrus polyphenols. *J. Food Compos. Anal.* **2014**, *33*, 85–104. [CrossRef]
7. Testai, L.; Calderone, V. Nutraceutical value of citrus flavanones and their implications in cardiovascular disease. *Nutrients* **2017**, *9*, 502. [CrossRef]
8. Yi, L.; Ma, S.; Ren, D. Phytochemistry and bioactivity of Citrus flavonoids: A focus on antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities. *Phytochem. Rev.* **2017**, *16*, 479–511. [CrossRef]
9. M'hiri, N.; Ioannou, I.; Ghoul, M.; Boudhrioua, N.M. Extraction methods of Citrus peel phenolic compounds. *Food Rev. Int.* **2014**, *30*, 265–290. [CrossRef]
10. Espino, M.; de los Ángeles Fernández, M.; Gomez, F.J.; Boiteux, J.; Silva, M.F. Green analytical chemistry metrics: Towards a sustainable phenolics extraction from medicinal plants. *Microchem. J.* **2018**, *141*, 438–443. [CrossRef]
11. Panzella, L.; Moccia, F.; Nasti, R.; Marzorati, S.; Verotta, L.; Napolitano, A. Bioactive phenolic compounds from agri-food wastes: An update on green and sustainable extraction methodologies. *Front. Nutr.* **2020**, *7*, 60. [CrossRef]
12. Park, S. Cyclic glucans enhance solubility of bioavailable flavonoids. *Molecules* **2016**, *21*, 1556. [CrossRef] [PubMed]
13. Suvarna, V.; Gujar, P.; Murahari, M. Complexation of phytochemicals with cyclodextrin derivatives–An insight. *Biomed. Pharmacother.* **2017**, *88*, 1122–1144. [CrossRef] [PubMed]
14. Cai, R.; Yuan, Y.; Cui, L.; Wang, Z.; Yue, T. Cyclodextrin-assisted extraction of phenolic compounds: Current research and future prospects. *Trends Food Sci. Technol.* **2018**, *79*, 19–27. [CrossRef]
15. Grigorakis, S.; Benchennouf, A.; Halahlah, A.; Makris, D.P. High-performance green extraction of polyphenolic antioxidants from *Salvia fruticosa* using cyclodextrins: Optimization, kinetics, and composition. *Appl. Sci.* **2020**, *10*, 3447. [CrossRef]
16. Kaltsa, O.; Lakka, A.; Grigorakis, S.; Karageorgou, I.; Batra, G.; Bozinou, E.; Lalas, S.; Makris, D.P. A green extraction process for polyphenols from elderberry (*Sambucus nigra*) flowers using deep eutectic solvent and ultrasound-assisted pretreatment. *Molecules* **2020**, *25*, 921. [CrossRef]
17. Lakka, A.; Karageorgou, I.; Kaltsa, O.; Batra, G.; Bozinou, E.; Lalas, S.; Makris, D.P. Polyphenol extraction from *Humulus lupulus* (hop) using a neoteric glycerol/L-alanine deep eutectic solvent: Optimisation, kinetics and the effect of ultrasound-assisted pretreatment. *AgriEngineering* **2019**, *1*, 403–417. [CrossRef]
18. Karakashov, B.; Grigorakis, S.; Loupassaki, S.; Makris, D.P. Optimisation of polyphenol extraction from *Hypericum perforatum* (St. John's Wort) using aqueous glycerol and response surface methodology. *J. Appl. Res. Med. Arom. Plants* **2015**, *2*, 1–8. [CrossRef]
19. Diamanti, A.C.; Igoumenidis, P.E.; Mourtzinos, I.; Yannakopoulou, K.; Karathanos, V.T. Green extraction of polyphenols from whole pomegranate fruit using cyclodextrins. *Food Chem.* **2017**, *214*, 61–66. [CrossRef]
20. Karageorgou, I.; Grigorakis, S.; Lalas, S.; Mourtzinos, I.; Makris, D.P. Incorporation of 2-hydroxypropyl β-cyclodextrin in a biomolecule-based low-transition temperature mixture (LTTM) boosts efficiency of polyphenol extraction from *Moringa oleifera* Lam leaves. *J. Appl. Res. Med. Arom. Plants* **2018**, *9*, 62–69. [CrossRef]
21. Lakka, A.; Lalas, S.; Makris, D.P. Development of a low-temperature and high-performance green extraction process for the recovery of polyphenolic phytochemicals from waste potato peels using hydroxypropyl β-cyclodextrin. *Appl. Sci.* **2020**, *10*, 3611. [CrossRef]
22. Bezerra, M.A.; Santelli, R.E.; Oliveira, E.P.; Villar, L.S.; Escaleira, L.A. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* **2008**, *76*, 965–977. [CrossRef] [PubMed]
23. M'hiri, N.; Ioannou, I.; Boudhrioua, N.M.; Ghoul, M. Effect of different operating conditions on the extraction of phenolic compounds in orange peel. *Food Bioprod. Proc.* **2015**, *96*, 161–170. [CrossRef]
24. M'hiri, N.; Irina, I.; Cédric, P.; Ghoul, M.; Boudhrioua, N. Antioxidants of Maltease orange peel: Comparative investigation of the efficiency of four extraction methods. *J. Appl. Pharmaceut. Sci.* **2017**, *7*, 126–135.

