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Response Surface Optimization of Inulin and Polyphenol Extraction from Artichoke (*Cynara scolymus* (L.)) Solid Wastes

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Featured Application: Recovery of bioactive compounds from artichoke solid wastes.

Abstract: Artichoke wastes after processing represent 60–70% of the raw material and are a potential source of inulin and polyphenols, bioactive compounds that can be valorized as food ingredients or nutraceutical products. The aim of this work was to assess and optimize the extraction of these compounds from artichoke wastes using water or water–ethanol mixtures as extracting agents. For simultaneous inulin and polyphenol extraction and to achieve high antioxidant activity in extracts, the best process conditions using water as an extracting agent were T = 89 °C and t = 139 min, where 80% of the inulin content, 60% of the total phenolic content (TPC) and 56% of the antioxidant activity (Aox) were obtained. For water–ethanol extractions, the best results were obtained with EtOH = 22.4%, T = 81 °C and t = 217 min, leading to extraction yields of 90% of TPC, 38% of Aox and 58% of inulin content. From these results, we recommend the use of water for the recovery of inulin and polyphenols from artichoke wastes. Although the extraction yield of polyphenols is lower in water treatments, the amount extracted is considerable and it is a greener option when compared with water–ethanol mixtures.

Keywords: bioactive compounds; green extraction; by-products; phenolic compounds; antioxidant activity; food waste valorization

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1. Introduction

Globe artichoke (*Cynara cardunculus* var. *scolymus*) is an herbaceous plant taxonomically included in a small genus [1] of the family *Asteraceae* [2,3]. *Cynara* species are originally from the Mediterranean region [4], and three Mediterranean countries (Italy, 367 kTon; Egypt 309 kTon and Spain, 197 kTon) generate over 50% of the world's production [5]. The production and intake of globe artichoke is increasing in South America [6]; in this sense, nowadays, almost 15% of the total world's production is concentrated in Perú and Argentina [5].

Globe artichoke presents a large capitula or head (immature flower) with edible bracts and receptacle, which represents 30–40% of the weight of the whole plant [4]. During artichoke processing to produce canned or frozen artichokes, non-edible parts with non-commercial use have traditionally been used as livestock feed [7].

Globe artichoke is a rich source of bio-compounds, mainly inulin and phenolic compounds [4]. Artichoke plants accumulate inulin as major reserve of carbohydrates, reaching up to 15% of the flowering part [2,4]. Inulin is a polysaccharide consisting of a chain of β(2-1) linear fructose molecules with a terminal glucose molecule [2,8] and shows water solubility strongly dependent on temperature [8]. Inulin improves health since its

consumption stimulates the growth and activity of desired bacteria in the colon; it has positive effects on blood glucose attenuation, lipid homeostasis, mineral bioavailability and immunomodulation [1,9]. It has been found that inulin can replace sugar and fat in food products, with the advantage of exhibiting low caloric value [4,10]. Additionally, inulin shows characteristic rheological properties: it increases viscosity, provides spreadability and improves texture [11,12]. Hence, due to its health benefits and rheological properties, inulin can be used in the formulation of different foods, such as yogurts, salad dressings, mousses, chocolates and bread [10].

The extraction of inulin from solid matrices is favored by the use of water as an extracting solvent, and different temperatures and extracting times have been studied [11–14].

Phenolic compounds are secondary metabolites that protect the plant against biotic and abiotic stress [15,16]. The phenolic compounds included in the globe artichoke are mainly chlorogenic acid, cynarin, apigenin-7-O-glucoside, luteolin, cynaroside, scolymoside, phenolic acids, mono- and dicaffeoylquinic acids and flavonoids [6,17–19]. The consumption of foods rich in polyphenols has proven health benefits, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [16,20].

For the solid–liquid extraction of polyphenols, several organic solvents and their mixtures with water have been used [21,22]. Due to the diverse chemical structures of polyphenolics, ranging from simple and free to conjugated and polymerized forms (lipophilic), which might consequently affect their solubility behavior, Sulaiman and coauthors stated that there is no specific or appropriate solvent recommended for the optimal extraction and recovery of total phenolic content from fresh sample matrices [23]. In spite of this, alcoholic solvents have been commonly employed to extract phenolics from natural sources: they give quite high yields of total extracts even though they are not highly selective for phenols. In particular, mixtures of alcohols and water have been revealed to be more efficient in extracting phenolic constituents than the corresponding mono-component solvent system [24].

The simultaneous extraction of inulin and polyphenols from artichoke wastes will lead to different results depending on the extracting agent. In this sense, it is expected that the use of water will lead to high inulin extraction yields but also some polyphenols in the extract; on the other hand, the use of water–ethanol mixtures will lead to high polyphenol extraction rates and moderate inulin extraction. Soto-Maldonado et al. [25] studied the co-extraction by maceration of these compounds at fixed operative conditions (T = 40 °C; ethanol:water = 75:25) from artichoke discards. They found that lyophilized samples gave better extraction results in terms of both inulin and phenolic content.

The aim of this work is to study and optimize the solid–liquid extraction of inulin and polyphenols from artichoke solid wastes, by using water or ethanol–water mixtures as extracting agents.

2. Materials and Methods

2.1. Raw Materials

Globe artichokes were purchased from a local supermarket in Valencia, Spain. Only pieces in good shape were selected for the experiments. Artichoke buds were cut into their different parts (stem, inner bracts, external bracts and heart) as follows: (i) stems were cut at 2 cm length from the bud base, and the rest was rejected; the stem still joined to the bud was then cut and kept apart; (ii) then, the bud was cut into halves and external bracts were separated by hand under color criteria; (iii) the hearts were separated from the halves. These four parts—stem, external bracts, inner bracts and heart—were packed separately under vacuum (Tecnotrip mod. EV-25-L-G-CD-SG 2006) and stored at −20 °C until use.

