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An Investigation into Apricot Pulp Waste as a Source of Antioxidant Polyphenols and Carotenoid Pigments

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Abstract: The interest in extracting bioactive compounds from food processing waste is growing unabated. Apricots are widely consumed worldwide, and many tons of waste are produced annually. Therefore, apricot pulp waste (APW) may serve as a rich source of bioactive compounds. In the present study, we investigated the extraction of antioxidant polyphenols and carotenoid pigments from APW. In both cases, a response surface methodology was employed, so as to optimize the extraction parameters. As regards polyphenols, it was found that optimum extraction yield (i.e., 28.6 mg gallic acid equivalents per g of dry weight) was achieved using a deep eutectic solvent (comprised of glycerol, citric acid, and L-proline at a molar ratio of 2:1:1), a liquid-to-solid ratio of 100 mL/g, and heating at 80 °C for 155 min. Similarly, optimum extraction of carotenoids (171.2 mg β-carotene equivalents per 100 g of dry weight) was achieved by extracting APW with an *n*-hexane: acetone: ethanol (2:1:1, *v*/*v*/*v*) mixture at 47 °C for 60 min. The proposed methods were highly efficient and can serve as an alternative to conventional methods employed to date.

Keywords: apricot pulp waste; antioxidants; polyphenols; carotenoids; pigments; canning fruit industry

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1. Introduction

Fruits are widely consumed, worldwide, either whole or they are processed to develop various products. Consumers choose to buy fruits that have the optimum appearance, shape, and size, according to their criteria [1]. However, it is common that fruits injured during harvest, transport, and storage are thrown away as waste, along with overripe fruits [2,3]. In addition, more waste is produced during and after the processing of fruits to prepare juices, jams, and other products. The amount of agri-food waste that is produced annually is estimated to be around 140 billion tons per year [4,5]. Handling and disposal of agri-food waste is not only costly but also poses a threat to the environment, since compounds contained in the waste may be toxic for (micro)organisms or contribute to the greenhouse effect after degradation [6]. As a result, industries nowadays try to reduce the usage of natural resources and their waste, to some extent, through integrated waste management. Moreover, more and more research focuses on the extraction of bioactive compounds from agri-food waste in order to reduce the environmental impact, while simultaneously retrieving valuable compounds [7].

Polyphenols are phytochemical compounds that come from secondary plant metabolism. More and more studies are being published, highlighting the importance of polyphenols in food and cosmetics, in order to promote human health [8]. Much emphasis is being placed on polyphenols since their consumption has been associated with reduced risk for cancers and heart diseases, achieved by their antioxidant potential [9]. Moreover, cellular components are protected from oxidative stress, when polyphenols are consumed [10]. The extraction of polyphenols is a topic of increasing interest. More and more studies are being published, aiming to extract polyphenols from various matrices. Extraction is carried out

either with conventional technologies (including Soxhlet extraction, maceration, percolation, etc.) or non-conventional technologies (including ultrasound-assisted extraction, pulsed electric field, microwave-assisted extraction etc.) [11]. Maceration is one the most commonly employed methods, since it is very simple, it has minimum requirements, is of low cost, and it has environmentally friendly characteristics [11]. In all these methods, various solvents are also being examined, in an effort to maximize the extraction yield.

Another important class of compounds is carotenoids. Carotenoids are contained in the leaves, stems, flowers, and fruits of plants and trees as well as in animals [12,13]. The conjugated double bonds of carotenoids absorb light at 400–500 nm, bestowing the yellow, orange, or red color to many types of plants [14]. Carotenoid content has a major role in the health benefits associated with the consumption of fruits and vegetables [15]. Consumption of fruits and vegetables with high carotenoid content contributes to the prevention of heart diseases, the prevention of cancers, and other diseases [16,17]. As regards the extraction of carotenoids, the same techniques as in the case of polyphenols are being used [18].

Apricot (*Prunus armeniaca* L.) is a stone fruit, that is widely consumed worldwide and as such, is one of the most commercialized fruits [19]. Many products are prepared from apricots, including juices and jams. Apricot has an intense color, which is attributed to the high content of carotenoids [20–22]. Studies have showcased that apricot peels contain 4–177 mg β-carotene/Kg, making it a very good source of carotenoids [23]. Moreover, as with most stone fruits, it contains plenty of polyphenols [24,25]. However, the apricot has a very short storage time and its ripening process is fast. As such, it has a limited shelf life. Therefore, due to the sensitivity of the fruit, the limited shelf life, and the many products produced from apricots, the amount of waste produced is increased.

To date, many studies report the extraction of such bioactive compounds from various plant matrices [26,27]. Advances in the field are achieved either by examining advanced extraction techniques or by examining new solvents, as an alternative to organic solvents [28]. One such type of novel, alternative solvent is deep eutectic solvents (DES). DES are mixtures of two or more components, that are liquid at or below the melting point of the single components. The unique combination of properties, such as low melting point, high solvation power, non-flammability, and high thermal stability, make them attractive for a wide range of industrial and environmental applications [29]. It has been demonstrated that DES are promising solvents for polyphenol extraction from plant-based sources [30]. In addition, it has been shown that DES are more effective than traditional solvents, such as ethanol, in terms of their ability to extract polyphenols from plant sources [31].

