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Research Article

Optimization of Bioactive Compound and Phytochemical Extraction From Prickly Pear (*Opuntia ficus-indica*) Fruit Through Ultrasound-Assisted Extraction (UAE)

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Opuntia ficus-indica (prickly pear fruit, PPF) as a rich source of natural antioxidants and colorants was aimed to be extracted (PPFE) through optimization by response surface methodology and central composite design (CCD) using ultrasound-assisted extraction (UAE). The effects of independent variables including ethanol concentration (0%, 24%, 48%, 72%, and 96%), citric acid concentration (0%, 0.25%, 0.50%, 0.75%, and 1%), and ultrasound power (0, 100, 200, 300, and 400 W) were investigated. Total phenol content (TPC), total flavonoid content (TFC), total betalain content (TBC), DPPH radical scavenging activity (RSA), and ferric reducing antioxidant power (FRAP) were determined. Based on the results, higher TPC, TFC, and TBC were obtained for PPFE when higher ultrasound power (300 W) was exerted. Ethanol concentration had an inverse relation with TPC and TFC, and higher concentration led to a reduction in respective properties. Acidifying the media by citric acid positively influenced the extraction of phenolic, flavonoid, and betalain compounds. DPPH RSA was negatively affected by ethanol concentration–ultrasound power and citric acid–ultrasound power interactions. Ethanol concentration negatively influenced FRAP, as well as observed for TPC. The optimal point was obtained at an ethanol concentration of 31.50%, citric acid concentration of 0.074%, and ultrasound power of 400 W, which rendered 1.97 mg GAE/g fresh fruit, 3.75 mg quercetin/g fresh fruit, 1.05 mg/100 g fresh fruit, 55.35%, and 5.60 mg Fe^{2+}/g fresh fruit for TPC, TFC, TBC, DPPH RSA, and FRAP value, respectively. Overall, PPFE can be incorporated into foods as natural antioxidants and colorants.

Keywords: betalains; bioactive compounds; flavonoids; phenolics; prickly pear extraction; ultrasound

1. Introduction

Opuntia ficus-indica belongs to the largest genus of the Cactaceae family and is well known for cactus fruits which are used for human consumption [1, 2]. They are widely distributed in Mexico and grow in Africa, Australia, the Mediterranean territory, and some parts of Asia [3]. *Opuntia ficus-indica* is not only used for the preparation of food products

but is also used as fodder for cattle, raw materials for some industries to produce plywood, adhesives and glue, and soap, and also in the pharmaceutical industry to develop nutritional drugs to treat disorders and diseases such as diabetes and in the cosmetic industry for the formulation of shampoo, body lotions, cream, and so on [2, 3]. The fresh fruits have excellent quality and flavor, and their young cladodes can be used as a vegetable and salad dish, while immature fruits can be used to make mock gherkins [2, 3].

Cactus pear fruit, well known as prickly pear berry, has a fleshy texture and hard seeds [4]. It is varied in shape, size, and color and has been characterized by a high sugar content (12%–17%) and low acidity (0.03%–0.12%) [1, 5]. The prickly pears contain high vitamin C, potassium, calcium, and phosphorus, while sodium is present in low amounts [2, 4]. They are also a rich source of betalains, which are water-soluble and nitrogen-containing pigments [2, 6]. The betalains belong to *Caryophyllales*, fulfilling the role of anthocyanins. They are divided into two groups: yellow–orange betaxanthins and red-violet betacyanins [2, 7]. The prickly pears with red and purple colors have a high content of total phenol, and those with purple skin have a high content of flavonoids with radical scavenging and reducing features [2, 4]. Based on the betalain content of the prickly pears, they can be used in low-acid foods as a natural color additive. Moreover, cactus pear fruit pulp indicates a high pH value, and its total soluble solid (TSS) content varies between 11°brix and 17°brix [2, 4]. Thus, prickly pears can be considered a natural source of phytochemicals, including antioxidants and color additives, which can be used in food formulations to extend the shelf life. In addition, due to the presence of ascorbic acid, phenolic compounds, and natural pigments with a mixture of betaxanthin and red betacyanin (betalains) that contribute to the antioxidant properties, cactus pear fruit can exhibit nutritional and health benefits [3]. The antioxidant activity of cactus pear fruit was highly correlated with total phenolics, betalains, and ascorbic acid concentrations [3, 8, 9]. The prickly pear has been introduced as a rich source of phenolic and flavonoid compounds [10]. Especially, catechin and gallic acid (GA) were found in the prickly pear fruit (PPF) seed, peel, and pulp. GA amounts in pulp and peel were found to be 17.46 and 27.70 mg/100 g, respectively. Also, catechin and rutin trihydrate contents of pulp were found to be 36.74 and 10.04 mg/100 g, respectively [11].

The extraction method of phytochemicals from vegetables, fruits, and plants is a critical issue [9, 12]. The conventional maceration method is a technique in which solid materials are immersed in a solvent media (commonly water) while pH, temperature, and time can be set [13, 14]. This method suffers from high consumption of solvent and energy since the extraction is usually prolonged [15, 16]. Recent studies have shown that the extraction of some phenolic compounds substantially depends on the solvent, and aqueous extraction has a low extraction yield [17, 18]. Therefore, using other solvents such as ethanol was suggested to increase the extraction yield of targeted compounds [4, 19, 20]. Following this issue, hydroethanolic extraction has been a successful procedure in the elicitation of phenolic compounds from the plant or fruit matrix [6, 19, 21]. It is mainly due to the chemical structure of phenolic compounds so that some of them are polar, some are semi-polar, and organic solvents can support the extraction of specific compounds [6, 13].

Novel alternative extraction processes such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) emerged to increase the extraction yield of phenolic or other nutraceutical compounds, while the extraction time is significantly reduced and less solvent is needed [19, 21–25]. Recent studies on the extraction of hemp seed oil highlight various advanced techniques that enhance yield and quality. MAE optimized conditions, such as 800 W power and 7.5% ethanol, achieved a yield of 30.69% and high oxidative stability. Supercritical fluid extraction (SFE) using CO_2 and ethanol demonstrated improved extraction efficiency, with yields reaching 30.13% and enriched phenolic content. Aqueous enzymatic extraction (AEE) achieved a 30.65% recovery rate while enhancing oil stability compared to traditional methods. Furthermore, screw press extraction revealed that temperature significantly impacts both yield (max of 21.82% at 100°C) and quality, emphasizing the need for optimizing extraction parameters to balance efficiency and the preservation of bioactive compounds [18, 19, 22, 23, 25]. Moreover, in alternative techniques, moderate extraction conditions (lower temperature and shorter time) are accompanied by generally recognized as safe solvents. The produced extracts are rich in phenolic compounds, and ethanol has been recommended for MAE and UAE methods [17]. PPF was extracted for its high source of betalains using conventional extraction (CE) and UAE [4]. The optimal conditions for CE were achieved at 2.05 h extraction time, 50°C, 80% ethanol concentration, and a 1/22.60 solid/solvent ratio, while for UAE, the optimal conditions were obtained at 30 min extraction time, 49.99°C, 40% ethanol concentration, and a 1/30 solid/solvent ratio [4]. It was reported that UAE proved more efficient, extracting betalain-rich bioactive compounds in 75% less time compared to CE [4]. In another study, *Opuntia ficus-indica* L. Mill. flowers were extracted with UAE and CE [26]. It was reported that the bioactive compound extraction efficiency was statistically higher using UAE than using CE (maceration and Soxhlet extraction) [26]. Similar results were reported for the extraction of betalains and phenolic compounds from PPF using the UAE method [24].

Response surface methodology (RSM) has been introduced as a powerful mathematical and statistical procedure to investigate the effects of multiple process factors, while their interaction impacts are also considered. RSM can support the determination of the effects of independent variables on dependent variables through building mathematical models, which can accurately describe the whole process [2, 4, 23]. Central composite design (CCD) and Box–Behnken design (BBD) are two common optimization methods that provide at least the effects of two independent variables on response factors, while the optimum conditions can be achieved based on maximizing or minimizing the respective factors [23].

This study's purpose was the optimization of phytochemical extractions from PPF through using the UAE method. The effects of process conditions, including citric acid and ethanol concentrations and ultrasound power, were studied on total

phenol content (TPC), total flavonoid content (TFC), and total betalain content (TBC), and consequently the antioxidant activity alterations. The optimization of process conditions to achieve an extract with the highest antioxidant activity through the UAE method has not been studied yet. Also, the betalain compounds of the optimum extract were identified by HPLC-DAD-ESI-MS analysis.

2. Materials and Methods

2.1. Materials. The fresh PPFs were purchased from a local market in Isfahan, Iran, and sent to the Systematic Herbarium in the Faculty of Agriculture at Islamic Azad University, Isfahan branch, for further identification. The harvesting time of fruits was reported as 98–112 days after flowering, implying full maturity. Also, the TSSs of fruits were determined using a refractometer, which was obtained at 15.5%, implying the matured conditions. The fruits were transferred into refrigerator conditions at 4°C until starting the project. Citric acid, Folin–Ciocalteu reagent, GA, quercetin, $FeCl_3$, sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, and ultrapure water were the chemical materials and solvents used in the present study. The chemical materials and reagents were purchased from Sigma-Aldrich (Missouri, United States). Ethanol 96% was provided by Mojallali Co. (Karaj, Iran). Double distilled water was provided by Sanjesh Co. (Tehran, Iran).

2.2. Fruit Preparation and UAE of PPF. The thick layer of skin and thorns was peeled from the fruits manually, and the fruits were washed thoroughly in running tap water to get rid of any impurities adhered to the surface of the fruit and cut into the required weight for the extraction of pigments from the fruits. The extraction of betalains was conducted according to Espinosa-Muñoz et al. [27] with some modifications. The fresh PPFs were mashed using a juicer, and seeds were removed by straining the pulp, and 15 g of prepared puree was dissolved in 50 mL of solvent containing ethanol, citric acid, and distilled water (simultaneous addition and mixing). The mixture solvent (ethanol and distilled water) was prepared based on the process conditions provided in Table 1. The prepared mixture was kept at 4°C for 1 h to get more hydrated. Then, the mixture was subjected to the sonication process using an ultrasound probe system (TOPSONICS, UHP-400, Iran) equipped with a horn (Titanium, 13 mm), outpower of 400 W, and input frequency of 20 KHz. The sonication was exerted for 5 min at the ultrasound power mentioned in Table 1, and the temperature of extraction was controlled using an ice bath, ensuring that temperatures did not exceed 30°C to prevent the degradation of thermolabile compounds. It should be pointed out that the ranges of ethanol and citric acid concentrations, and also ultrasound power, were considered based on preliminary tests and also surveying the studies published recently [4, 20, 26]. Ultrasound power of 0 W implied no exerting sonication process while constant agitation at 200 rpm for 5 min (same process time) was operated. The extract was filtered through a Buchner filter connected to a vacuum pump. The solvent of the filtrate was evaporated at 40°C using a rotary evaporator (IKA RV 10 digital V, Germany) under vacuum conditions. The produced extract was collected and poured into a dark glass, sealed, and stored at 4°C for further tests. The PPF extract was called PPFE.