25. El Kantar, S.; Boussetta, N.; Rajha, H.N.; Maroun, R.G.; Louka, N.; Vorobiev, E. High voltage electrical discharges combined with enzymatic hydrolysis for extraction of polyphenols and fermentable sugars from orange peels. *Food Res. Int.* **2018**, *107*, 755–762. [CrossRef]
26. Hernández-Carranza, P.; Ávila-Sosa, R.; Guerrero-Beltrán, J.; Navarro-Cruz, A.; Corona-Jiménez, E.; Ochoa-Velasco, C. Optimization of antioxidant compounds extraction from fruit by-products: Apple pomace, orange and banana peel. *J. Food Proc. Preserv.* **2016**, *40*, 103–115. [CrossRef]
27. Dalmau, E.; Rosselló, C.; Eim, V.; Ratti, C.; Simal, S. Ultrasound-assisted aqueous extraction of biocompounds from orange byproduct: Experimental kinetics and modeling. *Antioxidants* **2020**, *9*, 352. [CrossRef]
28. El Kantar, S.; Rajha, H.N.; Maroun, R.G.; Louka, N. Intensification of polyphenols extraction from orange peels using infrared as a novel and energy saving pretreatment. *J. Food Sci.* **2020**, *85*, 414–420. [CrossRef]
29. Saokham, P.; Muankaew, C.; Jansook, P.; Loftsson, T. Solubility of cyclodextrins and drug/cyclodextrin complexes. *Molecules* **2018**, *23*, 1161. [CrossRef]
30. López-Miranda, S.; Serrano-Martínez, A.; Hernández-Sánchez, P.; Guardiola, L.; Pérez-Sánchez, H.; Fortea, I.; Gabaldón, J.A.; Núñez-Delicado, E. Use of cyclodextrins to recover catechin and epicatechin from red grape pomace. *Food Chem.* **2016**, *203*, 379–385. [CrossRef]
31. Khan, M.K.; Abert-Vian, M.; Fabiano-Tixier, A.-S.; Dangles, O.; Chemat, F. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. *Food Chem.* **2010**, *119*, 851–858. [CrossRef]
32. Londoño-Londoño, J.; de Lima, V.R.; Lara, O.; Gil, A.; Pasa, T.B.C.; Arango, G.J.; Pineda, J.R.R. Clean recovery of antioxidant flavonoids from citrus peel: Optimizing an aqueous ultrasound-assisted extraction method. *Food Chem.* **2010**, *119*, 81–87. [CrossRef]
33. Dahmoune, F.; Boulekbache, L.; Moussi, K.; Aoun, O.; Spigno, G.; Madani, K. Valorization of *Citrus limon* residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction. *Ind. Crops Prod.* **2013**, *50*, 77–87. [CrossRef]
34. Dahmoune, F.; Moussi, K.; Remini, H.; Belbahi, A.; Aoun, O.; Spigno, G.; Madani, K. Optimization of ultrasound-assisted extraction of phenolic compounds from *Citrus sinensis* L. peels using response surface methodology. *Chem. Eng. Trans.* **2014**, *37*, 889–894.