In this study, we examined the extraction of bioactive compounds from apricot pulp waste (APW). More specifically, emphasis was placed on the extraction of antioxidant polyphenols and carotenoid pigments. The extraction solvent was examined, as a means of increasing the extraction yield. Organic solvents were employed, as well as deep eutectic solvents (DES). DES are innovatively designed liquids, which are composed of low-cost, recyclable, and non-toxic materials, such as certain natural substances (e.g., sugars, organic acids, salts, etc.) [32]. They possess many advantages over organic solvents and as such, they are more and more exploited as alternative solvents.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin-Ciocalteu reagent, gallic acid, ethanol, and *n*-hexane were purchased from Panreac Co. (Barcelona, Spain). Anhydrous sodium carbonate and anhydrous glycerol were obtained from Penta (Prague, Czech Republic). Iron chloride (hexahydrate) and choline chloride were purchased from Merck (Darmstadt, Germany). Methanol, acetone, petroleum ether, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), 2-propanol, hydrochloric acid, L-ascorbic acid, and 2,2-diphenylpicrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Darmstadt, Germany).

2.2. Apricot Pulp Waste (APW) Preparation

The APW was derived from the variety *Prunus armeniaca* 'Bebeco'. The sample was obtained from the waste of a fruit canning factory (ELBAK S.A., Larissa, Greece). The APW was collected and frozen at −40 °C using liquid nitrogen. Water was removed from the sample by freeze-drying using a Biobase BK-FD10P freeze-dryer (Jinan, Shandong, China). Next, the sample was placed in a blender and the resulting powder was placed in sieves (using a Fritsch Analysette 3, Idar-Oberstein, Germany). Powder with an average diameter of 180 μm was used in this study.

2.3. Synthesis of DES

To prepare the DES, the hydrogen bond donor was mixed with the hydrogen bond acceptor at a proper ratio. More specifically, according to our previous study [33], DES-1 was prepared by mixing glycerol and choline chloride at a molar ratio of 2:1 (w/w). DES-2 was prepared by mixing glycerol, citric acid, and L-proline at a ratio of 2:1:1 ($w/w/w$). In both cases, the mixture was heated in an amber glass, screw-capped vial at 80–90 °C for about 90 min, under stirring, until a transparent liquid was formed. Finally, 20% water was added to the DES and after thorough mixing, they were used for the extraction.

2.4. Extraction of Antioxidant Polyphenols

For the optimum extraction of antioxidant polyphenols from APW, in a 25-mL Duran™ glass bottle, 0.1 g of dried APW was transferred along with 10 mL of DES-2 solvent. Other solvents (water, ethanol, and DES-1) were also examined. The bottle was heated in an oil bath at 80 °C for 155 min, under stirring (500 rpm). Next, samples were centrifuged at 10,000 rpm for 10 min using a centrifuge NEYA 16R (Remi Elektrotechnik Ltd., Palghar, India). Supernatants were retracted and stored at –40 °C until analysis. For means of comparison, extracts were prepared in the same way, using other solvents (i.e., water, 60% (v/v) ethanol: water mixture, and DES-1). In the cases of water and the hydroethanolic mixture, heating was carried out under reflux, to avoid solvent evaporation.

2.5. Extraction of Carotenoid Pigments

For the optimum extraction of carotenoid pigments, in a 25-mL Duran™ glass bottle, 0.1 g of dried APW was transferred along with 10 mL of *n*-hexane: acetone: ethanol (2:1:1, $v/v/v$) solvent. Other solvents used were hexane, acetone, ethanol, propanol, and DES-2. The bottle was heated in an oil bath at 47 °C for 60 min, under reflux and under stirring (250 rpm). Next, samples were centrifuged at 10,000 rpm for 10 min. Supernatants were retracted and stored at −40 °C until analysis.

2.6. Design of Experiment and Response Surface Methodology (RSM) Optimization

The experimental design aimed to maximize the extraction yield for the tests for total polyphenol content (TPC) and total carotenoid content (TCC). This was accomplished for the TPC by optimizing the liquid-to-solid ratio (R, mL/g), extraction temperature (T, °C), and extraction time (t, min). On the other hand, the extraction time (t, min), temperature (T, °C), and speed (S, rpm) were tuned to achieve this for the TCC. An experiment using a Box–Behnken design with 15 design points, including 3 center points, served as the foundation for optimization. The process variables were constructed in 3 levels in accordance with the experimental design. The coded and actual levels are listed in Tables 1 and 2, respectively, for TPC and TCC. The overall model significance (R^2, p), as well as the significance of model (equations) coefficients, were assessed at a minimum level of 95% using analysis of variance (ANOVA) and lack-of-fit tests.

Table 1. Independent variables and their related actual and coded levels utilized to optimize the extraction of TPC.

Independent Variables	Code Units	Coded Variable Level		
		−1	**0**	**1**
R (mL/g)	X_1	40	70	100
T (°C)	X_2	50	65	80
t (min)	X_3	100	150	200

Table 2. Independent variables and their related actual and coded levels utilized to optimize the extraction of TCC.

Independent Variables	Code Units	Coded Variable Level		
		−1	**0**	**1**
t (min)	X_1	30	60	90
T (°C)	X_2	25	35	50
S (rpm)	X_3	250	500	750

Additionally, the response variable was predicted using a second-order polynomial model presented in the following Equation (1) as a function of the investigated independent factors:

$$Y_k = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

where Y_k is the predicted response variable; X_i and X_j are the independent variables; β_0, β_i, β_{ii}, and β_{ij} are the intercept, regression coefficients of the linear, quadratic, and interaction terms of the model, respectively. RSM was also used to calculate the greatest peak area and to examine the impact of a significant independent variable on response. To display the model equation visually, 3D surface response graphs were built.