TABLE 1: Independent variables and their levels used for central composite design.

Variables, unit	Factors	Levels				
		$-\alpha$	−1	0	+1	$+\alpha$
Ethanol concentration (%)	A	0	24	48	72	96
Citric acid (%)	B	0	0.25	0.50	0.75	1
Ultrasound power (Watts)	C	0	100	200	300	400

2.3. TPC. TPC of extracts was determined according to the Folin–Ciocalteu method described by Iftikhar et al. [9] with some modifications. Briefly, 0.3 mL of extract was poured into assay tubes, and 1.5 mL of Folin–Ciocalteu reagent was added while diluted using distilled water by 10 times. The prepared solution was mixed with 1.2 mL of 7.5% sodium carbonate and kept in the dark for 30 min to complete the reaction. The absorbance of the solution was read at 765 nm using a UV-vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The TPC of extracts was expressed based on the equivalent of GA standard by milligram GAE per gram of fresh fruit.

2.4. TFC. TFC of produced extracts was determined according to the method suggested by Iftikhar et al. [9] with some modifications. Briefly, 0.5 mL of extract was poured into an assay tube and mixed with 1.5 mL of $AlCl_3$ (2% *w*/*w*) reagent and incubated in the dark for 30 min. The absorbance of the reacted solution was measured at 430 nm. Also, quercetin was used as the standard material and its calibration curve was plotted. Thus, results were expressed based on the utilized standard as milligram quercetin per gram of fresh fruit.

2.5. TBC. The TBC of produced extracts was determined using a method described by Prakash Maran and Manikandan [2]. Briefly, 10 mL of extract was mixed with 10 mL of aqueous methanolic solution (50%) and agitated at 200 rpm for 30 min at room temperature. Then, the obtained dissolution was centrifuged at 4731 × g for 15 min. The supernatant was taken and filtered through a filter paper (Whatman No. 1, Whatman International Ltd., Maidstone, England) and analyzed using a UV-vis spectrophotometer. The betalain (betaxanthin/betacyanin) content was calculated using the equation below:

$$\text{Betalain}\left(\frac{\text{mg}}{100}\,\text{g fresh fruit}\right) = \frac{A \times DF \times M_W \times 100}{\varepsilon \times 1},$$
$$\text{Betalain} = \text{Betacyanin} + \text{Betaxanthin},$$

where in the above equation, A denotes the absorbance of the maximum absorbance corrected by the absorption at 600 nm, M_W indicates the molecular weight (*i*ndicaxanthin = 308 g/moL and betanin = 550 g/moL), ε is the molar attenuation coefficient (*i*ndicaxanthin = 48, 000

$L\,moL^{-1}\,cm^{-1}$ and betanin = 60, 000 $L\,moL^{-1}\,cm^{-1}$), and 1 is the cuvette path length (centimeters).

2.6. DPPH Radical Scavenging Activity (RSA). Free DPPH RSA was determined according to the method of Ghanbari et al. [28] with some modifications. Briefly, 750 μL of produced extract was mixed with 1.75 mL of 0.5 mM methanolic DPPH solution. The prepared mixture was stored in the darkness for 30 min. The absorbance of the solution was recorded at 517 nm against the control using a UV-vis spectrophotometer. DPPH RSA was computed using the equation below:

$$\text{DPPH RSA}\,(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100,$$

where A_{sample} is the absorption value of the PPFE and A_{control} is the absorption value of the control.

2.7. Ferric Reducing Antioxidant Power (FRAP) Assay. FRAP was determined based on the method described by Belwal et al. [29] and Aruwa et al. [30] with some modifications. Briefly, 0.1 mL of the produced extract was mixed with 3.9 mL of FRAP solution, which was made up of 2,4,6-tri-2-pyridyl-1,3,5-triazin (TPTZ) (10 mM), $FeCl_3.H_2O$ (20 mM), and sodium acetate buffer (pH = 3.6) at a ratio of 10:1:1. The obtained mixture was incubated at 37°C for 5 min, and then the absorption value was measured at 593 nm using a UV-vis spectrophotometer. The FRAP value was expressed as milligram Fe^{2+} per gram of fresh fruit.

2.8. Identification of Betalain Compounds. The optimum extract, based on the highest TPC, TFC, and TBC, as well as DPPH RSA and FRAP, was assessed for the identification of betalain compounds according to the method of Cejudo-Bastante et al. [31]. High performance liquid chromatography (HPLC, Agilent 1200 chromatographic system) was employed for separation, equipped with a quaternary pump, UV-vis diode array detector, automatic injector, and ChemStation software (Palo Alto, California, United States). The optimum extract was filtered through a 0.45 μm nylon filter (E0034, Análisis Vinícolas, Spain) prior to direct injection. Identification of betalains was performed using 1% formic acid in water (v/v, Eluent A) and methanol (Eluent B). The separation of betalains was achieved using a Zorbax C18 column (250 × 4.6 mm, 5 μm particle size), maintained at 25°C, with a flow rate of 1 mL/min and an injection volume of 20 μL. The separation was initiated with 100% Eluent A, followed by a linear gradient from 0% to 10% Eluent B over 20 min, then from 10% to 30% Eluent B over 10 min, and from 30% to 100% eluent B over 5 min. The initial conditions were restored using a linear gradient from 100% Eluent B to 100% Eluent A over 5 min. Detection of compounds was recorded at UV-vis spectra of 200–800 nm with a bandwidth of 1.0 nm. Betacyanins and betaxanthins, the main betalains, were monitored at 535 and 482 nm, respectively. Peaks were identified by comparing their spectral characteristics and retention times with standards. Semiquantification was performed based on the mean areas of individual betalains. To confirm the identity of individual betalains, mass spectrometry was applied. The separation of compounds was conducted using a Dionex Ultimate 3000RS U-HPLC (Thermo Fisher Scientific, Waltham, Massachusetts, United States) with the aforementioned column and mobile phases, employing a post-column split of 0.4 mL/min. Mass spectra were obtained using a microToF-QII high-resolution time-of-flight mass spectrometer (UHR-ToF) with Q-ToF geometry (Bruker Daltonics, Bremen, Germany), equipped with an electrospray ionization (ESI) source. The system was operated in positive ion mode with a scan range of m/z 50–1200. Nitrogen gas served as the purge gas at a flow rate of 8 mL/min, with a nebulizing pressure of 1.2 bar and a temperature of 200°C. Mass spectra were acquired in full scan mode.

2.9. Experimental Design and Statistical Analysis. RSM was used to optimize the process conditions (mentioned in Table 1). CCD was used for optimization, and three independent variables including ethanol concentration (A), citric acid concentration (B), and ultrasound power (C) were considered. The dependent variables were TPC, TFC, TBC, DPPH RSA, and FRAP antioxidant activity. The effects of factors were assessed at five levels. The numerical optimization was conducted based on the maximum values of dependent variables. Design-Expert Version 13 (State Ease, Inc.) was used to analyze data.

The analysis of variance (ANOVA) was carried out to determine linear, quadratic, and interaction regression coefficient. The fitness of the polynomial equation to the dependent variables was assessed using coefficient of determination (R^2). The significant effects of factors of the polynomial equation were trialed based on the calculation of the F-value at $p < 0.05$.

3. Results and Discussion

3.1. CCD Analysis. Fresh PPF was considered for the extraction of nutraceuticals. The fruits had TSS of 15.5%, which is in the range of matured fruits [32]. The RSM was applied to investigate the effects of three independent variables (ethanol concentration, citric acid concentration, and ultrasound power) on the TPC, TFC, TBC, and antioxidant activities of PPFE. The RSM can provide an empirical modeling technique to evaluate how factors influence the responses. In our present study, CCD was used to perform the experiments to reach the optimum conditions and study the effects of process variables on the mentioned dependent variables. The experimental results are provided in Table 2.

The obtained experimental data were fitted to various models (linear, interactive, and quadratic) to achieve the regression equations. The ANOVA analysis and statistical parameters are provided in Tables 3, 4, 5, 6, and 7. For each dependent variable, a specific model was rendered, and the linear, interaction, and quadratic effects were statistically studied.

3.2. Fitting the Model

3.2.1. TPC. The ANOVA statistical analysis of process variables for TPC is provided in Table 3. A significant quadratic model was suggested to study the relationship between the

TABLE 2: Central composite design (CCD) matrix and experimental responses.

Run	Ethanol concentration (%)	Citric acid (%)	Ultrasound power (Watts)	TPC (mg GAE/ g fresh fruit)	TFC (mg quercetin/g fresh fruit)	TBC (mg/100 g fresh fruit)	DPPH RSA (%)	FRAP (mg Fe^{2+} /g fresh fruit)
1	24	0.25	100	0.837	2.37	0.58	20.96	4.464
2	72	0.25	100	0.756	1.92	0.675	26.03	3.619
3	24	0.75	100	0.681	2.332	0.663	24.00	4.81
4	72	0.75	100	0.84	2.26	1.411	40.32	4.597
5	24	0.25	300	1.358	2.937	0.952	35.75	5.204
6	72	0.25	300	0.985	2.34	1.056	30.06	4.72
7	24	0.75	300	1.155	2.582	0.714	24.00	5.426
8	72	0.75	300	0.824	2.55	0.986	40.06	5.62
9	0	0.50	200	0.539	2.065	0.53	18.68	4.253
10	96	0.50	200	0.613	1.577	0.619	18.76	3.41
11	48	0.00	200	0.840	2.58	0.889	31.36	5.25
12	48	1.00	200	1	2.46	0.817	23.83	5.38
13	48	0.50	0	0.429	1.5	0.421	21.04	3.008
14	48	0.50	400	0.540	2.694	0.708	28.40	4.558
15	48	0.50	200	0.783	2.47	0.682	23.92	4.78
16	48	0.50	200	0.632	2.23	1.068	34.74	5.191
17	48	0.50	200	0.694	1.54	0.591	19.18	3.172

Note: TPC, TFC, TBC, DPPH RSA, and FRAP indicate total phenol content (milligram GAE per gram fresh fruit), total flavonoid content (milligram quercetin per gram fresh fruit), total betalain content (milligram betalain/100 g fresh fruit), antioxidant activity—DPPH radical scavenging activity (percent), and antioxidant activity—FRAP assay (milligram Fe^{2+} per gram fresh fruit), respectively.

TABLE 3: ANOVA analysis and statistical parameters of the model for the total phenol content (milligram GAE per gram fresh fruit).