35. 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. *Ultrason. Sonochem.* **2015**, *24*, 72–79. [CrossRef]
36. Alexandru, L.; Binello, A.; Mantegna, S.; Boffa, L.; Chemat, F.; Cravotto, G. Efficient green extraction of polyphenols from post-harvested agro-industry vegetal sources in Piedmont. *C. R. Chim.* **2014**, *17*, 212–217. [CrossRef]
37. Georgantzi, C.; Lioliou, A.-E.; Paterakis, N.; Makris, D.P. Combination of lactic acid-based deep eutectic solvents (DES) with β-cyclodextrin: Performance screening using ultrasound-assisted extraction of polyphenols from selected native Greek medicinal plants. *Agronomy* **2017**, *7*, 54. [CrossRef]
38. Shewale, S.; Rathod, V.K. Extraction of total phenolic content from *Azadirachta indica* or (neem) leaves: Kinetics study. *Prep. Biochem. Biotechnol.* **2018**, *48*, 312–320. [CrossRef]
39. Dong, H.; Li, Y.; Yu, J.; Song, Y.; Cai, X.; Liu, J.; Zhang, J.; Ewing, R.C.; Shi, D. A versatile multicomponent assembly via β-cyclodextrin host–guest chemistry on graphene for biomedical applications. *Small* **2013**, *9*, 446–456. [CrossRef]
40. Zhang, Q.F.; Cheung, H.Y.; Shangguan, X.; Zheng, G. Structure selective complexation of cyclodextrins with five polyphenols investigated by capillary electrokinetic chromatography. *J. Sep. Sci.* **2012**, *35*, 3347–3353. [CrossRef]
41. Cai, C.; Liu, M.; Yan, H.; Zhao, Y.; Shi, Y.; Guo, Q.; Pei, W.; Han, J.; Wang, Z. A combined calorimetric, spectroscopic and molecular dynamic simulation study on the inclusion complexation of (E)-piceatannol with hydroxypropyl-β-cyclodextrin in various alcohol+ water cosolvents. *J. Chem. Thermodyn.* **2019**, *132*, 341–351. [CrossRef]
42. Zhang, M.; Li, J.; Zhang, L.; Chao, J. Preparation and spectral investigation of inclusion complex of caffeic acid with hydroxypropyl-β-cyclodextrin. *Spectrochim. Acta A Mol. Biomol. Spectr.* **2009**, *71*, 1891–1895. [CrossRef] [PubMed]

43. Aksamija, A.; Polidori, A.; Plasson, R.; Dangles, O.; Tomao, V. The inclusion complex of rosmarinic acid into beta-cyclodextrin: A thermodynamic and structural analysis by NMR and capillary electrophoresis. *Food Chem.* **2016**, *208*, 258–263. [CrossRef] [PubMed]
44. Liu, M.; Zheng, Y.; Wang, C.; Xie, J.; Wang, B.; Wang, Z.; Han, J.; Sun, D.; Niu, M. Improved stability of (+)-catechin and (−)-epicatechin by complexing with hydroxypropyl-β-cyclodextrin: Effect of pH, temperature and configuration. *Food Chem.* **2016**, *196*, 148–154. [CrossRef] [PubMed]
45. Goulas, V.; Manganaris, G.A. Exploring the phytochemical content and the antioxidant potential of Citrus fruits grown in Cyprus. *Food Chem.* **2012**, *131*, 39–47. [CrossRef]
46. Chen, X.-M.; Tait, A.R.; Kitts, D.D. Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. *Food Chem.* **2017**, *218*, 15–21. [CrossRef]
47. Lachos-Perez, D.; Baseggio, A.M.; Mayanga-Torres, P.; Junior, M.R.M.; Rostagno, M.; Martínez, J.; Forster-Carneiro, T. Subcritical water extraction of flavanones from defatted orange peel. *J. Supercrit. Fluids* **2018**, *138*, 7–16. [CrossRef]