2.7. Determinations

2.7.1. Total Polyphenol Content (TPC)

In an Eppendorf tube, 100 µL of appropriately diluted sample was transferred along with 100 µL Folin-Ciocalteu reagent [34]. After 2 min of incubation, the reaction was terminated by adding 800 µL Na_2CO_3 solution (5% w/v). After vortex mixing, the solutions were heated in a water bath at 40 °C for 20 min. Finally, the absorbance was recorded at 740 nm using a spectrophotometer (Shimadzu UV-1700 PharmaSpec Spectrophotometer; Kyoto, Japan). Results (C_{TP}) were expressed as mg gallic acid equivalents (GAE) per L, using a calibration curve with standard gallic acid solutions (10–100 mg/L in methanol). Next, the extraction yield of total polyphenols (Y_{TP}) was expressed as mg GAE per g dry weight (dw), using the following equation:

$$Y_{TP}\ (\text{mg GAE/g dw}) = \frac{C_{TP} \times V}{w} \quad (2)$$

where V is the volume of solvent (in L) and w is the dry weight of the sample (in g).

2.7.2. Ferric Reducing Antioxidant Power (FRAP) Assay

A previously reported method was employed for the study of FRAP [33]. In an Eppendorf tube, 50 µL of an appropriately diluted polyphenolic extract sample was mixed with 50 µL of $FeCl_3$ solution (4 mM in 0.05 M HCl) and incubated for 30 min in a water bath at 37 °C. Next, 900 µL TPTZ solution (1 mM in 0.05 M HCl) was added, and the absorbance was recorded at 620 nm after 5 min. Results (P_R) were expressed as µmoL

ascorbic acid equivalents (AAE) per g of dw, using an ascorbic acid calibration curve (C_{AA}, 50–500 μmoL/L in 0.05 M HCl), using the following equation:

$$P_R\ (\mu\text{moL AAE/g dw}) = \frac{C_{AA} \times V}{w} \quad (3)$$

where V is the volume of the extraction medium (in L) and w is the dry weight of the sample (in g).

2.7.3. Antiradical Activity (DPPH Assay)

A volume of 25 μL polyphenolic extract sample was mixed with 975 μL DPPH solution (100 μmoL/L in methanol) and the absorbance at 515 nm was read immediately after mixing ($A_{515(i)}$) and after 30 min ($A_{515(f)}$) [35]. The capacity to scavenge the DPPH radical was expressed as:

$$\text{Inhibition}\ (\%) = \left(\frac{A_{515(i)} - A_{515(f)}}{A_{515(i)}} \right) \times 100 \quad (4)$$

Antiradical activity (A_{AR}) was determined as μmoL ascorbic acid equivalents (AAE) per g of dw, using an ascorbic acid calibration curve (C_{AA}, 100–1000 μmoL/L in methanol), using the following equation:

$$A_{AR}\ (\mu\text{moL AAE/g dw}) = \frac{C_{AA} \times V}{w} \quad (5)$$

where V is the volume of the extraction medium (in L), and w is the dry weight of the sample (in g).

2.7.4. Total Carotenoid Content (TCC)

For the determination of the TCC of the extracts, the method of Ayour et al. [36] was employed. In brief, the samples were ten-fold diluted with the solvent that was used for their preparation, and the absorbance was measured at 450 nm. Results (TCC) were expressed as mg β-carotene equivalents per 100 g of dw, using a β-carotene calibration curve.

2.8. Statistical Analysis

The experimental design, statistical analysis related to the response surface methodology, and distribution analysis were all created using the JMP® Pro 16 (SAS, Cary, NC, USA) software. All extracts were prepared 3 times and each analysis was carried out 3 times from each extract, resulting in a total of 9 measurements per condition. Variability was expressed with the standard deviation of the nine measurements. Results were expressed as mean values ± standard deviation. The normality of the distribution of the results was examined with the Kolmogorov–Smirnov test. Statistically significant differences ($p < 0.05$) between samples were examined with the Kruskal–Wallis test. All statistical analysis was carried out using SPSS (version 26) (SPSS Inc., Chicago, IL, USA) software.

3. Results and Discussion

For the optimization of the extraction procedures, two DES were examined, along with organic solvents. The selected DES consisted of glycerol and proline or choline chloride. Both proline and choline chloride are low-cost solvents that are commonly used in the food and cosmetics industries. Moreover, previous studies have showcased that they can exhibit better performance, compared to commonly employed organic solvents [37,38].