Source	Sum of squares	df	Mean square	*F* -value	*p* value
Model	0.4986	5	0.0997	11.05	0.0032
A—Ethanol concentration (%)	0.0526	1	0.0526	5.83	0.0464
B—Citric acid concentration (%)	0.024	1	0.024	3.62	0.106
C—Ultrasound power (W)	0.1824	1	0.1824	20.20	0.0028
AC	0.0764	1	0.0764	8.47	0.0227
B^2	0.0638	1	0.0638	7.06	0.0326
C^2	0.0601	1	0.0601	6.66	0.0364
Residual	0.0632	7	0.0090	—	—
Lack of fit	0.0517	5	0.0103	1.79	0.3954
Pure error	0.0115	2	0.0058	—	—
Cor total	0.5618	12	—	—	—
R^2	—	—	0.8875	—	—
Adjusted R^2	—	—	0.8072	—	—
Predicted R^2	—	—	0.7805	—	—
Adequate precision	—	—	10.39	—	—
CV (%)	—	—	11.07	—	—

empirical results obtained by CCD and the input variables. The linear terms of ethanol concentration (A) and ultrasound power (C), the interaction term of AC, and the quadratic terms of citric acid and ultrasound power were obtained significant ($p < 0.05$). The suggested model was well fitted with the experimental results, so the lack of fit value was insignificant ($p > 0.05$). Also, a high coefficient of determination ($R^2 = 0.88$) indicated that the quadratic model had an acceptable fitness with empirical results. The obtained polynomial equation in terms of coded factors to

TABLE 4: ANOVA analysis and statistical parameters of the model for the total flavonoid content (milligram quercetin per gram fresh fruit).

Source	Sum of squares	df	Mean square	*F* -value	*p* value
Model	1.84	3	0.6132	8.87	0.0028
A—Ethanol concentration (%)	0.2828	1	0.2828	4.09	0.0682
B—Citric acid concentration (%)	0.003	1	0.003	0.041	0.8442
C—Ultrasound power (W)	0.9580	1	0.9580	13.85	0.0034
B^2	0.5989	1	0.5989	8.66	0.0134
Residual	0.7607	11	0.0692	—	—
Lack of fit	0.2945	9	0.0327	0.1404	0.9860
Pure error	0.4662	2	0.2331	—	—
Cor total	2.60	14	—	—	—
R^2	—	—	0.7074	—	—
Adjusted R^2	—	—	0.6277	—	—
Predicted R^2	—	—	0.5170	—	—
Adequate precision	—	—	9.333	—	—
CV (%)	—	—	11.82	—	—

TABLE 5: ANOVA analysis and statistical parameters of the model for the total betalain content (milligram betalain/100 g fresh fruit).

Source	Sum of squares	df	Mean square	*F* -value	*p* value
Model	0.3236	3	0.1079	8.79	0.0049
A—Ethanol concentration (%)	0.0437	1	0.0437	3.56	0.0917
B—Citric acid concentration (%)	0.000	1	0.000	0.000	0.9797
C—Ultrasound power (W)	0.1582	1	0.1582	12.89	0.0058
B^2	0.0824	1	0.0824	6.71	0.0292
Residual	0.1105	9	0.0123	—	—
Lack of fit	0.1063	8	0.0133	3.21	0.4080
Pure error	0.0041	1	0.0041	—	
Cor total	0.4341	12	—	—	—
R^2	—	—	0.7455	—	—
Adjusted R^2	—	—	0.6607	—	—
Predicted R^2	—	—	0.6102	—	—
Adequate precision	—	—	8.45	—	—
CV (%)	—	—	15.25	—	—

investigate the relationship between the results and factors was given as below:

$$\mathrm{TPC}\left(\mathrm{mg}\frac{\mathrm{GAE}}{\mathrm{g\ fresh\ fruit}}\right) = +0.7151 - 0.0692A + 0.1510C - 0.0977AC + 0.0712B^2 + 0.1432C^2.$$

Based on the mentioned equation, the linear term of ethanol concentration (A) and the interaction term of AC negatively affected TPC. To interpret the effects, it was shown that high ethanol concentration and high ultrasound power led to lower TPC compared to low ethanol concentration and high ultrasound power, which resulted in high TPC. In contrast, the linear term of ultrasound and the quadratic terms of citric acid and ultrasound power positively influenced the TPC.

3.2.2. TFC. The suggested model for TFC results was a quadratic model ($p < 0.05$). In this regard, the linear term of ultrasound power and the quadratic term of citric acid concentration were significant ($p < 0.05$), while the linear term of ethanol concentration was insignificant ($p > 0.05$) (Table 8). The insignificant value of lack of fit indicated that the model can be efficiently used to be fitted with experimental data. The coefficient of determination was obtained as 0.7, while the adjusted R^2 and predicted R^2 were obtained

TABLE 6: ANOVA analysis and statistical parameters of the model for the antioxidant activity—DPPH radical scavenging activity (percent).

Source	Sum of squares	df	Mean square	*F* -value	*p* value
Model	372.13	3	124.04	9.70	0.0035
A—Ethanol concentration (%)	62.20	1	62.20	4.87	0.0548
B—Citric acid concentration (%)	7.72	1	7.72	0.67	0.4384
C—Ultrasound power (W)	21.61	1	21.61	1.89	0.2116
AC	169.83	1	169.83	13.29	0.0054
BC	231.79	1	231.79	18.13	0.0021
Residual	115.05	9	12.78	—	—
Lack of fit	103.82	8	12.98	1.16	0.6206
Pure error	11.23	1	11.23	—	—
Cor total	487.18	12	—	—	—
R^2	—	—	0.7638	—	—
Adjusted R^2	—	—	0.6851	—	—
Predicted R^2	—	—	0.6694	—	—
Adequate precision	—	—	10.38	—	—
CV (%)	—	—	13.41	—	—

TABLE 7: ANOVA analysis and statistical parameters of the model for the antioxidant activity—FRAP assay (milligram Fe^{2+} per gram fresh fruit).

Source	Sum of squares	df	Mean square	*F* -value	*p* value
Model	4.78	5	0.9560	28.88	0.0004
A—Ethanol concentration (%)	0.5753	1	0.5753	17.38	0.0059
B—Citric acid (%)	0.7479	1	0.7479	22.59	0.0031
C—Ultrasound power (W)	1.51	1	1.51	45.73	0.0005
AB	0.2145	1	0.2145	6.48	0.0437
AC	0.074	1	0.074	2.41	0.2185
BC	0.005	1	0.005	0.17	0.7106
A^2	1.73	1	1.73	52.22	0.0004
B^2	0.028	1	0.028	0.91	0.4102
C^2	0	0			
Residual	0.1986	6	0.0331	—	—
Lack of fit	0.1141	5	0.0228	0.2703	0.8876
Pure error	0.0845	1	0.0845	—	—
Cor total	4.98	11	—	—	—
R^2	—	—	0.9601	—	—
Adjusted R^2	—	—	0.9269	—	—
Predicted R^2	—	—	0.8745	—	—
Adequate precision	—	—	16.00	—	—
CV (%)	—	—	3.89	—	—

as 0.62 and 0.51, implying reasonable data. The rendered polynomial equation was as below:

$$\text{TFC}\left(\text{mg}\frac{\text{quercetin}}{\text{g fresh fruit}}\right) = +2.01 - 0.1329A + 0.2447C + 0.4005B^2.$$

Based on the equation, the linear term of ethanol concentration (A) negatively influenced the TFC, while the respective effect was insignificant. The linear term of ultrasound power and the quadratic term of citric acid concentration positively influenced the TFC.

3.2.3. TBC. The statistical effects of process variables were studied on TBC, and ANOVA results are provided in Table 9. In this regard, a significant quadratic model was suggested to forecast the TFC considering the process

TABLE 8: Numerical optimization conditions and optimum point.

Responses	Optimization conditions	Optimum point		
		Ethanol concentration (%) 31.50	Citric acid (% w/v) 0.074	Ultrasound power (W) 400
		Predicted value	Actual value	Relative errors (%)
Total phenol content (mg GAE/g fresh fruit)	Maximize	1.97	1.94	1.52^{ns}
Total flavonoid content (mg quercetin/g fresh fruit)	Maximize	3.75	3.70	1.33^{ns}
Total betalain content (mg betalain/100 g fresh fruit)	Maximize	1.05	1.03	1.90^{ns}
Antioxidant activity—DPPH radical scavenging activity (%)	Maximize	55.35	54.53	1.48^{ns}
Antioxidant activity—FRAP assay (mg Fe^{2+}/g fresh fruit)	Maximize	5.60	5.50	1.79^{ns}
Desirability	—	0.99	—	—

Abbreviation: ns, not significant.

TABLE 9: Molecular formula, retention times, UV-Vis data, and mass spectral data of betalains identified in *Opuntia ficus-indica* fruit by HPLC-DAD-ESI-TOF-MS.

Peak	Compound	Molecular formula	t_r (min)	UV-vis maxima (nm)	m/z$[M+H]^+$	MS ion
	Betaxanthins					
1	Histidine–betaxanthin (muscarine)	$C_{15}H_{16}N_4O_6$	5.7	476		
2	Glutamine–betaxanthin (vulgaxanthin I)	$C_{14}H_{17}N_3O_7$	10.3	472		
3	Aminobutyric acid–betaxanthin	$C_{13}H_{16}N_2O_6$	19.1	462	295.22	252.10
4	Proline–betaxanthin (indicaxanthin)	$C_{14}H_{16}N_2O_6$	22.5	479	305.14	261.12
5	Valine–betaxanthin	$C_{14}H_{18}N_2O_6$	28.9	472	308.41	136.51
6	Valine–betaxanthin isomer	$C_{14}H_{18}N_2O_6$	30.2	471	308.74	136.41
7	Isoleucine–betaxanthin	$C_{15}H_{20}N_2O_6$	34.1	472	319.31	218.25
8	Leucine–betaxanthin (vulgaxanthin IV)	$C_{15}H_{20}N_2O_6$	34.5	471	319.41	208.31
9	Phenylalanine–betaxanthin	$C_{18}H_{18}N_2O_6$	35.3	468		
	Betacyanins					
10	Betanin	$C_{24}H_{26}N_2O_{13}$	26.3	534	549.21	388.74
11	Isobetanin	$C_{24}H_{26}N_2O_{13}$	28.4	534	551.21	388.57
12	Gomphrenin	$C_{24}H_{26}N_2O_{13}$	29.5	535	550.14	389.15

variables ($p < 0.05$). The linear term of ethanol concentration was insignificant, while the linear term of ultrasound power had a significant effect on TFC. Moreover, the quadratic term of citric acid concentration was significant ($p < 0.05$). The obtained insignificant lack of fit exhibited that the obtained model can be accepted to be fitted with the experimental data. The coefficient of determination was 0.74, and the obtained polynomial equation was as below:

$$\text{Total } BC\left(\frac{\text{mg}}{100\text{ g fresh fruit}}\right) = +0.6678 + 0.066A + 0.1035C + 0.0782B^2.$$

Based on the above coded equation, the ultrasound linear term and citric acid quadratic term positively influence the TBC.