When necessary, packets were taken from the freezer and naturally defreezed. A part of the defreezed material was dehydrated in a vacuum oven (SELECTA, mod. Vacioterm-T) at 60 °C and −0.95 bar until constant weight was reached. Wet and dried samples were separately ground (KRUPS, mod. GVX 242) and sieved with a 1 mm sieve. Particles larger

than 1 mm were refused. Then, wet and dried samples were separately stored in hermetic flasks and kept at 4–5 °C until their use.

2.2. Compositional Analysis

In order to assess the extraction yields, compositional analysis was performed for dried samples. As commented by other authors, vegetable residues suffer quick decomposition in an uncontrolled manner during their storage prior to their use. Hence, almost any recycling process should start with drying, size reduction and fractionation in such a way that the process creates a product with reproducible conditions [26].

The weight distribution of the four artichoke parts (stem, inner bracts, external bracts and heart) was determined, and, according to these data, a reconstituted solid waste (RSW) sample was prepared, containing stem and external bracts. The compositional analysis was performed in terms of moisture, inulin and phenolic content, as well as antioxidant activity. All measurements were conducted in triplicate.

All chemicals used in this work were purchased from Sigma-Aldrich (Sigma-Aldrich, Madrid, Spain).

2.2.1. Moisture

Moisture content was determined by introducing samples into a vacuum oven at 60 °C and −0.95 bar until constant weight.

2.2.2. Inulin Content

Inulin content (mg glucose eq./g sample) was calculated by using the equation (Equation (1)) proposed by Lou et al., 2009 [27], based on the previous determination of total carbohydrates and reducing sugars.

$$\text{Inulin} = \text{Total carbohydrate} - \text{Reducing sugars} \quad (1)$$

For the determination of total carbohydrates [28], 100 mL of water with a pH between 6.5 and 8.0 was added to 1 g of sample. The mixture was then stirred at 85 °C for 1 h, and evaporated water was replaced when necessary. After the extraction, the mixture was left to stand till room temperature and then was vacuum-filtered, obtaining a liquid extract. Then, 1 mL of this extract was introduced into a test tube and 1 mL of a 5% aqueous phenol solution and 5 mL of concentrated sulfuric acid were added, and the mixture was well mixed using a vortex. The homogenized mixture was left stand for 10 min. Then, the test tube was stirred again and introduced into a thermostatic bath at 30 °C for 20 min. Finally, the carbohydrate content was measured at 490 nm. Results were expressed in mg of glucose/mL.

For the reducing sugars analysis [29], 3 mL of the liquid extract was introduced into a test tube and 3 mL of 1% DNS reagent (10 g of 3,5-dinitrosalicilic acid, 2 g of phenol, 0.5 g of sodium sulfite and 10 g of sodium hydroxide dissolved in 1 L of water) was added. The test tube was covered and introduced into a thermostatic bath at 90 °C for 15 min. Then, 1 mL of Rochelle salt (40% aqueous solution) was added and was left to stand until room temperature. Finally, the reducing sugars content was measured by spectrophotometry at 575 nm. Results were expressed in mg of glucose/mL and then converted into mg glucose equivalent/g sample (d.b.).

2.2.3. Total Polyphenol Content (TPC)

According to the method described by Jimenez-Escrig et al. (2003) [30], 1 g of sample was ground and mixed with 40 mL of methanol/water solution 1:1 (v/v) at pH = 2. The mixture was then stirred at room temperature for 1 h. Afterwards, the mixture was filtered using a vacuum pump (Rocker mod. 400). The extracted liquid was kept apart while the solid was extracted again at room temperature for 1 h with a methanol/acetone solution (70:30 (v/v)) and it was then vacuum-filtered. Filtered liquids from the first and second extractions were mixed and used for the spectrophotometric determination.

Total polyphenol analysis was carried out using the Folin–Ciocalteu method [31]. This method consisted of the addition of 0.5 mL of the extract to 2 mL of an aqueous solution of sodium carbonate (7.5%) and 2.5 mL of Folin–Ciocalteu reactant previously diluted 10-fold. The mixture was stirred in a vortex, and it was left to stand for 15 min at room temperature. Afterwards, the absorbance at 765 nm was measured. Since a previous calibration curve was generated using solutions of known concentrations of gallic acid as standard, results were expressed initially in terms of mg of gallic acid equivalents/mL (mg GAE/mL) and then converted into mg GAE/g sample.

2.2.4. Antioxidant Activity (Aox)

The ability of samples to scavenge DPPH (1,1-diphenil-2-picrilhydracil radical) was used to determine their antioxidant activity [32,33]. In test tubes, 0.2 mL of the extract was added to 3.8 mL of a solution of DPPH in methanol 60 µM. The antioxidant activity was determined as the inhibition percentage of the DPPH due to the antioxidant compounds present in the sample, measuring the absorbance at 515 nm from t = 0 min to t = 30 min of reaction. The inhibition percentage was calculated using Equation (2).

$$\%\Delta Abs_{515} = \frac{Abs_{515(0)} - Abs_{515(30)}}{Abs_{515(0)}} \cdot 100 \quad (2)$$

A previous calibration curve was generated using standard Trolox solutions. Thus, results of antioxidant activity were expressed as equivalents of mM of Trolox/g sample.