3.1. Optimization of Polyphenols Extraction

One of the most well-known types of compounds that is abundant in natural product wastes is polyphenols. Therefore, our first aim was to maximize the recovery of polyphenols from APW. Before optimizing the parameters that affect the extraction yield, preliminary experiments were carried out in order to determine the most suitable solvent. To this end,

water, 60% (v/v) ethanol, and the two synthesized DES were evaluated. The hydroethanolic solution was tested for means of comparison, since it is reported to achieve enhanced extraction of polyphenols, compared to other ratios [39–41]. Results can be seen in Figure 1. It can be seen that the use of a 60% (v/v) ethanol: water mixture resulted in extracts that contained twice the amount of polyphenols than the extracts prepared with water. As regards the use of DES, it can be seen that DES-1 (glycerol: choline chloride, 2:1) not only did not increase the extraction of polyphenols but resulted in extracts that only marginally contained any polyphenols. It is reported that the polarity of the DES is the most important parameter that affects extraction potential. The better that the polarity of the DES matches the polarity of the target compounds, the higher the extraction yield. Therefore, it can be inferred that the observed results may be due to the physicochemical characteristics of the DES-1, whose polarity is higher than that of water [42]. The opposite may hold true for DES-2 (glycerol: citric acid: L-proline, 2:1:1) since its usage resulted in extracts with 540% increased TPC compared to aqueous extracts. Therefore, it is obvious that the use of DES-2 is not only beneficial in terms of avoiding organic solvents but also achieves a three-times higher extraction yield, compared to ethanol which is usually used. It is noteworthy that the DES does not need to be removed from the obtained extracts, and can be consumed directly since previous studies have demonstrated that DES-2 exhibits a low toxicity [43].

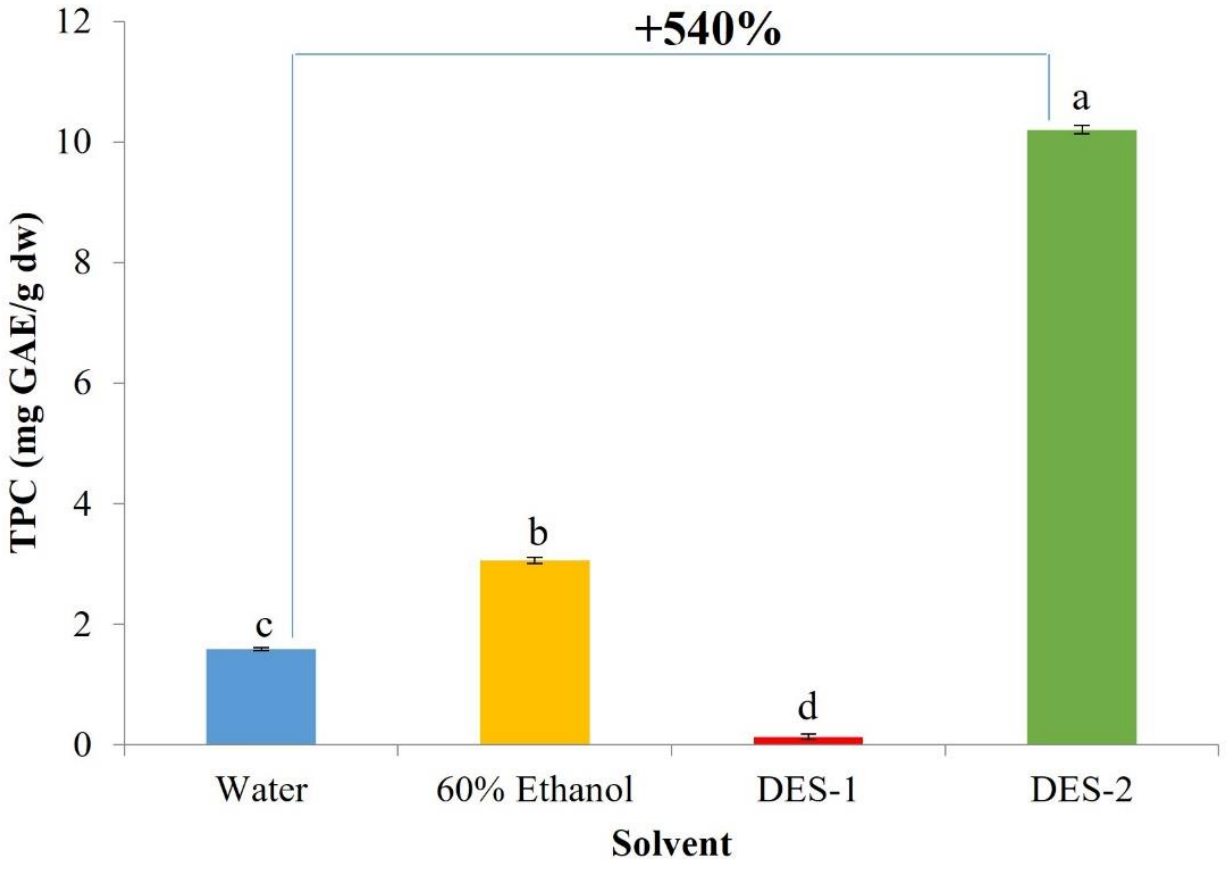

Figure 1. Total polyphenol content (TPC, mg GAE/g dw) of the extracts from APW using different solvents; Standard deviation is presented with error bars and means with different superscript letters are significantly ($p < 0.05$) different.