3.2.4. DPPH Antioxidant Activity. Regarding the effects of process variables on DPPH RSA, an interactive 2FI model was suggested ($p < 0.05$) and ANOVA results are represented in Table 10. The interaction terms of ethanol concentration-ultrasound power (AC) and citric acid concentration-ultrasound power (BC) were obtained to be significant ($p < 0.05$). The insignificant value of lack of fit indicated that the model can be statistically fitted with experimental data. The coefficient of determination was obtained as 0.76. The interactive coded model was provided as below:

$$\text{DPPH RSA}\,(\%) = +25.05 + 2.54A - 5.99AC - 6.99BC.$$

Based on the equation, the interactive terms (AC and BC) negatively influence DPPH RSA.

3.2.5. FRAP. ANOVA results for effects of process variables on FRAP value are provided in Table 7. In this regard, a significant quadratic model was obtained which well fitted with

TABLE 10: Mean areas and standard deviations of betalain compounds (betacyanins and betaxanthins) belonging to *Opuntia ficus-indica* fruit.

Compound	Mean areas and standard deviation
Betanin	402.21 ± 13.10
Isobetanin	103.61 ± 12.50
Gomphrenin I	23.41 ± 1.31
Sum of betacyanins	529.23 ± 14.60
Histidine–betaxanthin (muscarine)	184.32 ± 5.40
Glutamine–betaxanthin (vulgaxanthin)	87.65 ± 10.40
Aminobutyric acid–betaxanthin	149.74 ± 21.40
Proline–betaxanthin (indicaxanthin)	9061.10 ± 54.30
Valine–betaxanthin	65.10 ± 2.10
Valine–betaxanthin isomer	48.32 ± 3.10
Isoleucine–betaxanthin	98.32 ± 4.10
Leucine–betaxanthin (vulgaxanthin)	92.12 ± 3.20
Phenylalanine–betaxanthin	75.61 ± 4.80
Sum of betaxanthins	9862.28 ± 105.65
Total betalains	10,391.51 ± 110.47

experimental data by rendering a high coefficient of determination ($R^2 = 0.96$). The linear terms of ethanol concentration (A), citric acid concentration (B), and ultrasound power (C) were obtained significant ($p < 0.05$). Also, the interaction term of AB and the quadratic term of ethanol concentration were attained significant ($p < 0.05$). The insignificant value of lack of fit exhibited that the model was well matched with empirical data. The established coded equation was as below:

$$\text{FRAP}\left(\text{mg}\frac{\text{Fe}^{2+}}{\text{g fresh fruit}}\right) = +5.08 - 0.1896A + 0.3057B + 0.4350C + 0.1638AB - 0.3043A^2.$$

Based on the above equation, the linear terms of B and C positively affected the FRAP value, while the linear term of A had negative impacts on the FRAP value. Besides, the interaction term of AB and the quadratic term of A exhibited positive and negative impacts on the FRAP value, respectively.

3.3. Effects of Process Variables

3.3.1. Effects on TPC. 3D surface plots and one factor effects of process variables are represented in Figure 1. According to ANOVA results, ethanol concentration and ultrasound power significantly affected TPC. Based on the illustration of Figure 1 and experimental data presented in Table 2, when ethanol concentration was increased, TPC indicated a decreasing trend. In contrast, when ultrasound power was increased, the TPC exhibited a rising trend (Table 2 and Figure 1). Ultrasound power had a polynomial effect on extraction of phenolic compounds from PPF, which was shown in the 3D surface plot and also in one factor effect (Figure 1). By considering the simultaneous changes in ethanol concentration and ultrasound power, for instance, the lower ethanol concentration and the higher ultrasound power, the higher level of extraction of phenolic compounds occurred. These results showed that ethanol concentration and ultrasound power had critical effects so that to efficiently extract the phenolic compounds, a moderate concentration of ethanol along with a moderate ultrasound power should be considered. Phenolic compounds have a versatile tendency to different solvents. Using a single solvent in the extraction of phenolic compounds would lead to low extraction efficiency, which is associated with their chemical structure. This is in agreement with other reports [33–36]. In contrast, using mixed solvents such as water and ethanol can increase their extraction efficiency [34, 35, 37]. Thus, the ethanol concentration should be importantly taken into consideration. Using a hydroethanolic extraction method can result in a high extraction content of phenolic compounds, although the ethanol concentration should not be exceeded [36]. Pistachio hull was extracted using a mixture of ethanol and water through ohmic-assisted extraction. It was reported that using ethanol and water in a ratio of 1:1 (v/v) resulted in an efficient extraction of phenolic and flavonoid compounds [34]. In another study, walnut husk and pomegranate peel were extracted using the UAE method [35]. The mixture of ethanol:water (80:20) resulted in the extraction yield of 10.97 and 11.87% for walnut husk extract and pomegranate peel extract, respectively [35].

Ultrasound power indicated positive impacts on the extraction of phenolic compounds; although due to extraction with mixed ethanol and water, its intensity was influenced. Higher TPC was obtained when higher ultrasound power and lower ethanol concentration were considered. Therefore, our results implied that ultrasound can efficiently extract the phenolic compounds in a short time, which has been reported in many studies [23, 35]. Moreover, using hydroethanolic extraction is a good and efficient technique in increasing the extraction yield of phenolic compounds [13, 34, 35]. Despite this, ethanol concentration, due to having direct effects on washing the PPF, should be importantly taken into consideration. The southern African *Opuntia* fruit was extracted with polar (ethanol, methanol, and water) and nonpolar (hexane) solvents [30]. The respective results implied that the polar solvents led to higher extraction yields [30]. The TPC of extracts from Southern African *Opuntia ficus-indica* using polar and nonpolar solvents generally decreased in the order of ethanol > methanol > hexane > water. The highest TPC was obtained for oven-dried peel ethanol and freeze-dried peel methanol extracts at 17.59 and 16.51 mg GAE/g, respectively. The higher TPC was achieved for hydroalcoholic solvents, likely due to the reason that hydroethanolic solvents have a high polarity index, which allows the expression of polar and nonpolar compounds [30]. It was reported that polar compounds such as

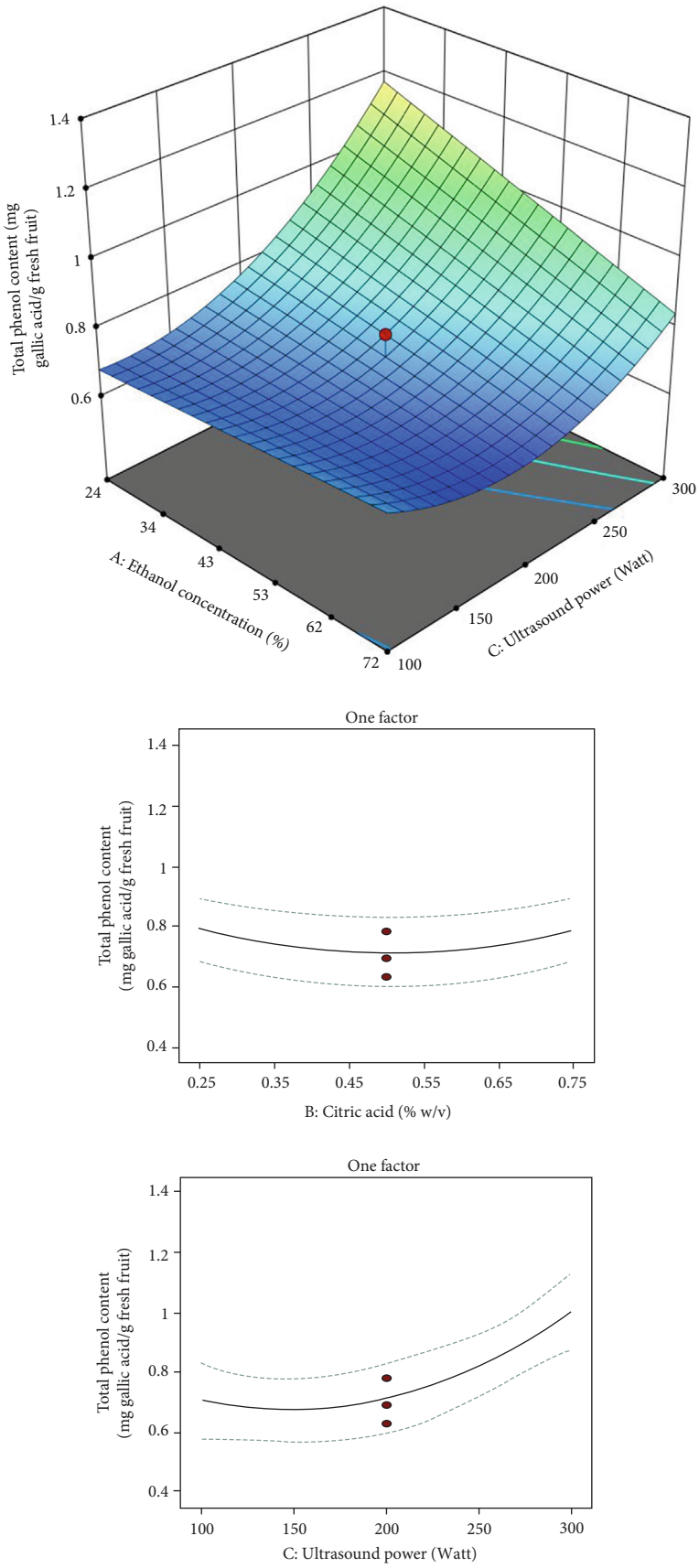

Figure 1: 3D surface and one factor plots of response surface methodology for total phenol content of prickly pear fruit extract (PPFE) produced by ultrasound-assisted extraction.