2.3. Extraction of Inulin and Polyphenols from the Reconstituted Solid Waste (RSW)

Two independent experimental sets were performed following the response surface methodology (RSM). In the first one, the main objective was to optimize inulin recovery using water as a solvent from RSM, whereas in the second set, the experimental conditions were selected so as to maximize the extraction of polyphenols by the use of mixtures of ethanol and water as an extracting solvent.

2.3.1. Water Extraction and Optimization: Experimental Design

The aim of this study was to determine the extraction conditions (temperature and time) to reach the maximum content of inulin. According to the literature, inulin presents high water solubility depending on the temperature [8], and therefore, for inulin extraction, water was used as an extracting agent. A two-factor and two-level central composite design consisting of 13 experimental runs with five replications at the central point (Table 1) was used. The ranges for temperature and time were 60–90 °C and 30–120 min, respectively. Solid:liquid ratio of 1:100 (w/v) and stirring at 300 rpm were kept constant for all the experiments. Temperature and time were selected on the basis of Lou et al. (2009) [27], who used water as an extracting solvent, a temperature of 85 °C and a time of 1 h as a method to determine inulin content in foods.

After each extraction, the mixture was vacuum-filtered and the extracts were kept apart and stored at −20 °C for later analysis. All extracts were analyzed in terms of inulin content, total polyphenol content and antioxidant activity. Analyses were performed in triplicate and results were expressed as averages with their confidence intervals.

2.3.2. Ethanol–Water Extraction and Optimization: Experimental Design

In this set of experiments, the purpose was to determine the best conditions that favor the extraction of polyphenols from the RSW. A three-factor (ethanol concentration, temperature and time) and two-level central composite design consisting of 19 experimental runs with five replications at the central point (Table 2) was used. The ranges for ethanol concentration (w/w), temperature and time were 20–80%, 38.2–76.8 °C and 97–293 min, respectively. All extractions were carried out with a solid:liquid ratio of 1:100 (w/v). The stirring was kept constant at 300 rpm for all the experiments. For ethanol–water extraction,

temperature and time ranges were selected based on previous studies on polyphenol extraction in grapefruit solid wastes [34].

Table 1. Experimental design for water extraction.

Run No.	Variables		Coded Levels	
	Temperature (°C)	Time (min)	X_1	X_2
1	75	75	0	0
2	60	30	−1	−1
3	90	30	+1	−1
4	54	75	−1.414	0
5	75	11	0	−1.414
6	75	75	0	0
7	60	120	−1	+1
8	75	75	0	0
9	96	75	+1.414	0
10	90	120	+1	+1
11	75	75	0	0
12	75	139	0	+1.414
13	75	75	0	0

Table 2. Experimental design for ethanol–water extraction.

Run No.	Variables			Coded Levels		
	Ethanol Concentration (%) (*w*/*w*)	Temperature (°C)	Time (min)	X_1	X_2	X_3
1	50.1	57.5	195.0	0	0	0
2	50.1	57.5	195.0	0	0	0
3	20.4	38.2	97.0	−1	−1	−1
4	50.1	57.5	360.0	0	0	+1.682
5	50.1	57.5	195.0	0	0	0
6	50.1	57.5	195.0	0	0	0
7	20.4	76.8	97.0	−1	+1	−1
8	79.8	38.2	293.1	+1	−1	+1
9	50.1	57.5	195.0	0	0	0
10	0.2	57.5	195.0	−1.682	0	0
11	50.1	90.0	195.0	0	+1.682	0
12	79.8	38.2	97.0	+1	−1	−1
13	50.1	25.0	195.0	0	−1.682	0
14	79.8	76.8	96.9	+1	+1	−1
15	20.4	76.8	293.1	−1	+1	+1
16	20.4	38.2	293.1	−1	−1	+1
17	100.0	57.5	195.0	+1.682	0	0
18	50.1	57.5	30.0	0	0	−1.682
19	79.8	76.8	293.1	+1	+1	+1

After each extraction, the mixture was vacuum-filtered, and the liquid extracts were kept apart and stored at −20 °C for later analysis of inulin, polyphenol content and antioxidant activity. Analyses were carried out in triplicate and results were expressed as averages with their confidence intervals.

2.4. Statistical Analysis

Inulin content, polyphenol content and antioxidant activity were fitted to a second-order polynomic equation (Equation (3)). This equation considers lineal and quadratic effects as well as interaction effects among the experimental factors studied.

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

where Y is the studied response and β_0, β_i, β_{ii} and β_{ij} are the independent, lineal, quadratic and interaction coefficients, respectively.

The non-linear fitting as well as the ANOVA analysis of Equation (3) coefficients were performed by using the software STATGRAPHICS Centurion XVI (Statpoint Technologies Inc., Warrenton, VA, USA). The goodness of fit was evaluated in terms of the squared regression index, r^2, for a significance level of $p \leq 0.05$. The optimum values from the response surfaces were obtained using a numeric optimization pack in the same software.

Two multiple-response optimizations were carried out for both inulin and phenolic experimental designs. Multiple response optimization allows one to find a compromise situation in order to simultaneously optimize all considered responses. To determine the goodness of this multiple response optimization, the desirability function D was used, with values ranging between 0 and 1, being D = 1 the most desirable situation [35].

3. Results and Discussion

3.1. Compositional Analysis

Table 3 shows compositional data and weight distributions for different parts (heart, inner bracts, external bracts and stem) considered of the vacuum dried artichoke.

Table 3. Compositional data of different artichoke parts.