Results were in accordance with previous studies [44,45]. In the study of Cheaib et al. [44], the extraction of polyphenols from apricot pomace was discussed. The authors examined the effect of extraction techniques, by comparing mixing under heating, ultrasound-assisted extraction, microwave-assisted extraction, and infrared-assisted extraction. When three out of the four techniques were employed, a content of 3–4 mg GAE/g dw was

recorded; meanwhile, only in the case of infrared-assisted extraction was a polyphenol content of 10 mg GAE/g dw recorded. Similar to our case, extraction with common solvents resulted in 2–4 mg GAE/g dw in the extracts, leaving significant amounts of polyphenols unextracted. Instead of using complicated prototype devices, as in the case of Cheaib et al. [44], using an easily synthesized DES, the same TPC can be achieved. In the study of Dulf et al. [45], the extraction of polyphenols from apricot pomace was also discussed. For the preparation of the extract, the authors used a hydrochloric acid: methanol: water mixture (1:80:19), and the extraction was assisted by ultrasounds. The authors found that the extract contained ~1.2 mg GAE/g dw polyphenols. This content is similar to that obtained in our case using water. Based on the above, it can be inferred that the proposed preparation of extracts is superior to the methods reported so far, because organic solvents are avoided and at the same time a higher extraction yield is achieved, without the need for assistance from additional techniques, such as ultrasound and infrared.

After the selection of the optimum solvent, a RSM was employed to optimize the parameters that affect the extraction (the selection of the parameters was based on preliminary experiments). More specifically, the liquid-to-solid ratio (ranging from 40 to 100 mL/g), the extraction temperature (ranging from 50 to 80 °C), and time (ranging from 100 to 200 min) were examined. The experimental conditions and the recorded responses (i.e., TPC) are given in Table 3. The statistical parameters, second-order polynomial equation (model), and coefficients derived for the model are shown below in Equation (6):

$$Y = 31.6 - 0.92X_1 - 0.69X_2 + 0.25X_3 + 0.004{X_1}^2 + 0.003{X_2}^2 - 0.0004{X_3}^2 + 0.01X_1X_2 - 0.0005X_1X_3 - 0.0007X_2X_3 \ (R^2 = 0.99, p = 0.0003) \quad (6)$$

Table 3. The design points included in the experimental design, the corresponding coded values of the process variables, and the TPC values for the measured and predicted responses were optimized for APW extraction with DES-2.

Design Point	Independent Variables			Response (TPC, mg GAE/g dw)	
	X_1 (*R*, mL/g)	X_2 (*T*, °C)	X_3 (*t*, min)	Measured	Predicted
1	1	0	1	20.5	20.4
2	0	−1	1	9.8	10.3
3	−1	0	1	12.9	11.7
4	0	1	1	15.1	15.9
5	−1	−1	0	10.9	11.6
6	0	0	0	11.9	11.6
7	1	1	0	29.1	28.4
8	1	−1	0	13.5	13.2
9	−1	0	−1	6.0	6.1
10	1	0	−1	16.8	18.0
11	0	1	−1	13.4	12.9
12	0	−1	−1	6.1	5.3
13	0	0	0	11.6	11.6
14	0	0	0	11.2	11.6
15	−1	1	0	9.2	9.6

It can be seen that the coefficients were >0.99, suggesting a good fit for the developed models. In addition, the actual versus predicted response, desirability function, and 3D response graphs are given in Figure S1 (Supplementary Materials) and Figure 2. Using the desirability function, it was found that for the optimum extraction a solvent-to-solid ratio of 100 mL/g should be employed, and the extraction should be carried out at 80 °C for 155 min. Using these conditions, the predicted TPC was calculated to be 28.5 ± 2.4 mg GAE/g dw. In order to validate the calculations, extraction was carried out, using the proposed parameters. The TPC in the extract was found to be 28.4 ± 2.2 mg GAE/g dw, suggesting a good prediction from the model.

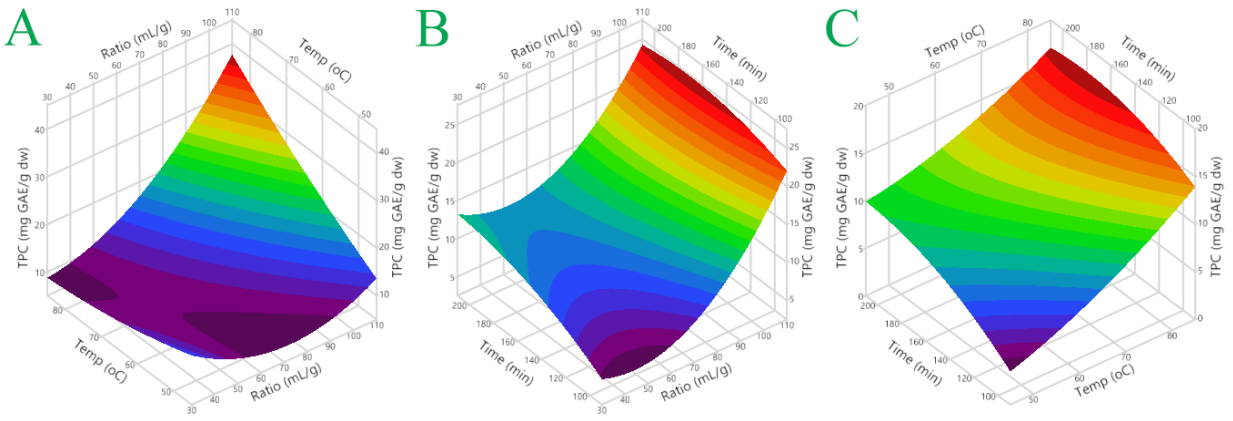

Figure 2. For the extraction of APW using DES-2, 3D graphs showing the impact of the process variables taken into consideration on the response. Plots (**A**,**B**) show the covariation of X_1 (R) and X_2 (T), X_1 (R), and X_3 (t), and (**C**) the covariation of X_2 (T) and X_3 (t).