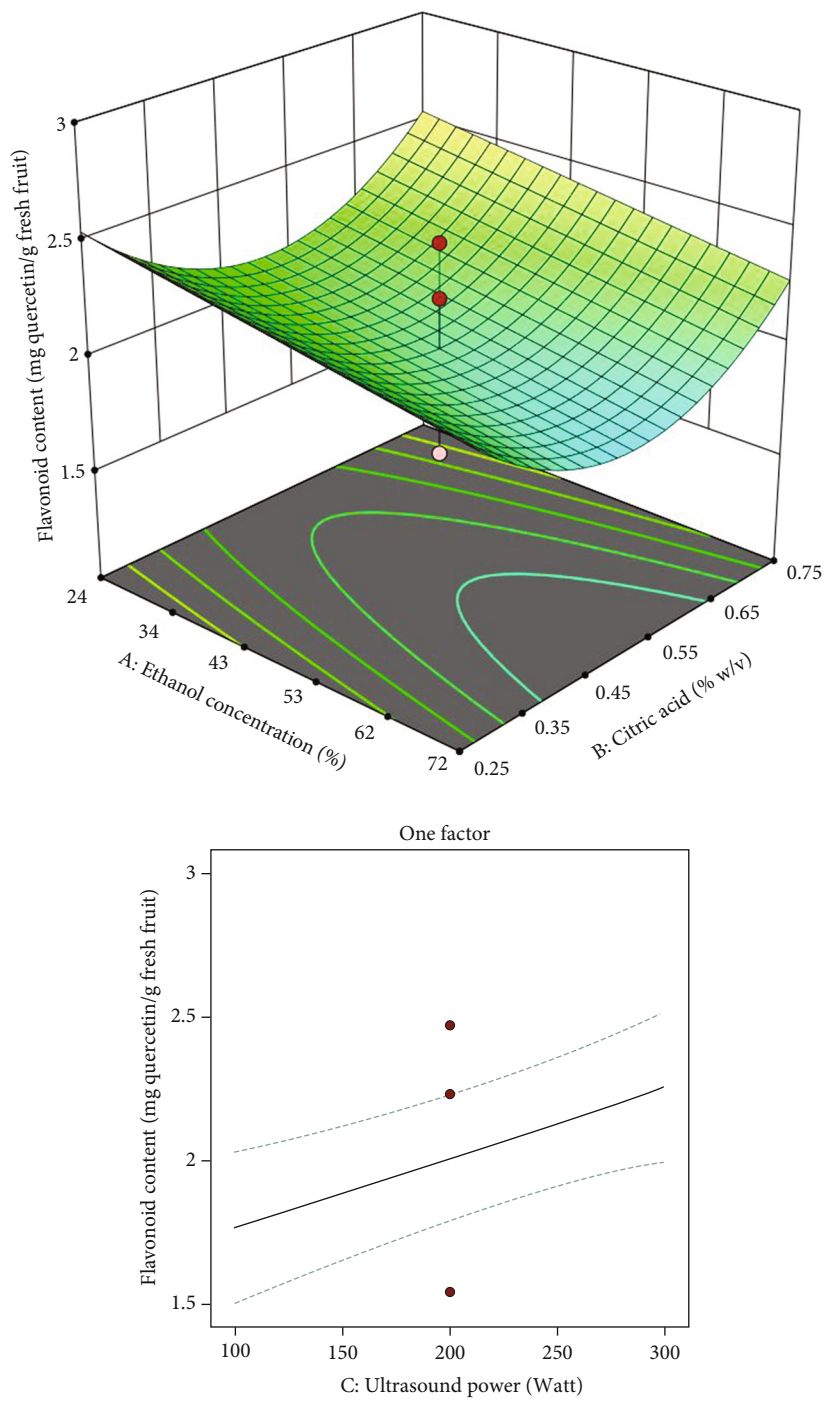

Figure 2: 3D surface and one-factor plots of response surface methodology for total flavonoid content of prickly pear fruit extract (PPFE) produced by ultrasound-assisted extraction.

polyphenols are more likely to be expressed in solvents with a high polarity index, such as water, ethanol, and methanol. Indeed, the polarity index determines the relative ability of a solvent to allow the expression of polar solutes [30].

Based on the literature, the important phenolics that were extracted from the fruit, pulp, and peel of Mexican and Spanish prickly pear (*Opuntia ficus-indica* L. Mill.) corresponded mostly to flavonoid (isorhamnetin, quercetin, and kaempferol) glycosides and a phenolic acid, piscidic acid. The highest TPC was found in the Spanish *Morada* cultivar (49,012 μg/g dry peel) [38].

There are various reports that have shown UAE is a novel technique that can facilitate the extraction of bioactive compounds through reducing the time and energy [13, 35]. UAE can produce microstreams in solvents that can stimulate the formation of microbubbles that can emerge together and make bigger bubbles. The produced bubbles can explode in the media and release energy, which can disrupt the cell

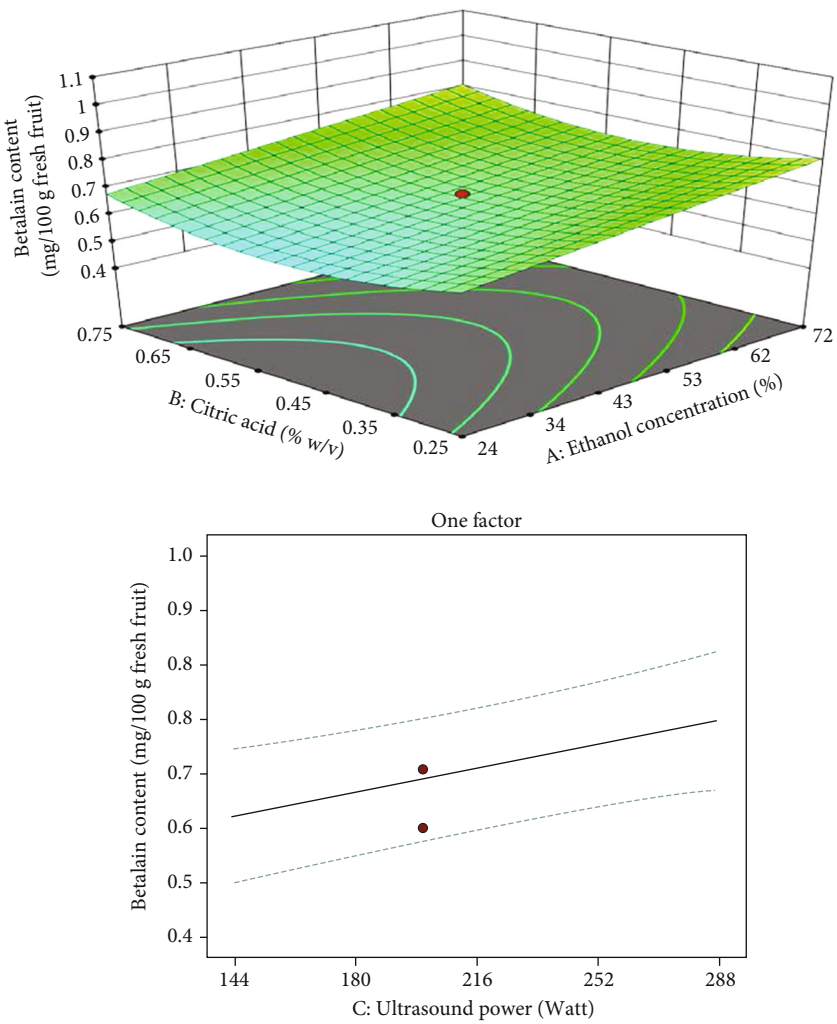

Figure 3: 3D surface and one factor plots of response surface methodology for total betalain content of prickly pear fruit extract (PPFE) produced by ultrasound-assisted extraction.

walls of plant tissues. It is namely called bubble cavitation [21, 39]. In a study, *Opuntia ficus-indica* peel was extracted for phenolic compounds using ultrasonic-assisted solvent extraction [20]. BBD was used to optimize the extraction conditions. The maximum TPC (5.95 mg GAE/g dry weight) and TFC (9.79 mg rutin equivalent/g dry weight) were obtained at 17 min of time, 40°C bath temperature, and 1:24 g/mL solid solvent ratio [20]. It was reported that ultrasonic cavitation caused morphological changes, which accelerated the rate of extraction. Also, the phytochemical analysis of extracts from UAE and the Soxhlet method revealed that the components that possessed pharmacological activity were about 68.08% in the UAE extract while 61.72% in the Soxhlet extract [20].

Based on citric acid effect (Figure 1), it was observed that moderate concentrations lowered the extraction of phenolic compounds. At lower and higher citric acid concentrations, lower TPC was obtained. Defatted Hom Nin rice bran (DHRB) as a rich source of phenolic compounds and anthocyanins was extracted with various solvents (water, methanol, and ethanol), while the addition of citric acid was investigated [40]. The obtained extract using methanol and 0.1 mol/dm^3 citric acid had the highest yield (43.72%), and phenolic content was 86.63 mg GAE/g, while using 0.05 mol citric acid led to lower extraction of phenolic compounds. Phenolic compounds are usually found in plant and fruit tissues in three different groups, that is, they are in free form, they are in conjugated form, and also bound form. The bound form is mainly extracted using citric acid or alkaline hydrolysis from the lignin layer. When citric acid is used, the plant's cell walls are disrupted which facilitates the release of entrapped compounds. For any type of solvent, the citric acid concentration can vary [40]. Also, when ethanol and water were compared, using sole ethanol led to lower extraction since most of the phenolic compounds are water-soluble components. Methanol has been known as an efficient solvent while it is not as safe as ethanol. Thus, using ethanol along with water (hydroethanolic system) can be more efficient than individual solvents (water or ethanol) [40]. According to the previous reports, using acidified polar solvents (ethanol, methanol, and water) facilitated the release of phenolic compounds from Southern *Opuntia*