	Heart	Inner Bracts	External Bracts	Stem
Weight distribution (%) (w.b.) [1]	12.8 ± 3.0	44.2 ± 7.5	36.9 ± 10.7	6.1 ± 2.8
Moisture (%) (w.b.) [1]	86.1 ± 0.62	88.7 ± 0.64	83.7 ± 0.99	88.3 ± 0.56
Inulin (mg eq glucose/g artichoke) (d.b.) [2]	158.8 ± 24.4	73.8 ± 5.3	45.4 ± 8.7	21.6 ± 5.4
Polyphenols (mg GAE/g artichoke) (d.b.) [2]	25.3 ± 0.2	32.6 ± 5.0	17.8 ± 0.2	44.8 ± 1.6
Aox (mM trolox/g artichoke) (d.b.) [2]	172.2 ± 86.1	213.8 ± 77.1	81.5 ± 9.7	213.1 ± 62.3

[1], Wet basis; [2], Dry basis.

Solid wastes (stem and external bracts) represent around 43% of the total weight (w.b.). These results are different to data provided by other authors (70–80% (w.b.)) [1,10], likely because, at industrial scale, the term "solid waste" includes the stem and external bracts of the capitula itself (our data) but also those artichokes that do not satisfy the quality specifications and leaves.

The highest content of inulin was present in the heart part, decreasing as the portion became more external. These results are similar to those obtained by Lattanzio et al., who studied the inulin content in the edible portion (heart and inner bracts) of artichoke for different varieties and found that the inulin content was within the range of 189–362 mg eq glu/g (d.b.) [4].

Regarding the TPC content, the trend found was stem > inner bracts > heart > external bracts. Fratianni et al. evaluated the polyphenol content in different parts and varieties of globe artichokes and found that the phenolic content distribution did not follow a common pattern and was dependent on the variety studied [3]. Pandino et al. studied the phenolic

profile of globe artichoke and found that the heart and inner bracts presented similar phenolic content and 2.5-fold higher values of TPC than external bracts [16].

The assessment of the inulin and total polyphenol extraction and optimization has been performed on RSW.

3.2. Water Extraction Experiments and Response Surface Analysis

3.2.1. Water Extraction

In Table 4 are listed the results obtained for the inulin extraction experiments from the dried RSW.

Table 4. Results of water extraction experiments from dried RSW.

Run No.	Inulin Content	Total Polyphenol Content, TPC	Antioxidant Activity, Aox
	mg glu eq/g (d.b.)	mg GAE/g (d.b.)	mM trolox/g (d.b.)
1	41.89 ± 5.50	10.89 ± 0.13	37.84 ± 2.83
2	11.03 ± 1.47	13.18 ± 0.97	48.33 ± 0.89
3	19.13 ± 5.03	12.87 ± 0.32	45.14 ± 0.88
4	1.60 ± 0.35	10.17 ± 0.23	39.17 ± 1.56
5	13.74 ± 4.12	12.49 ± 0.35	48.56 ± 1.53
6	42.99 ± 5.37	10.22 ± 0.23	37.31 ± 1.08
7	23.88 ± 3.32	9.50 ± 0.23	39.55 ± 1.80
8	42.00 ± 3.51	10.30 ± 0.23	37.97 ± 1.07
9	24.19 ± 4.90	12.03 ± 0.81	47.06 ± 1.54
10	39.44 ± 5.25	11.16 ± 0.40	47.30 ± 2.39
11	52.00 ± 8.31	10.27 ± 0.26	39.24 ± 2.10
12	37.69 ± 5.31	11.66 ± 0.67	46.54 ± 2.40
13	44.72 ± 3.20	11.00 ± 0.13	49.04 ± 0.90

It is observed that the maximum inulin content was obtained at the experimental conditions of the center point (75 °C and 75 min; runs No. 1, 6, 8, 11 and 13), with an average value of 44.72 mg glu eq/g artichoke (d.b.). On the contrary, the lowest inulin content was obtained at the lowest temperature, 54 °C. For TPC and Aox, average values for the central points were 10.54 mg GAE/g artichoke (d.b.) and 40.28 mM trolox/g artichoke (d.b.), respectively. Maximum values of Aox are associated with the maximum TPC content. Table 5 shows the ANOVA results considering the quadratic model indicated in Equation (3).

For inulin content, it is observed that for a confidence level of 95%, the only non-significant term is the combined term for time and temperature (Figure 1a). The response surface based on the quadratic model (Equation (4)) is shown in Figure 1b. This trend for inulin extraction is agreement with the results obtained by Redondo-Cuenca et al. [36], who reported that, initially, an increase in the extraction temperature facilitates the recovery of carbohydrates, but that overly high temperatures lead to a reduction in the extraction yield, likely due the hydrolysis of inulin [37]. This fact may explain the negative quadratic effect of temperature (Equation (4)). The optimum value of inulin content is 47.8 mg glu eq/g artichoke (d.b.) for the experimental conditions of 98.8 min and 79 °C. Fit results of the quadratic model are shown in Equation (4), which explains 96% of the total variability (r^2 = 96%; r^2(adj) = 93%).

$$\text{Inulin content (mg gluc eq/g (d.b.))} = -389.849 + 10.34 \cdot T + 0.5971 \cdot t - 0.0672 \cdot T^2 + 0.0028 \cdot T \cdot t - 0.0041 \cdot t^2 \quad (4)$$