3.2. Evaluation of the Antioxidant Properties of the Polyphenolic Extracts

The next step was to evaluate the antioxidant properties of the optimum extract. To this end, two commonly employed assays were employed.

Results from the FRAP assay of the extract are given in Figure 3. It was found that the extract exhibited a FRAP antioxidant activity of 67.2 μmoL AAE/g dw (590% more antioxidant activity compared to water). The DES-2 was also examined in case it exhibited any antioxidant activity. The results showed that DES-2 did not exhibit any antioxidant activity in the FRAP assay (as occurred with water and the hydroethanolic mixture), signifying that the observed value is attributed solely to the extracted compounds. It is known that, aside from the polyphenols that are contained in apricots that exhibit antioxidant properties, apricots also contain other organic acids, such as citric acid and malic acid which also possess antioxidant properties. For means of comparison, extracts from APW were prepared under optimum conditions using water and a 60% ethanol: water mixture. From the results, it was obvious that DES-2 exhibited much higher FRAP activity, compared to the other two examined solvents, suggesting its superiority in terms of FRAP activity.

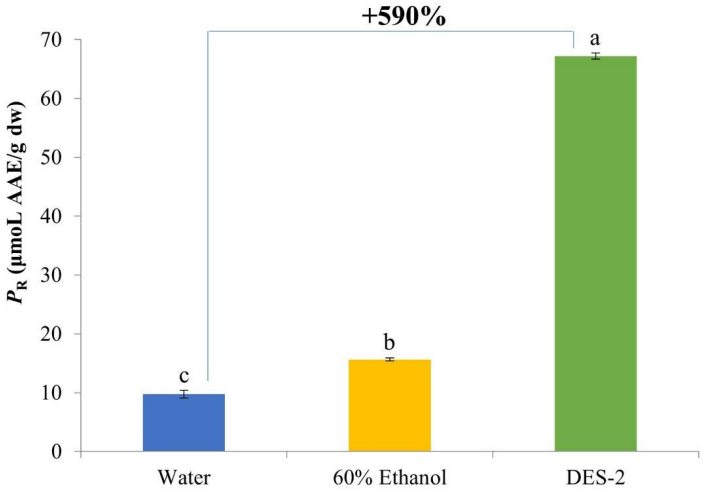

Figure 3. FRAP antioxidant activity (P_R, μmoL AAE/g dw) of extracts from APW using various solvents and DES-2 obtained by the optimum extraction conditions; Standard deviation is presented with error bars and means with different superscript letters are significantly ($p < 0.05$) different.

The abovementioned extracts were further evaluated for their DPPH antioxidant activity. The results are summarized in Figure 4. Results were similar to that obtained

from the FRAP assay. However, DES-2 yielded an extract with 330% more antioxidant activity, compared to water. When the optimum extraction conditions were employed, the obtained extract exhibited three times higher activity compared to the ethanol-based extract. The DES-2 was also evaluated for its DPPH antioxidant activity, before being used for extractions. A value of 0.29 μmol AAE/g was recorded for DES-2. Water and the hydroethanolic mixture did not exhibit any activity. This value was subtracted from the results, and as such, Figure 4 depicts the value attributed only to the extracted compounds.

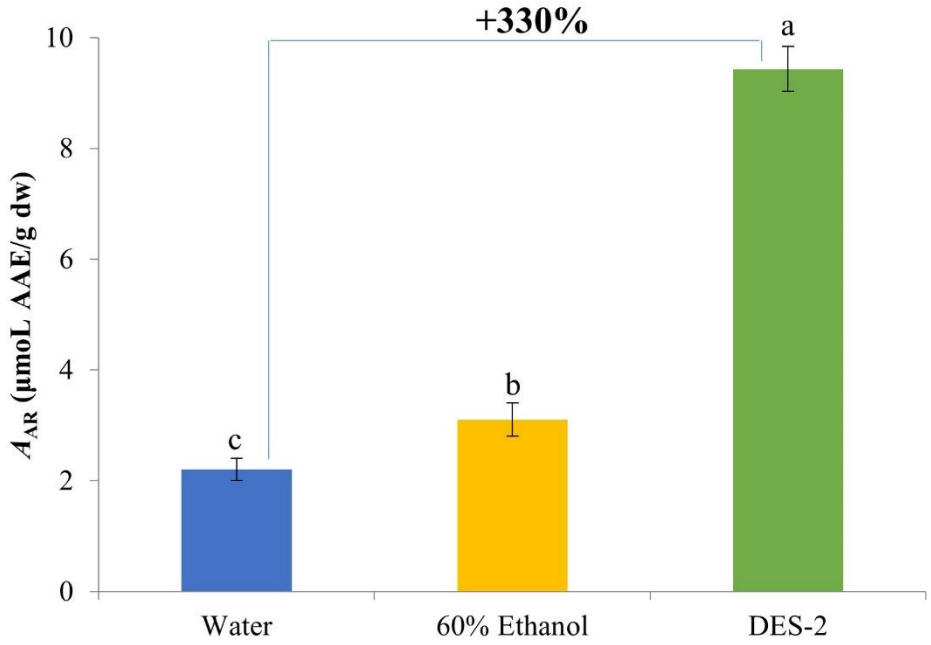

Figure 4. DPPH antioxidant activity (A_{AR}, μmoL AAE/g dw) of extracts from APW using various solvents and DES-2 obtained by the optimum extraction conditions; Standard deviation is presented with error bars and means with different superscript letters are significantly ($p < 0.05$) different.