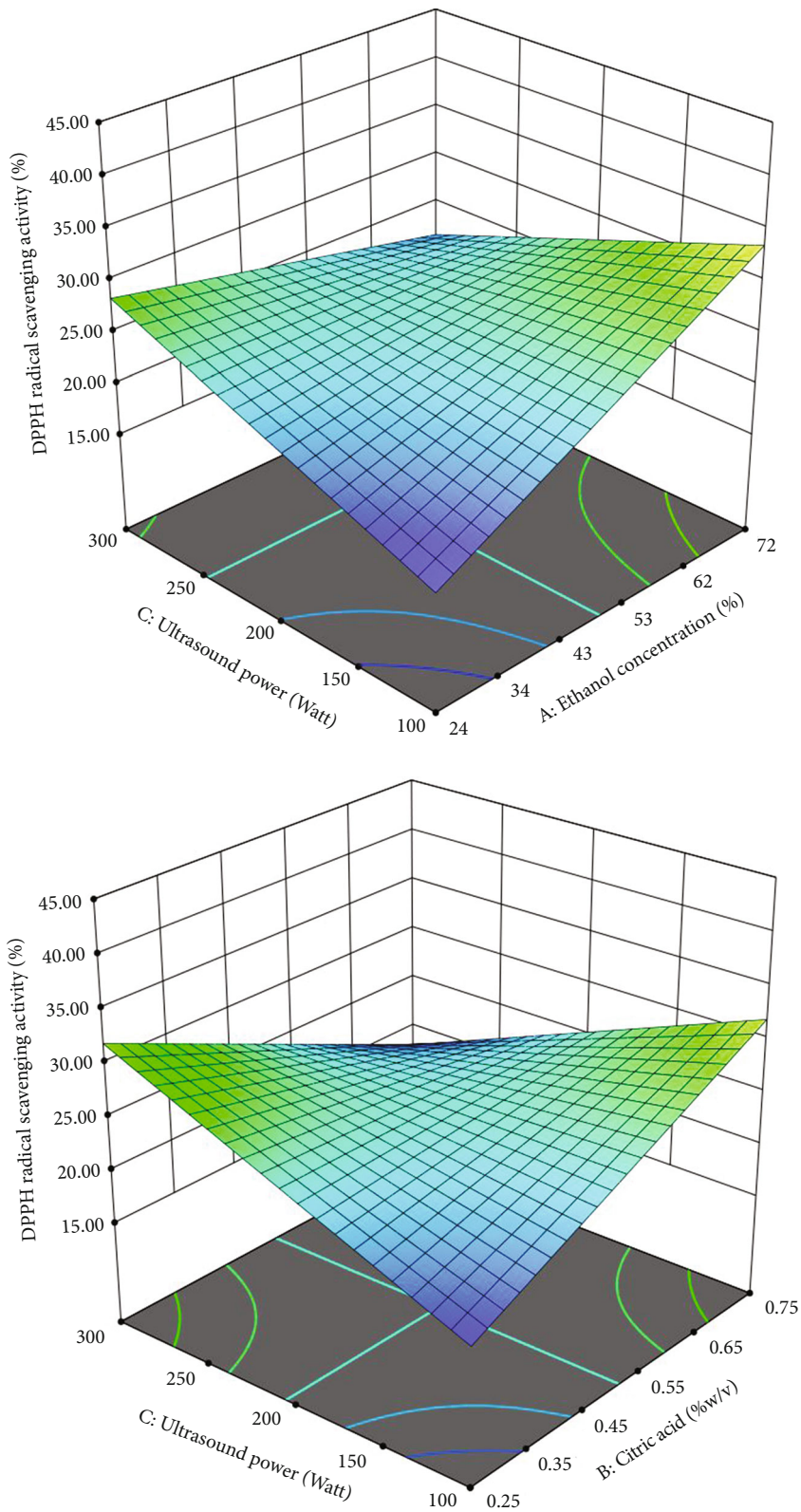

Figure 4: 3D surface illustration of response surface methodology for DPPH RSA (percent) of prickly pear fruit extract (PPFE) produced by ultrasound-assisted extraction. The effects of ethanol concentration, citric acid concentration, and ultrasound power were investigated.

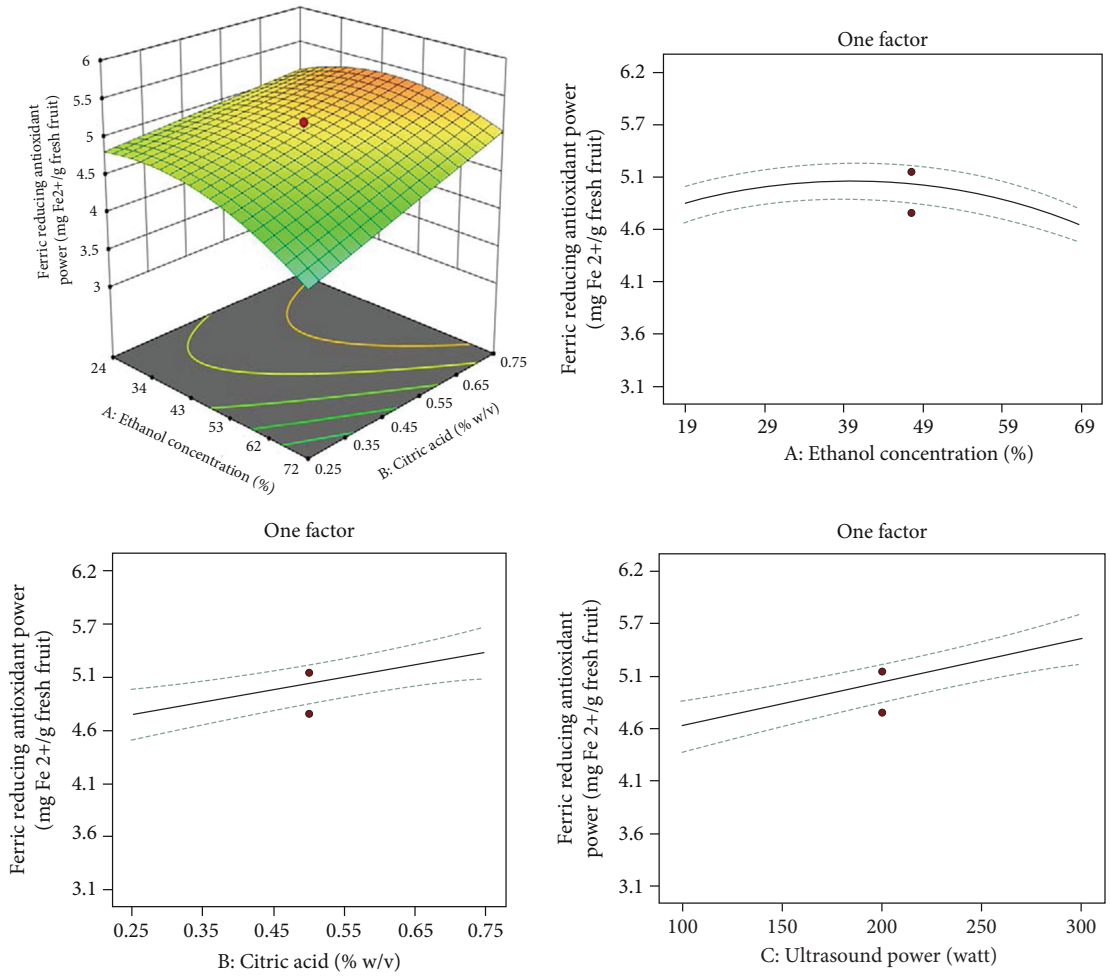

Figure 5: 3D surface and one factor plots of response surface methodology for ferric reducing antioxidant power influenced by ethanol concentration, citric acid concentration, and ultrasound power.

ficus-indica fruit pulp and peels [30]. Based on the findings of previous reports, the yield of freeze-dried *Opuntia* fruit pulp extracts was as follows: ethanol (40.76%, *w*/*w*) > acidified methanol (34.27%, *w*/*w*) > acidified water (25.10%, *w*/*w*) > hexane (0.64%, *w*/*w*). Also, TPC of extracts from freeze-dried fruit pulp was as follows: ethanol (14.83 mg GAE/g) > acidified methanol (9.94 mg GAE/g) > hexane (8.61 mg GAE/g) > acidified water (4.25 mg GAE/g) [30]. Thus, it was observed that ethanol had a high efficiency in the extraction of phenolic compounds while the acidification of methanol resulted in facilitating the extraction of polyphenolic compounds [30].

3.3.2. Effects on TFC. The effects of process variables on TFC are represented in Figure 2. Results showed that TFC depended on citric acid concentration and ultrasound power. The highest TFC was obtained at lower and higher citric acid concentrations. The middle citric acid concentration decreased flavonoid extraction. Moreover, TFC was linearly influenced by ultrasound power, and whatever ultrasound intensity was increased, the higher TFC was obtained. Addition of acids clearly altered the extraction of phenolic and flavonoid components. However, the extraction was critically influenced by the citric acid concentration. Based on reports, using the UAE technique supported the efficient extraction of flavonoids from walnut husk and pomegranate peel and increased TFC [35]. Indeed, UAE can generate microstreams in solvent media which result in "bubble cavitation." This phenomenon can proceed until the bubbles emerge and make larger bubbles, which eventually explode and release higher energy. This can affect the cell wall of plant tissues, which accelerates the extraction of entrapped flavonoids [21, 35, 41, 42]. The presence of acids in the extraction media can soften the cell walls, which can support the acceleration of flavonoid extraction. *Opuntia ficus-indica* was extracted for its phenolics, flavonols, and flavonoids using acidified solvents, and results showed that acidifying led to efficient extraction due to effects on changes in cell membrane and cell wall structures [30].

3.3.3. Effects on TBC. The effects of process variables on TBC are illustrated in Figure 3. The results were as similar as TFC, and TBC was significantly influenced by ultrasound power and citric acid concentration. The citric acid concentration indicated a polynomial relation with TBC, and higher BC was obtained at lower and higher concentrations of citric

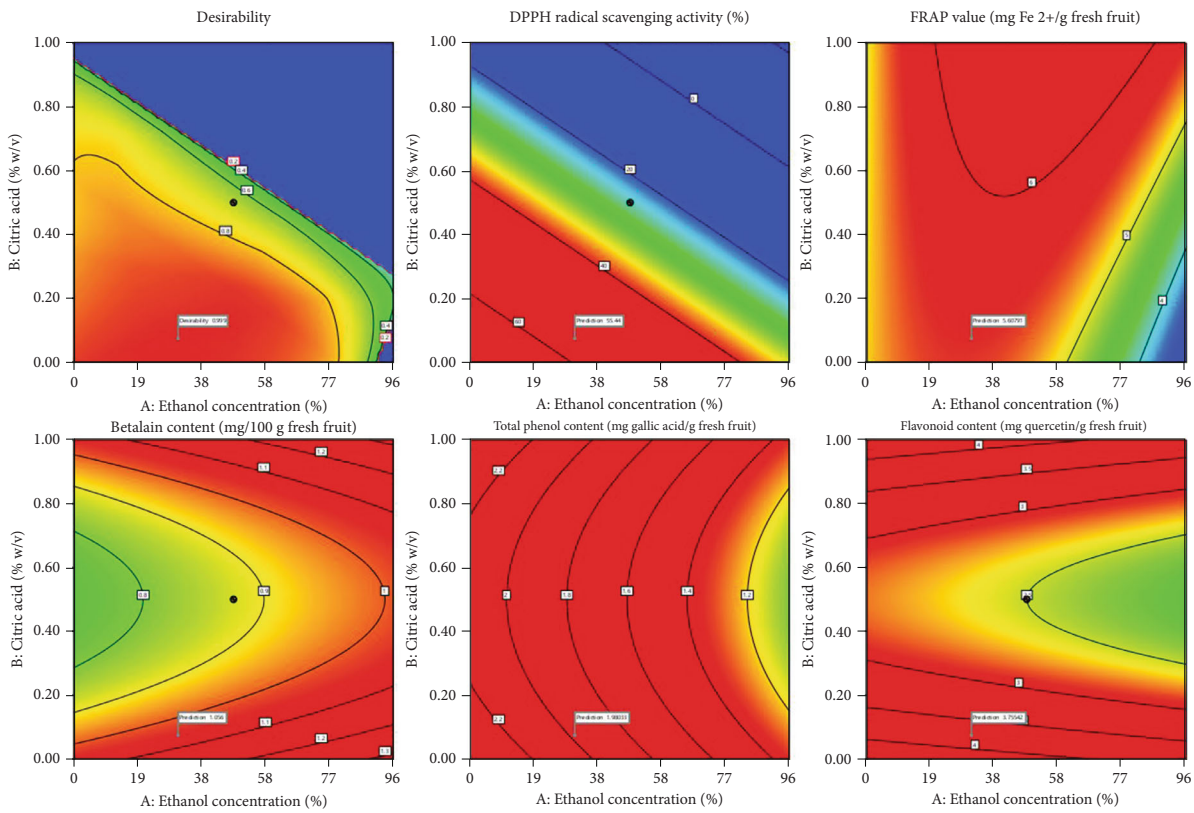

Figure 6: Optimal conditions based on the achievement of the highest values of total phenol content, total flavonoid content, total betalain content, DPPH RSA, and FRAP value. The effects of ethanol concentration (A) and citric acid concentration (B) were investigated.

