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Extraction of Soluble Phenols and Flavonoids from Native Mexican Pigmented Corn Kernel Powder by Ultrasound: Optimization Process Using Response Surface Methodology

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Abstract: This study focused on optimizing ultrasound-assisted extraction (UAE) conditions (X_{PC}: pulse cycle of 1:1, 2:1, and 3:1 s on/off; X_{UP}: ultrasound power of 80, 90, and 100%; and X_{ET}: extraction time of 2, 4, and 6 min) for maximizing the content of soluble phenols (TSPs) and flavonoids (FLAs) from a native Mexican pigmented corn kernel powder through response surface methodology (RSM). Under the Box–Behnken design conditions, the UAE of TSPs ranged from 27.72 to 34.87 mg/g, while FLA content ranged from 16.59 to 27.28 mg/g. The highest content for TSPs was under 4 min X_{ET}, 1:1 s on/off X_{PC}, and 100% X_{UP}, while for flavonoids it was under 6 min X_{ET}, 2:1 s on/off X_{PC}, and 80% X_{UP}. According to RSM analysis, the optimal UAE conditions for TSPs were found to be X_{ET} 3.15 min, 1.58 s on/off X_{PC}, and 100% X_{UP}, and an X_{ET} of 4.18 min, 3 s on/off X_{PC}, and 80% X_{UP} were the best experimental conditions for FLAs with a predictive TSP of 35.07 mg/g and FLA of 27.51 mg/g. These data were adjusted in a second-order polynomial model and experimentally validated (TSP = 34.06 mg/g and 27.04 mg/g). Furthermore, the extracts demonstrated antioxidant activity (ABTS, FRAP, and DPPH methods) for optimal UAE for TSPs and FLAs. The antioxidant extract from the native Mexican pigmented corn kernel powder can be used for diverse industrial applications. Thus, the UAE is an effective and sustainable technology for recovering bioactive compounds from maize-based materials.

Keywords: native maize; pigmented corn; bioactive compounds; ultrasound; Box–Behnken design

1. Introduction

Zea mays, known as maize, is a plant belonging to the Gramineae family, and Mexico is recognized as the origin center. It is cultivated worldwide and is considered one of the most essential crops after rice and wheat [1]. Mexico ranks as the seventh largest producer of corn globally [2]. In Mexico, maize is a vital crop for the country's diet and economy, with significant social and cultural importance [3,4]. In Mexican cuisine, corn kernels are primarily consumed by humans as food and beverage products such as tortillas, tlayudas, tamales, atole, and tejuino [5]. Additionally, they can be used as an ingredient to prepare other food products, mainly in powder form. In this context, the white and yellow

hybrid corns are highly used at the industrial scale due to their higher production yields. On the other hand, Mexico is also known for its diversity of pigmented corn cultivars (comprising 68 from 350 cultivars reported in the Americas) that include white, yellow, red, pink, blue, purple, and black corn kernel colors [5,6], which are considered native and are produced and consumed locally due to their low production yields [7]. These pigmented corn kernels (mainly darker pigmented corns) contain phytochemicals (phenolic acids, flavonoids, carotenes, and anthocyanins) that exhibit a broad spectrum of biological properties (inflammatory, antibacterial, antidiabetic, antiproliferative, and antioxidant effects), which can be potentially used for diverse industrial applications [1,8].

Traditional technologies for extracting bioactive compounds from plant materials include maceration, hydro-distillation, and Soxhlet extraction [9]. However, these methods may degrade bioactive compounds and produce low extraction yields due to their harsh conditions, including long extraction times and high temperatures [1,10]. Diverse authors have utilized different extraction methods such as magnetic stirring (5 g powder/5 mL acetone water 70:30 for 60 min), constant agitation at 500 rpm (1 g powder/10 mL 80% ethanol for 15 min), and infrared-assisted extraction (1 g powder/70 mL water for 132 min at 73 °C) to extract phenolic compounds from pigmented corn kernels. These extraction methods exhibited advantages and limitations, mainly because they were time-consuming and recovered low yields. In contrast, non-conventional extraction technologies, particularly ultrasound, have emerged as an efficient alternative for extracting bioactive compounds from plant materials [1].