Table 5. ANOVA results for the water extraction experiments.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*-Value *
		Inulin content			
A:T (°C)	386.052	1	386.052	23.07	0.0020
B:t (min)	561.996	1	561.996	33.58	0.0007
AA	1542.62	1	1542.62	92.17	0.0000
AB	13.9128	1	13.9128	0.83	0.3922
BB	494.137	1	494.137	29.52	0.0010
Residual	117.162	7	16.7374		
Total	2922.23	12			
		Total polyphenol content			
A:T (°C)	1.97400	1	1.97400	4.55	0.0704
B:t (min)	5.36587	1	5.36587	12.37	0.0098
AA	0.65622	1	0.65622	1.51	0.2585
AB	0.97023	1	0.97023	2.24	0.1785
BB	4.34977	1	4.34977	10.03	0.0158
Residual	3.03712	7	0.43387		
Total	15.996	12			
		Antioxidant activity			
A:T (°C)	30.7509	1	30.7509	2.04	0.1961
B:t (min)	11.2008	1	11.2008	0.74	0.4170
AA	12.9178	1	12.9178	0.86	0.3852
AB	29.9209	1	29.9209	1.99	0.2015
BB	88.5585	1	88.5585	5.88	0.0458
Residual	105.414	7	15.0592		
Total	271.639	12			

* Terms showing a *p*-value lower than 0.05 (in red) are significant. Terms with a *p*-value higher than 0.05 (in black) are non-significant.

For the total polyphenol content, the only significant terms are the lineal and quadratic time coefficients ($p < 0.95$) (Table 5 and Figure 1c). The related response surface is shown in Figure 1d. It has been found that the optimized TPC in the studied ranges of time and temperature would be 14.18 mg acid gallic eq/g artichoke (d.b.) at 11 min and 54 °C. Fitted results are shown in Equation (5) and explain 81% of the total variability (r^2 = 81%; r^2(adj) = 67.5%).

$$\text{TPC (mg ac.gallic eq/g(d.b.))} = 23.2465 - 0.2236 \cdot T - 0.1301 \cdot t + 0.0013 \cdot T^2 + 0.0007 \cdot T \cdot t + 0.0004 \cdot t^2 \quad (5)$$

The response surface for the antioxidant activity is shown in Figure 1f. The optimized Aox would be 54.5 mM trolox/g artichoke (d.b.) for the experimental conditions of 11 min and 54 °C. Table 5 and Figure 1e show that only the quadratic factor of time is significant ($p < 0.95$). Fit results are shown in Equation (6), and the model explains 61% of the total variability (r^2 = 61%; r^2(adj) = 34%)

$$\text{Aox (mM trolox/g(db))} = 128.244 - 1.8373 \cdot T - 0.6719 \cdot t + 0.011 \cdot T^2 + 0.0041 \cdot T \cdot t + 0.0023 \cdot t^2 \quad (6)$$

3.2.2. Optimization for the Water Extraction

The main goal of the experiments described in Section 3.2.1 was to determine the best process conditions for inulin extraction, although it was proven that polyphenols were also extracted using water as an extracting solvent. Then, it was possible to perform a multiple-response optimization, in order to find the experimental conditions that maximize the extraction of inulin and polyphenols with the highest antioxidant activity in the extracts.

In this sense, it was found that T = 89 °C and t = 139 min give a desirability factor of 0.805 and result in expected values of inulin content = 34.47 mg glu eq/g (d.b.); TPC = 12.45 mg GAE/g (d.b.) and Aox = 52.89 mM trolox/g (d.b.). These values would

represent 80% of the inulin content, 60% of the TPC and 56% of the Aox, when compared to those obtained in the compositional analysis.