acid, while lower values were obtained at middle concentration. Based on the reports, the methanolic extract with 0.1 mol of citric acid provided the highest anthocyanin content (23.01 mg/g) [40]. Also, it was reported that the number of anthocyanins was significantly increased when acidified ethanol was used along with a higher citric acid concentration (0.1 mol) [40]. Using water led to a lower extraction of anthocyanins, even when citric acid was added. Based on the data present in the literature, anthocyanins are more stable in an acid solution. Using acid in the extraction process can assist the phytochemical elicitation and can support the digestion of cell walls of the plant tissues. Therefore, the anthocyanins can be efficiently released in an acidic media [40]. Although ethanol concentration indicated an insignificant effect on TBC, it was an important factor in the extraction of betalains. Ultrasound power linearly influenced the TBC, so that the content of extraction was significantly increased ($p < 0.05$). UAE has been introduced as an efficient technique in the extraction of phytochemicals due to reducing the extraction time and energy [24, 35]. Besides, it softens the plant tissues, and the entrapped compounds can be easily diffused out [20, 26]. Thus, a higher power of ultrasound remarkably influenced the rate and yield of extraction.

UAE demonstrated effective recovery of betalain compounds from PPF. The optimal conditions were 23.15% glycerol, a 1:10 sample to solvent ratio, 10.43 min treatment time, and 31.15°C temperature. Under these conditions, the betalain content was 858.25 mg/L [24]. In Mexican and Spanish prickly pear's pulp, peel, and whole fruit, the highest level was found in pulp and whole fruit compared to peel [38]. In terms of pulp, the values of betalains were in the range of 240.5 to 2273.6 μg/g dry weight, and the greatest content was detected in the purple Mexican *Pelota* and Spanish *Morada* cultivars, followed by Spanish *Sanguinos* (red), Mexican *Vigor* (red), and Mexican *Diamante* (yellow), while the lowest level was found in the yellow Spanish *Verdal* cultivar [38].

3.3.4. Effects on DPPH RSA. The effects of process variables on DPPH RSA are illustrated in Figure 4, and experimental data are presented in Table 2. Two interaction terms, including AC and BC, exhibited significant effects. According to Figure 4, when ethanol concentration and ultrasound power were increased, the DPPH RSA indicated a decreasing trend. This could be due to high ethanol concentration, which negatively influenced the extraction of phenolic compounds. These components are water-soluble, while their extraction can be facilitated when organic solvents are used. However, the concentration of organic solvents, and especially ethanol, is crucial in withdrawing the respective components. There are various reports that have mentioned using mixed solvents to increase the extraction yield of antioxidants and phytochemicals. Hydroethanolic extraction of Algerian prickly pear led to the achievement of the highest percentage in terms of antioxidant activity [43]. Also, the same extract

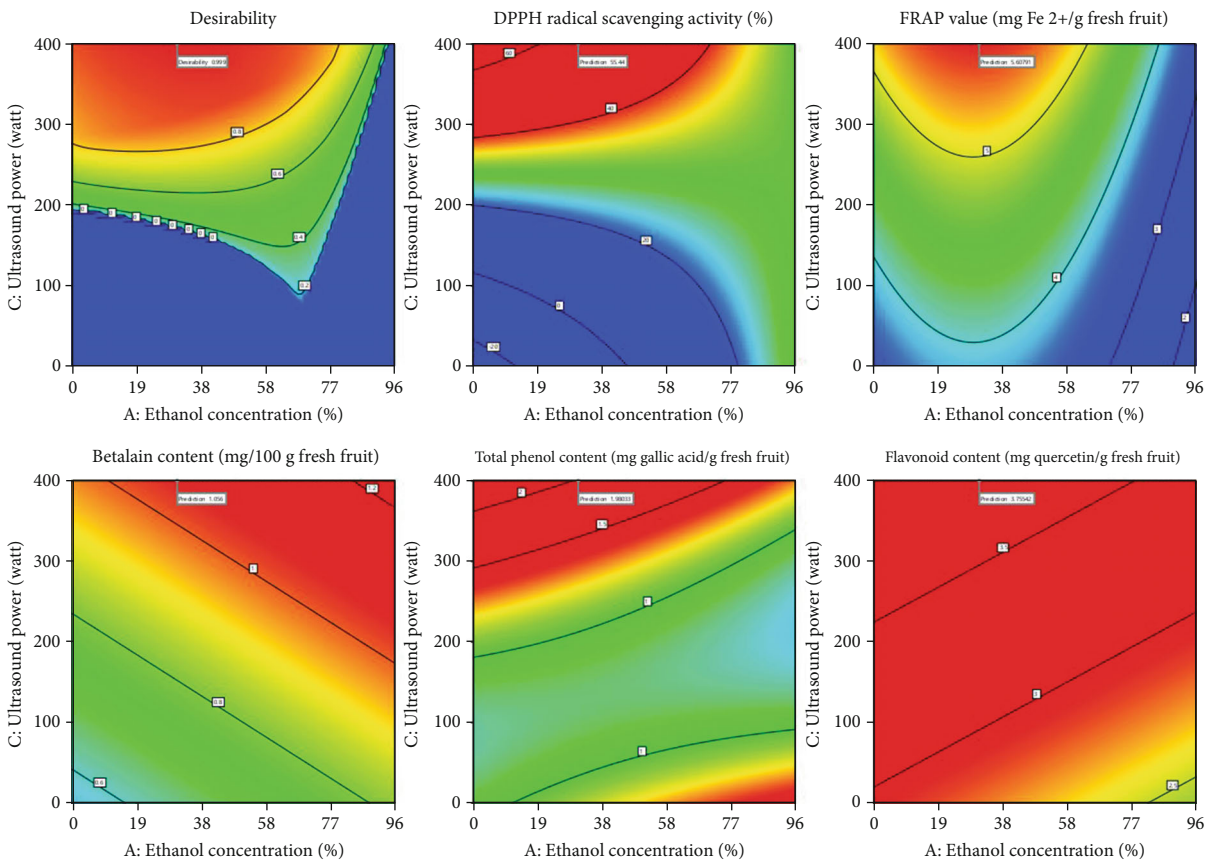

Figure 7: Optimal conditions based on the achievement of the highest values of total phenol content, total flavonoid content, total betalain content, DPPH RSA, and FRAP value. The effects of ethanol concentration (A) and ultrasound power (C) were investigated.

had a high content of phenolic compounds with 144.5 mg GAE/100 g dry matter, while the aqueous extract had TPC of 114.87 mg GAE/100 g. Polyphenols in *Opuntia* fruit are responsible for antioxidant activity [30]. They can block oxidative reactions caused by free radical generation through the ability to transfer hydrogen atoms or electrons. The site of the hydroxyl group and also their number can affect the antioxidant activity. The higher the number of hydroxyl groups, the stronger the antioxidant power [30, 38].

3.3.5. Effects on FRAP. The effects of process variables on FRAP value are presented in Figure 5. Also, the experimental data are provided in Table 2. Based on the ANOVA analysis, the linear terms of A, B, and C had significant effects on FRAP value. Regarding the ethanol concentration, whatever it was increased, FRAP indicated a declining trend. Ethanol concentration indicated a polynomial effect on the FRAP, while citric acid concentration and ultrasound power had linear impacts on FRAP value. When PPFE was obtained with high ethanol concentration, lower phenolic compounds were extracted due to the high polarity of polyphenols and their intention to be extracted with water. Albeit, it should be pointed out that polyphenols can be extracted with aqueous media, and the issue is their lower extraction efficiency. When organic solvents are used, the extraction of polyphenols with versatile polarity is facilitated. These results are in high agreement with the findings of previous studies [6, 30, 43]. Addition of citric acid and acidifying increased the FRAP values. Indeed, the addition of citric acid can facilitate the extraction efficiency of polyphenols, and higher FRAP antioxidant values can be rendered (Figure 5). It was observed that citric acid linearly increased FRAP value. *Opuntia ficus-indica* from Southern Africa was extracted using polar (ethanol, methanol, and water) and nonpolar (hexane) solvents. Regarding the extract from freeze-dried pulp, acidified water showed a higher FRAP value (22.95 μmol Fe^{2+}/g) compared to acidified methanol (19.83 μmol Fe^{2+}/g) and ethanol. The extracts from oven-dried pulp indicated different results. Acidified methanol indicated 58.66 μmol Fe^{2+}/g, followed by 27.01 μmol Fe^{2+}/g for ethanolic extract, and 15.49 μmol Fe^{2+}/g for aqueous extract. It was reported that polyhydroxylated flavonoids and phenolic acids occurred frequently in ethanolic and methanolic *Opuntia* extract. The presence of flavonoid glycosides in ethanolic and methanolic pulp and peel extracts may have contributed to their reduced antioxidant activities. Glycosylated polyphenols have a low ability to donate hydrogen and are less effective as antioxidants compared to their free aglycone forms. In addition, it has been reported that high TPC does not translate into high bioactivity. Based on the literature, Mexican *Opuntia robusta* fruits with high phenol content exhibited low antioxidant activity, and yellow *Opuntia albicarpa* fruits with reduced TPC demonstrated weak antioxidant activity. Indeed, the biological