3.3. Optimization of Carotenoids Extraction

Our second aim was to determine the optimum extraction conditions for carotenoids from APW. Since carotenoids are less polar compounds, solvents of medium polarity and non-polar solvents were examined. Moreover, DES-2 was also examined for its extraction potential. DES-1 was not examined, since it is highly polar, and thus it would be unsuitable for the extraction of carotenoids. The results are presented in Figure 5. As can be seen, propanol, ethanol, and acetone resulted in extracts with nearly the same carotenoid content, whereas hexane increased the extraction yield of β-carotene (statistically significant for $p < 0.05$. The use of DES-2 resulted in an extract with similar β-carotene content to those prepared with acetone and hexane. Therefore, the use of DES-2 was not proved to be more beneficial, as occurred in the case of polyphenols. Finally, a mixture of *n*-hexane: acetone: ethanol (2:1:1, *v*/*v*/*v*) was examined and found to increase the carotenoid content by nearly 10%, compared to hexane. The use of the mixture is beneficial compared to the use of pure organic solvents, both in terms of handling, and in terms of safety/cost. Selection of this mixture was based on previous studies [36,46]. Although safety issues may arise with heating a mixture of organic solvents, the use of reflux may lower the risk of accidents.

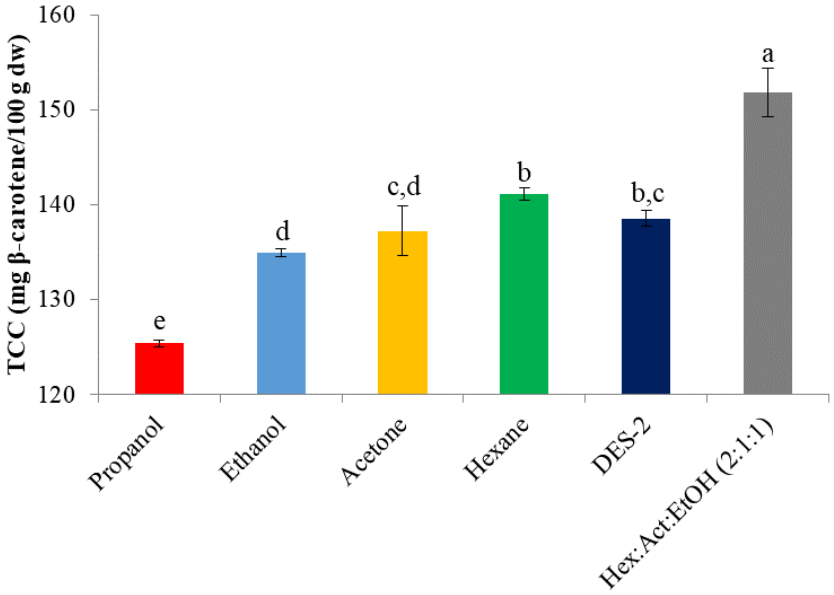

Figure 5. Total carotenoid content (TCC, mg β-carotene/100 g dw) of the extracts from APW using different solvents; Standard deviation is presented with error bars and means with different superscript letters are significantly ($p < 0.05$) different.

Using the optimum solvent solution, the extraction parameters were further examined, so as to achieve maximum recovery of carotenoids. Selection of the parameters was based on preliminary experiments. Optimization was carried out by a response surface methodology. The extraction time (ranging from 30 to 90 min), the extraction temperature (between 25 and 50 °C), and the stirring rate (ranging between 250 and 750 rpm) were examined. The experimental conditions and the recorded responses (i.e., TCC) are given in Table 4. The statistical parameters, second-order polynomial equation (model), and coefficients derived for the model are shown below in Equation (7):

$$Y = -23.45 + 1.73X_1 + 6.54X_2 - 0.02X_3 - 0.008{X_1}^2 - 0.05{X_2}^2 + 0.0001{X_3}^2 - 0.02X_1X_2 - 0.00007X_1X_3 - 0.002X_2X_3 \ (R^2 = 0.93, p = 0.0236) \quad (7)$$

Table 4. The design points included in the experimental design, the corresponding coded values of the process variables, and the TCC values for the measured and predicted responses were optimized for APW extraction with *n*-hexane: acetone: ethanol (2:1:1) solvent.

Design Point	Independent Variables			Response (TCC, mg β-Carotene/100 g dw)	
	X_1 (*t*, min)	X_2 (*T*, °C)	X_3 (*S*, rpm)	Measured	Predicted
1	−1	−1	0	131.6	130.2
2	−1	1	0	156.8	154.1
3	1	−1	0	145.4	149.0
4	1	1	0	148.5	149.1
5	0	−1	−1	143.1	145.4
6	0	−1	1	166.1	161.7
7	0	1	−1	167.4	170.7
8	0	1	1	161.5	160.4
9	−1	0	−1	151.7	151.0
10	1	0	−1	166.3	161.4
11	−1	0	1	152.9	157.8
12	1	0	1	165.3	166.0
13	0	0	0	159.3	159.9
14	0	0	0	160.2	159.9
15	0	0	0	160.0	159.9

It can be seen that the coefficients were >0.93, suggesting a good fit for the developed models. In addition, the actual versus predicted response, desirability function, and 3D response graphs are given in Figure S2 and Figure 6. From the desirability function, it was found that the optimum extraction should be carried out at 47 °C for 60 min with a 250 rpm stirring rate. Using these conditions, the predicted TCC was calculated to be 171.2 ± 9.2 mg/100 g dw. Extraction was carried out using the optimum conditions and it was found that the TCC was 173.1 ± 5.4 mg/100 g dw, suggesting a good prediction from the model.