Ultrasound-assisted extraction (UAE) is an advanced, innovative, popular, simple-to-operate, cheap, fast, environmentally friendly, efficient, and sustainable alternative for extracting bioactive compounds from plant sources with good recovery yield [11]. UAE is based on the propagation of ultrasound waves (frequency of 20–100 kHz) in a liquid medium, causing a cavitation phenomenon (transient or stable) where, under compression and rarefaction of the acoustic field, cavitation bubbles grow and eventually collapse, promoting physical and chemical effects, breaking down the plant cell wall. After the implosion of the cavitation bubbles, the UAE involves solvent diffusion through the plant cell wall that facilitates the release of cellular components (phytochemicals) following the breakdown of the cell's outer layer [12]. This method has been successful in extracting phenolic compounds from various plant sources [13] and has been applied to maize-based materials such as corn pericarp [14], maize filaments [15,16], and corn husk [17]. In contrast, anthocyanins have been extracted by ultrasound from pigmented corn kernel samples [18,19] and carotenoids from yellow corn meal [20]. However, UAE process optimization is essential, as various factors influence the extraction process. In this context, diverse statistical and mathematical strategies, including Box–Behnken design (BBD) and response surface methodology (RSM) analysis, have been successfully used to examine UAE process optimization for extracting bioactive compounds from plant sources [21–24]. These methods help understand the effects and interactions of extraction factors, reduce the number of experiments required, and find the optimal experimental conditions for maximizing the recovery yield [13].

This work aimed to investigate the effect of extraction time (2, 6, and 10 min), pulse cycle (1:1, 2:1, and 3:1 s on/off), and ultrasound power (80, 90, and 100%) on the ultrasound-assisted extraction of soluble phenols and flavonoids from a Mexican native pigmented corn powder using the BBD and RSM.

2. Materials and Methods

2.1. Plant Material and Chemicals

The native pigmented corn kernel (*Zea mays*) was donated by a local producer in San Marcos, Jalisco, Mexico (latitude: 20°46′21.6″, longitude: W 104°17′8.36″, and altitude: 1524 m.a.s.l.). The pigmented corn kernel was chosen for its regular size, avoiding material with injuries (Figure 1a). The corn kernel was dried (50 °C/24 h) and ground in a rotatory impact mill (Retsch SR300, Haan, Germany). Then, the powder was sieved

to a particle size of 500 µm (stainless-steel mesh no. 35; Humboldt, AASHTO M92, IL, USA), as shown in Figure 1b. The pigmented corn kernel powder was protected from light and kept at 25 °C until analysis. All reagents and chemicals were analytical- or HPLC-grade. Methanol, Folin–Ciocalteu reagent, sodium acetate, gallic acid, 2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, catechin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferric chloride hexahydrate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hydrochloric acid, 2,4,6-tripyridyl-s-triazine (TPTZ), and water were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA) and aluminum chloride ($AlCl_3$) and sodium nitrite ($NaNO_3$) from Golden Bell (Guanajuato, Mexico).

Figure 1. Visual appearance of corn kernel (**a**) and corn kernel powder (**b**) of native Mexican pigmented corn.

2.2. Experimental Design

A three-level, three-factor Box–Behnken design (BBD) was used in a randomized experiment to find the best conditions for extracting soluble phenols and flavonoids from native Mexican pigmented corn kernel powder using ultrasound-assisted extraction. The design considered extraction time (X_{ET}; 2, 4, and 6 min), pulse cycle (X_{PC}; 1:1, 2:1, 3:1 s on/off), and ultrasound power (X_{UP}; 80, 90, and 100%). Fifteen combinations of these parameters (factors and levels) were examined (Table 1).

2.3. Ultrasound-Assisted Extraction (UAE) Procedure

To extract soluble phenols and flavonoids, a high-intensity ultrasonic processor with a nominal output power of 550 Watts and a frequency of 20 kHz (XMSJ, PZ-550LI, Zhengzhou City, China) coupled with a 6 mm diameter ultrasonic probe was employed [15]. Corn kernel powder (0.5 g) was mixed with a methanol/water solution (80:20, 25 mL) acidified with 2% *v/v* 2 M HCl. The mixture was sonicated and then centrifuged (8000× *g* for 10 min at 4 °C, Hermle Z32HK, Wehingen, Germany), and the resulting supernatants were stored at −20 °C until analysis [25]. The extraction temperature was maintained (25 ± 2 °C) using an ice bath.

2.4. Total Soluble Phenols (TSP)

TSP content was determined in a 96-well microplate applying a modified version of the Montreau method [26], as recommended [27]. The mixture reaction comprises corn extract (12 µL), Folin–Ciocalteu reagent at 2N (12 µL), sodium bicarbonate solution at 7.5% *w/v* (116 µL), and distilled water (164 µL). The mixture was homogenized in darkness (15 min), and the absorbance (750 nm) was recorded in a microplate reader (ACCURIS Instruments, SmartReader MR-9600, Nankín, China). Gallic acid was used as a standard (R^2 = 0.998), and the results are given as milligrams of gallic acid equivalents per gram of dry sample.