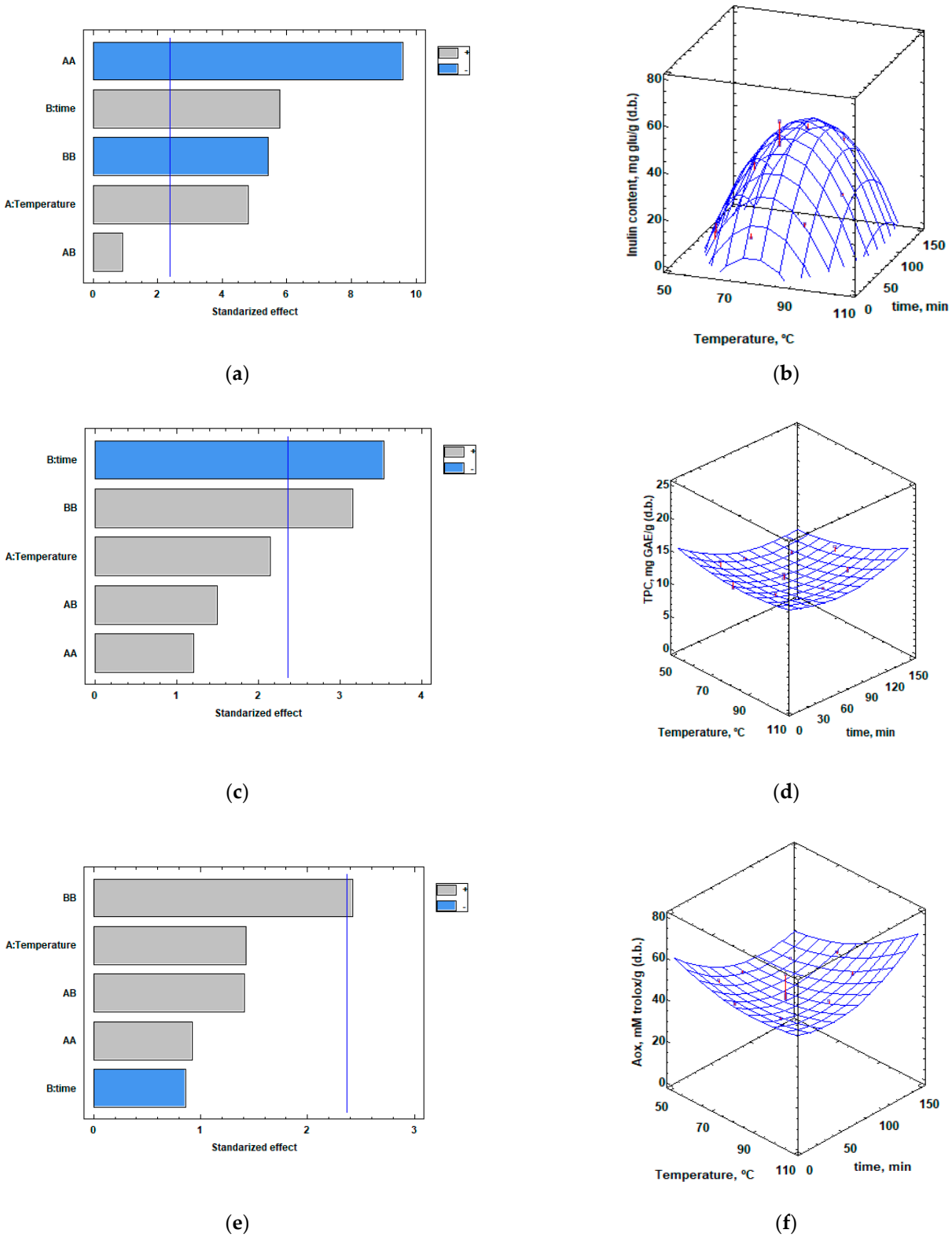

Figure 1. Pareto and surface plots for the water extraction experiments: (**a**,**b**) inulin content; (**c**,**d**) polyphenol content; (**e**,**f**) antioxidant activity.

3.3. Ethanol–Water Extraction and Optimization

3.3.1. Ethanol–Water Extraction

Results of the ethanol–water extraction experiments are shown in Table 6.

Table 6. Results of ethanol–water extractions from dried RSW.

Run No.	Total Polyphenol Content, TPC	Antioxidant Activity, Aox	Inulin Content
	mg GAE/g (d.b.)	mM trolox/g (d.b.)	mg glu eq/g (d.b.)
1	11.82 ± 0.02	36.21 ± 1.70	8.65 ± 1.65
2	12.51 ± 1.09	38.83 ± 0.85	4.59 ± 0.76
3	12.89 ± 0.25	36.57 ± 1.72	2.89 ± 0.69
4	11.94 ± 0.87	37.33 ± 1.63	10.10 ± 2.32
5	12.60 ± 0.01	39.78 ± 2.20	0.93 ± 0.09
6	13.02 ± 0.98	39.78 ± 2.20	3.89 ± 1.01
7	14.82 ± 0.25	28.06 ± 3.06	29.47 ± 4.37
8	8.57 ± 0.74	10.97 ± 1.44	1.42 ± 0.28
9	13.28 ± 0.66	35.04 ± 4.25	7.49 ± 1.55
10	14.82 ± 0.90	37.37 ± 5.10	7.07 ± 1.62
11	18.83 ± 0.43	21.74 ± 0.84	20.76 ± 4.93
12	5.99 ± 0.42	11.71 ± 2.47	5.19 ± 0.91
13	10.43 ± 0.86	34.12 ± 1.68	15.34 ± 3.12
14	9.15 ± 0.49	13.83 ± 2.2.	9.04 ± 1.54
15	19.27 ± 0.43	25.74 ± 2.25	22.41 ± 4.98
16	14.80 ± 0.24	39.95 ± 0.82	4.65 ± 0.81
17	3.28 ± 0.01	9.55 ± 0.83	4.23 ± 0.85
18	10.86 ± 0.50	34.99 ± 3.03	5.25 ± 1.12
19	10.54 ± 0.75	19.63 ± 3.40	3.33 ± 0.74

It was found that for the five replicates of the center point (runs 1, 2, 5, 6 and 9), the polyphenol content and antioxidant activity in the ethanol–water extracts showed low dispersion: 12.64 ± 0.53 mg GAE/g (d.b.) and 37.93 ± 2.07 mM trolox/g (d.b.), respectively. On the contrary, the inulin content showed higher variability (5.11 ± 2.92 mg glu eq/g (d.b.)), probably due to the presence of ethanol in the extraction media.

These results show that TPC in ethanol–water extracts was slightly lower than values obtained for water extracts. However, there was a significant difference in terms of inulin content in both types of extracts, in such a way that the presence of ethanol in the extracting media significantly reduced the inulin extraction.

According to the ANOVA shown in Table 7, it was found for TPC that all single factors were significant with a 95% confidence level. The response surface for the TPC is shown in Figure 2a.

For a confidence level of 95%, quadratic effects of ethanol concentration and temperature are significant for TPC (Figure 2a). In view of the response surface shown in Figure 2b, TPC in the extracts decreases with ethanol concentration and increases with temperature. This tendency is in agreement with results obtained by other authors that used mixtures of ethanol–water for polyphenol extraction, so that the ethanol's presence promotes the higher extraction of polyphenols; however, from a maximum ethanol concentration, dependent on the food matrix, the extraction yields decrease [38–40].