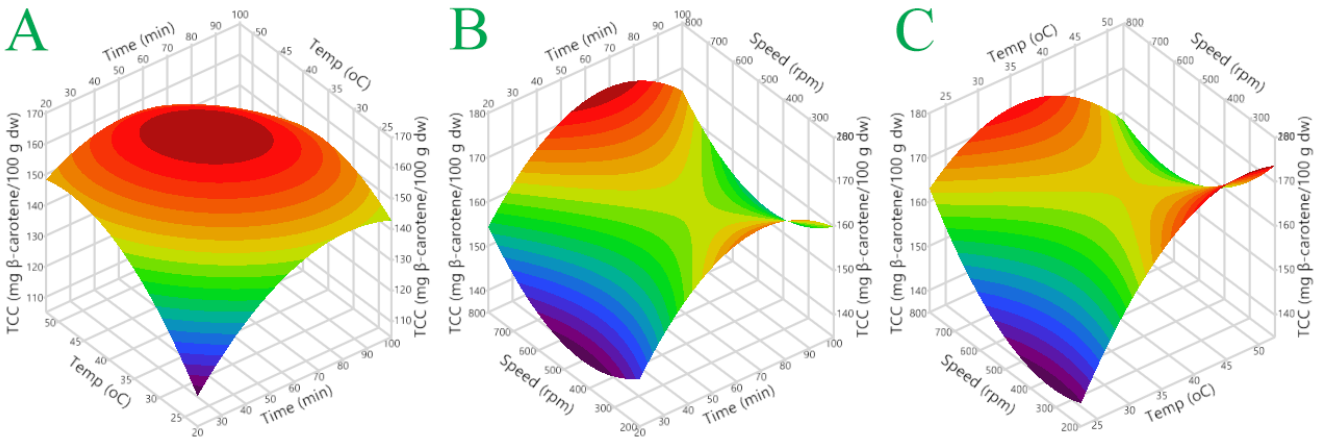

Figure 6. For the extraction of APW using *n*-hexane: acetone: ethanol (2:1:1), 3D graphs showing the impact of the process variables taken into consideration on the response. Plots (**A**,**B**) show the covariation of X_1 (*t*) and X_2 (*T*), X_1 (*t*) and X_3 (*S*), and (**C**) the covariation of X_2 (*T*) and X_3 (*S*).

In the study of Lima et al. [47] the supercritical fluid extraction of carotenoids from various vegetable waste matrices was discussed. The authors achieved an extraction of ~132.7 and ~212.4 mg β-carotene/100 g dw from apricot flesh and apricot peels, respectively. Similarly, Sanal et al. [48] achieved an extraction yield of 100.4 mg β-carotene/100 g dw from apricot pomace. Finally, in the study of Koutsoukos et al. [31], the extraction of β-carotene from apricot pulp was described. The authors examined the use of ultrasound-assisted extraction and microwave-assisted extraction for the extraction of β-carotene and it was found that the extracts obtained in the two cases contained 11.51 and 26.5 mg β-carotene/100 g dw, respectively. When a DES comprised of choline chloride and tartaric acid was used as the extraction solvent, the content of the extracts in β-carotene was 41.3 and 76.11 mg β-carotene/100 g dw, respectively. From the abovementioned studies, it is evident that the optimization of the extraction parameters employed herein can achieve comparable, or even better extraction of β-carotene from APW, without the need for additional techniques, as pretreatment steps.

4. Conclusions

In this study, the extraction of antioxidant polyphenols and carotenoid pigments from APW was described. In order to maximize the extraction yield, optimization of the extraction parameters was carried out. In the case of polyphenols, the use of DES-2 (glycerol: citric acid: L-proline, 2:1:1) proved to be superior, not only to commonly employed organic solvents and water but the obtained results were also found to be better compared to previously reported methods. This was also the case with carotenoids, which were found to be extracted at a higher degree when a *n*-hexane: acetone: ethanol (2:1:1) mixture was used. Based on the above, it was validated that APW is a rich source of bioactive compounds that can be retrieved, so as to be used for other purposes. Moreover, the proposed methods can substitute, or serve as an alternative, to commonly employed methods, so as to simplify and reduce the cost, while also maximizing the extraction yield.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomass2040022/s1, Figure S1: Plot comparing actual vs. predicted values for the response (TPC, mg GAE/g dw) (plot A) and desirability function (plot B) for the DES-2-optimized extraction of APW polyphenols; Statistics for the evaluation of the model that was developed are provided in the inset tables; Asterisks and colored values indicate statistically significant values; Figure S2: Plot comparing actual vs. predicted values for the response (TCC, mg β-carotene/100 g dw) (plot A) and desirability function (plot B) for the *n*-hexane: acetone: ethanol (2:1:1)-optimized extraction of APW carotenoids; Statistics for the evaluation of the model that was developed are provided in the inset tables; Asterisks and colored values indicate statistically significant values.

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