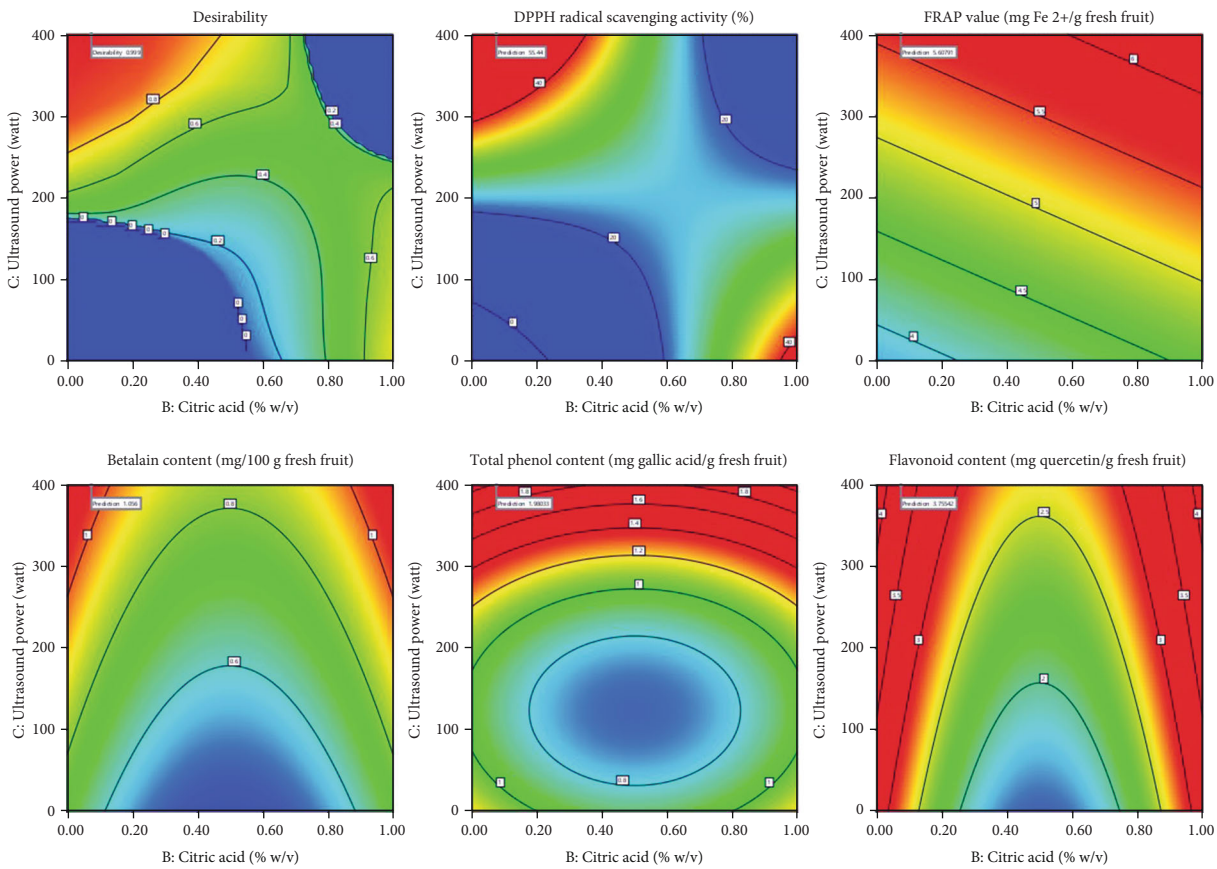

Figure 8: Optimal conditions based on the achievement of the highest values of total phenol content, total flavonoid content, total betalain content, DPPH RSA, and FRAP value. The effects of citric acid concentration (B) and ultrasound power (C) were investigated.

activity of extracts is associated with a single extract component or the synergistic effect of several extract components [30].

Another effective factor was ultrasound power, which had a positive effect on FRAP value. As illustrated in Figure 5, whenever ultrasound power was increased, FRAP value was increased. High power of ultrasound had destructive effects on the plant tissue that was softened by sonication. The cavitation phenomenon caused the explosion of bubbles, and the released energy resulted in the destruction of cell walls of plant tissues [4]. There are similar results that have declared remarkable effects of sonication on the cell walls of plant cells, enhancing the extraction efficiency [21, 44–46].

3.4. Numerical Optimization. The numerical optimization of the process was conducted based on the achievement of the maximum values of TPC, TFC, TBC, DPPH RSA, and FRAP. The results are provided in Table 8 and Figures 6, 7, and 8. Accordingly, the optimum point based on the maximum values was obtained at an ethanol concentration of 31.50%, a citric acid concentration of 0.074%, and an ultrasound power of 400 W, which rendered 1.97 mg GAE/g fresh fruit, 3.75 mg quercetin/g fresh fruit, 1.05 mg/100 g fresh fruit, 55.35%, and 5.60 mg Fe^{2+}/g fresh fruit for TPC, TFC, TBC, DPPH RSA, and FRAP value, respectively. This point has a high desirability (0.99) and performed under the experimental condition, and no significant difference was observed ($p > 0.05$). The optimum extract was assessed for betalain compounds through identification by HPLC-DAD-ESI-TOF-MS.

3.4.1. Validation Test. When the weight and significance values for five responses were determined to be equal, the numerical optimization approach was employed to optimize the extraction conditions (Table 8). In this research, the level of optimization was to be maximized for the TPC (1.97 mg GAE/g fresh fruit), TFC (3.75 mg Quercetin/g fresh fruit), TBC (1.05 mg betalain/100 g fresh fruit), antioxidant activity in terms of DPPH RSA (55.35%), and antioxidant activity in terms of FRAP assay (5.60 mg Fe^{2+}/g fresh fruit) of the fresh fruit extract samples (Table 8). The best optimal formula for maximum bioactive compound extraction from fresh fruit includes ethanol concentration (31.50%), citric acid concentration (0.074% *w/v*), and ultrasound power (400 watts) as the predicted results, whose desirability values were equal to 0.99 (Table 8). Furthermore, the disparity between predicted numbers (State-Ease Inc., Minneapolis, Minnesota, United States) and actual (performed in the laboratory) data was negligible and not significant.

3.5. Betalain Compounds. Betalains including betaxanthins and betacyanins were identified for the optimum extract by HPLC-DAD-ESI-TOF-MS, and predominant bioactive

compounds are presented in Tables 9 and 10. The molecular formula, retention times, UV-vis peak detection, scan *m/z*, and MS ion properties are displayed in Table 9. In this regard, histidine–betaxanthin (muscarine), glutamine–betaxanthin (vulgaxanthin), aminobutyric acid–betaxanthin, proline–betaxanthin (indicaxanthin), valine–betaxanthin, valine–betaxanthin isomer, isoleucine–betaxanthin, leucine–betaxanthin (vulgaxanthin), and phenylalanine–betaxanthin were the predominant betaxanthins detected in the optimum extract. The corresponding mean areas were 184.32, 87.65, 149.74, 9061.10, 65.10, 48.32, 98.32, 92.12, and 75.61, respectively, with the total value of 9862.28 (Table 10). Also, the predominant betacyanins were betanin, isobetanin, and Gomphrenin with the corresponding mean areas of 402.21, 103.61, and 23.41, respectively, with the total value of 529.23. These compounds, along with flavonoids and phenolic compounds, are responsible for antioxidant activity. Similar results were found in a study that obtained the extract by the conventional agitation extraction method [31]. In the respective study, a mixture of methanol and water (50:50) or methanol 50% and also 50 mM sodium ascorbate (for avoiding possible oxidations) were used while the obtained mixture was agitated at 225 rpm for 10 min in darkness [31]. In the mentioned study, the mean areas for histidine–betaxanthin (muscarine), glutamine–betaxanthin (vulgaxanthin), aminobutyric acid–betaxanthin, proline–betaxanthin (indicaxanthin), valine–betaxanthin, valine–betaxanthin isomer, isoleucine–betaxanthin, leucine–betaxanthin (vulgaxanthin), and phenylalanine–betaxanthin were obtained as 155.6, 48.7, 100.2, 7665.1, 43.9, 33, 72.9, 69.7, and 51.1, respectively, with the total value of 8231.3, which was significantly lower than that obtained in the present study ($p < 0.05$). Moreover, the mean areas of betacyanins including betanin (226.3), isobetanin (55.4), and gomphrenin I (11) were significantly lower than those obtained in the present study, with a total value of 292.6. The differences in mean areas can be attributed to the extraction method. UAE has been known as an efficient technique in facilitating and improving the extraction of bioactive compounds from plant and fruit tissues [23, 24, 47]. *Opuntia stricta* var. *dillenii*'s wild fruits were extracted for betalains and phenolic compounds using UAE as a "green" extraction method [21]. Betalains were identified by HPLC-DAD-ESI-MS. Betanin and 5″-O-E-sinapoyl-2′-apyosil-phyllocactin were the most abundant betalains, with 2.74 ± 0.02 g/g dry weight and 2.77 ± 0.01 mg/g dry weight, respectively. *Opuntia stricta* var. *dillenii* whole fruits were also a source of isobetanin with 1.68 ± 0.01 mg/g dry weight, neobetanin with 1.64 ± 0.00 mg/g dry weight, and 2′-O-apiosyl-4-O-phyllocactin with 1.22 ± 0.01 mg/g dry weight [21].

4. Conclusion

PPFE was optimized for its TPC, TFC, TBC, and antioxidant activities through UAE and RSM. The results showed that ethanol concentration, citric acid concentration, and ultrasound power can affect the extraction of phenolics, flavonoids, and betalains. Higher TPC, TFC, and TBC were obtained when a lower concentration of ethanol was applied. The addition of citric acid and acidifying the extraction media accelerated the extraction of phytochemicals. Higher ultrasound power (300 and 400 W) supported higher extraction efficiency of phytochemicals. The optimal point was obtained at an ethanol concentration of 31.50%, a citric acid concentration of 0.074%, and an ultrasound power of 400 W, which rendered 1.97 mg GAE/g fresh fruit, 3.75 mg quercetin/g fresh fruit, 1.05 mg/100 g fresh fruit, 55.35%, and 5.60 mg Fe^{2+}/g fresh fruit for TPC, TFC, TBC, DPPH RSA, and FRAP value, respectively. Histidine–betaxanthin (muscarine), glutamine–betaxanthin (vulgaxanthin), aminobutyric acid–betaxanthin, proline–betaxanthin (indicaxanthin), valine–betaxanthin, valine–betaxanthin isomer, isoleucine–betaxanthin, leucine–betaxanthin (vulgaxanthin), and phenylalanine–betaxanthin were the predominant betaxanthins identified by HPLC-DAD-ESI-TOF-MS. Also, the predominant betacyanins were betanin, isobetanin, and gomphrenin. Thus, PPFE can be introduced as a rich source of natural antioxidants and colorants for food fortification.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: S.P., M.G., N.S., H.A.A., and M.J. Data curation: S.P., M.G., H.A.A., and M.J. Formal analysis: S.P., M.G., and N.S. Funding acquisition: S.P. and M.G. Investigation: S.P., M.G., N.S., H.A.A., and M.J. Methodology: S.P., M.G., N.S., H.A.A., and M.J. Project administration: S.P., M.G., and N.S. Resources: S.P. and M.G. Software: S.P. and M.G. Supervision: M.G. and N.S. Advisor: H.A.A. and M.J. Validation: S.P., M.G., and N.S. Visualization: S.P., M.G., and N.S. Writing—original draft: S.P. and M.G. Writing—review and editing: S.P. and M.G.

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