The optimum value of TPC would be 22.64 mg GAE/g artichoke (d.b.) for the experimental conditions with an ethanol concentration of 2.0% (*w*/*w*), 360 min and 90.0 °C. The response surface in Equation (7) explains 96.1% of the total variability (r^2 = 96.1%; r^2(adj) = 92.3%).

$$\begin{aligned}\text{TPC}\ (\text{mg ac.gallic eq/g(d.b.)}) &= 12.351 + 0.0558\cdot\text{EtOH} - 0.1466\cdot\text{T} + 0.0237\cdot\text{t} - 0.0014\cdot\text{EtOH}^2 - 0.0003\cdot\text{EtOH}\cdot\text{T} - 0.0001 \\ &\cdot\text{EtOH}\cdot\text{t} + 0.0021\cdot\text{T}^2 + 0.0001\cdot\text{T}\cdot\text{t} - 0.00004\cdot\text{t}^2\end{aligned} \quad (7)$$

In Figure 2d is shown the response surface for the antioxidant activity. It has been found that the optimized Aox would be 43.08 mM trolox/g artichoke (d.b.) for the experimental conditions with a 22.5% ethanol concentration, 200 min and 44 °C. Table 7 and Figure 2c show that the quadratic effects of EtOH concentration and temperature, and their combination, are significant ($p < 0.95$). The experimental data were fitted to Equation (8) and explained 93% of the total variability (r^2 = 93.2%; r^2(adj) = 86.4%).

$$\begin{aligned}\text{Aox (mM trolox/g(db))} &= 15.9372 - 0.0398\cdot\text{EtOH} + 0.9376\cdot\text{T} + 0.0672\cdot\text{t} - 0.0071\cdot\text{EtOH}^2 + 0.0073\cdot\text{EtOH}\cdot\text{T} + 0.0002 \\ &\cdot\text{EtOH}\cdot\text{t} - 0.0125\cdot\text{T}^2 + 0.00006\cdot\text{T}\cdot\text{t} - 0.00018\cdot\text{t}^2\end{aligned} \quad (8)$$

Table 7. ANOVA results for the ethanol–water extraction experiments.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*-Value *
Total polyphenol content					
A: EtOH (%)	0.228	1	0.228	0.20	0.6651
B: T (°C)	2.632	1	2.632	2.32	0.1624
C: t (min)	5.579	1	5.579	4.91	0.0540
AA	19.405	1	19.405	17.07	0.0026
AB	0.201	1	0.201	0.18	0.6839
AC	0.715	1	0.715	0.63	0.4481
BB	8.308	1	8.308	7.31	0.0242
BC	0.227	1	0.227	0.20	0.6656
CC	1.780	1	1.780	1.57	0.2424
Residual	10.29	9	1.137		
Total	265.209	18			
Antioxidant activity					
A: EtOH (%)	49.63	1	49.63	3.02	0.1164
B: T (°C)	39.72	1	39.72	2.41	0.1546
C: t (min)	25.81	1	25.81	1.57	0.2419
AA	534.76	1	534.76	32.51	0.0003
AB	140.32	1	140.32	8.53	0.0170
AC	2.00	1	2.00	0.12	0.7355
BB	297.64	1	297.64	18.09	0.0021
BC	0.09	1	0.09	0.01	0.9431
CC	42.46	1	42.46	2.58	0.1426
Residual	148.06	9	16.45		
Total	2180.73	18			
Inulin content					
A: EtOH (%)	0.48	1	0.48	0.02	0.8895
B: T (°C)	154.31	1	154.31	6.53	0.0309
C: t (min)	1.43	1	1.43	0.06	0.8114
AA	0.004	1	0.004	0.00	0.9902
AB	186.09	1	186.09	7.87	0.0205
AC	2.19	1	2.19	0.09	0.7678
BB	260.22	1	260.22	11.01	0.0090
BC	14.45	1	14.45	0.61	0.4545
CC	6.67	1	6.67	0.28	0.6081
Residual	212.76	9	23.64		
Total	1091.58	18			

* Terms showing a *p*-value lower than 0.05 (in red) are significant. Terms with a *p*-value higher than 0.05 (in black) are non-significant.

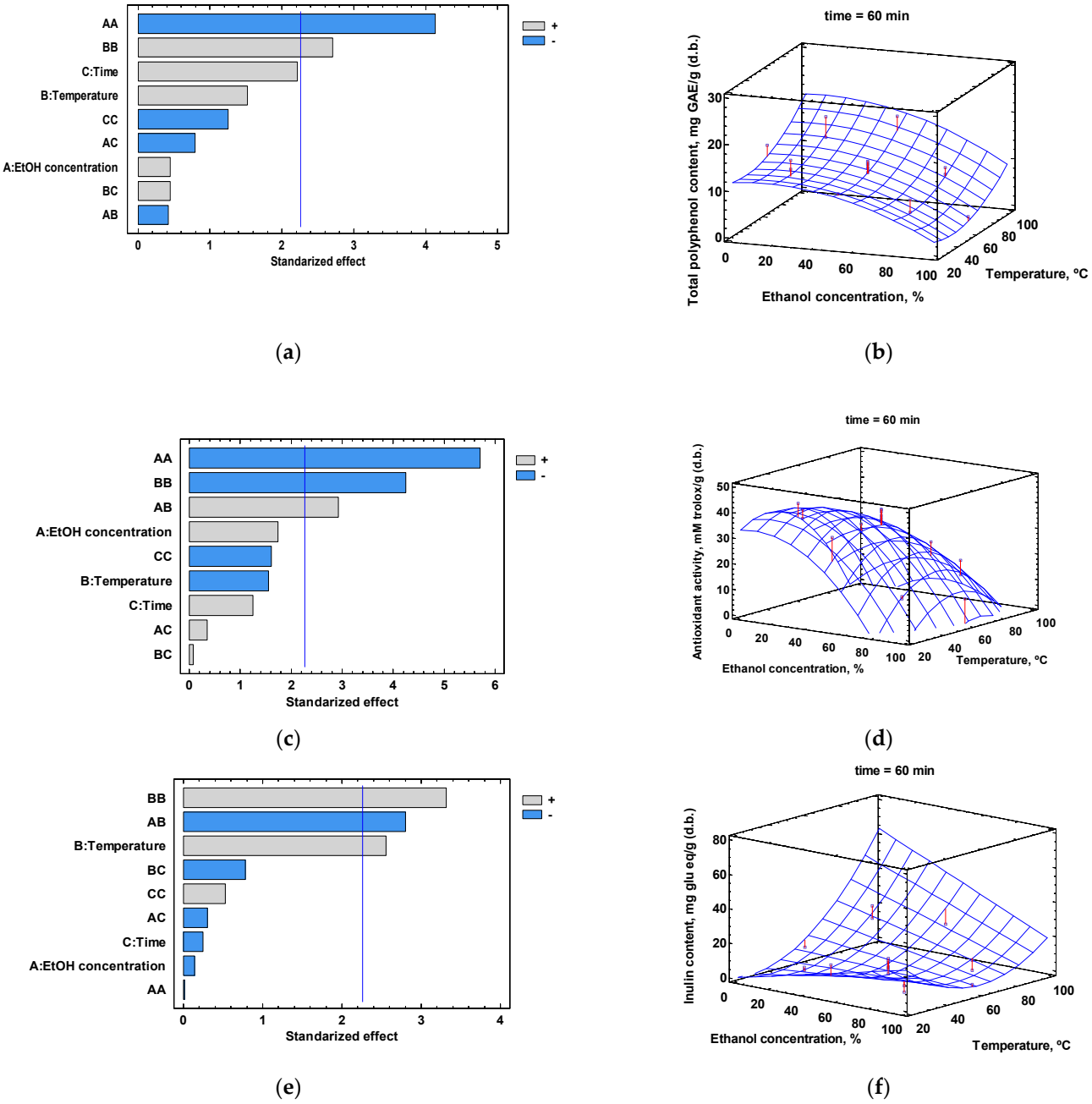

Figure 2. Pareto and surface plots for the ethanol–water extractions: (**a**,**b**) polyphenol content; (**c**,**d**) antioxidant activity; (**e**,**f**) inulin content.

Regarding the inulin content in ethanol–water extracts, linear and quadratic effects for temperature and the mixed effect with EtOH concentration–temperature were shown for a confidence level of 95%, significant regarding the inulin content (Figure 2e). Equation (9) explains 81% of the total variability (r^2 = 80.5%; r^2(adj) = 61.0%) The response surface is shown in Figure 2f. The expected optimum value of inulin content in the studied range of time and temperature is 49.06 mg glu eq/g artichoke (d.b.) for the experimental conditions with a 0.2% ethanol concentration, 90 °C and 30 min.

$$\text{Inulin content (mg gluc eq/g (d.b.))} = 6.2309 + 0.0409 \cdot \text{EtOH} - 0.5608 \cdot \text{T} + 0.0165 \cdot \text{t} - 0.00002 \cdot \text{EtOH}^2 - 0.0084 \cdot \text{EtOH} \cdot \text{T} - 0.0002 \cdot \text{EtOH} \cdot \text{t} + 0.0117 \cdot \text{T}^2 - 0.0007 \cdot \text{T} \cdot \text{t} + 0.00007 \cdot \text{t}^2 \quad (9)$$

3.3.2. Optimization for Ethanol–Water Extraction

The ethanol–water extraction experiments had the main objective of identifying those extraction conditions (ethanol concentration, temperature and time) that achieve the maximum content of polyphenols, although it was proven that inulin was also extracted using water–ethanol mixtures as extracting solvents. Then, it was possible to perform a multiple-response optimization, in order to determine the experimental conditions that maximize the extraction of inulin and polyphenols with the highest antioxidant activity in these ethanol–water extracts. In this sense, it has been found that EtOH = 22.4%, T = 81 °C and t = 217 min give a desirability factor of 0.767 and result in expected values of TPC = 18.71 mg GAE/g (d.b.); Aox = 26.36 mM trolox/g (d.b.) and inulin content = 25.09 mg glu eq/g (d.b.). These values, compared those obtained in the compositional analysis, would represent 90% of the TPC, 38% of the Aox and 28% of the inulin content.

4. Conclusions

The objective of this work was to optimize the process conditions to extract inulin and polyphenols from artichoke wastes, by using water or water–ethanol mixtures as extracting agents.

The best process conditions using water as an extracting agent were T = 89 °C and t = 139 min, where 80% of inulin content, 60% of total phenolic content and 56% of antioxidant activity were obtained. For water–ethanol extractions, the best results were obtained with EtOH = 22.4%, T = 81 °C and t = 217 min, leading to extraction yields of 90% total phenolic content, 38% antioxidant activity and 58% inulin content.

From these results, we recommend the use of water for the recovery of inulin and polyphenols from artichoke wastes. Although the extraction yield of polyphenols is lower in water treatments, the amount extracted is considerable and it is a greener option when comparted with water–ethanol mixtures.

Future work must focus on increasing extraction yields (inulin, polyphenols, antioxidant activity) by using water as an extracting solvent and assisted extraction techniques, such as those using microwave or ultrasound.

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