Table 1. Experimental runs and experimental and predicted values of the total soluble phenol and total flavonoid contents, error rate, and yield after ultrasound-assisted extraction from native Mexican pigmented corn kernel powder.

Run	Predictors [1]			Response Variables		Relative Error (%)	Yield (%)	Response Variables		Relative Error (%)	Yield (%)
	X_{ET} (min)	X_{PC} (s)	X_{UP} (%)	Experimental TSP [2]	Predicted TSP [3]			Experimental FLA [4]	Predicted FLA [5]		
1	2	3	90	27.72 ± 0.52 ^{ef}	28.50	–2.73	5.54	16.59 ± 0.43 ^{h}	17.64	–5.95	3.31
2	6	3	90	28.28 ± 0.68 ^{ef}	29.09	–2.78	5.65	18.34 ± 0.75 ^{g}	17.71	3.55	3.66
3	4	2	90	29.97 ± 0.59 ^{de}	31.20	–3.94	5.99	22.48 ± 0.37 ^{de}	20.95	7.30	4.49
4	6	2	100	29.86 ± 0.39 ^{de}	29.06	2.75	5.97	22.99 ± 0.33 ^{cd}	24.14	–4.76	4.59
5	6	1	90	29.91 ± 0.39 ^{de}	30.71	–2.60	5.98	24.57 ± 0.45 ^{bc}	23.94	2.63	4.91
6	4	2	90	30.17 ± 0.56 ^{de}	31.20	–3.30	6.54	20.35 ± 0.55 ^{f}	20.95	–2.86	4.07
7	2	2	100	32.04 ± 0.59 ^{bcd}	31.27	2.46	6.40	23.51 ± 0.33 ^{cd}	23.32	0.81	4.70
8	4	3	80	31.20 ± 0.52 ^{cd}	31.99	–2.46	6.24	26.60 ± 0.33 ^{a}	27.49	–3.23	5.32
9	4	1	80	27.35 ± 0.19 ^{f}	28.13	–2.77	5.47	25.76 ± 0.57 ^{ab}	26.66	–3.37	5.15
10	6	2	80	34.75 ± 0.68 ^{a}	33.95	2.35	6.95	27.28 ± 0.37 ^{a}	27.94	–2.36	5.45
11	4	1	100	34.87 ± 0.86 ^{a}	35.67	–2.24	6.97	27.05 ± 0.79 ^{a}	26.58	1.76	5.41
12	2	2	80	33.43 ± 1.09 ^{abc}	32.66	2.35	6.68	26.61 ± 0.18 ^{a}	24.71	7.68	5.32
13	4	3	100	34.69 ± 0.52 ^{a}	35.47	–2.19	6.93	23.86 ± 0.11 ^{cd}	23.38	2.05	4.77
14	4	2	90	30.07 ± 0.59 ^{de}	31.20	–3.63	6.81	20.87 ± 0.33 ^{ef}	20.95	–0.38	4.17
15	2	1	90	31.45 ± 0.98 ^{cd}	32.22	–2.38	6.29	19.33 ± 0.70 ^{fg}	20.38	–5.15	3.86

All values are means ± standard deviation (n = 9). Different letters in each file indicate significant statistical differences between treatments ($\alpha = 0.01$). [1] Extraction time (X_{ET}); pulse cycle (X_{PC}); ultrasonic power (X_{UP}); [2] gallic acid equivalents (mg GAE/g dry basis); [3] the values were predicted using a second-order polynomial equation, $R^2 = 0.8260$. [4] Catechin equivalents (mg CE/g dry basis); [5] the values were predicted using a second-order polynomial equation, $R^2 = 0.9648$.

2.5. Total Flavonoids

Total flavonoids (FLA) were determined in a test tube at room temperature [28]. An aliquot of corn kernel extract (125 µL) was combined with a 5% sodium nitrite solution (37.5 µL) and allowed to stand for five minutes. Subsequently, 10% aluminum chloride solution (37.5 µL) was added, followed by distilled water (300 µL) and 1M NaOH solution (250 µL) after six minutes. The mixture was homogenized in a vortex (60 s), and the resulting solution (200 µL) was transferred to a 96-well microplate. The absorbance was measured at 450 nm in a microplate reader. The results were calculated using a calibration curve of catechin standard (R^2 = 0.999), and results are reported as milligrams of catechin equivalents per gram of dry sample.

2.6. Ultrasound-Assisted Extraction Yield of Soluble Phenols and Flavonoids

The UAE yield was determined using Equation (1) and expressed as a percentage [29].

$$\text{Yield} = \frac{\text{Phenolic compound (g)}}{\text{Dried corn powder (g)}} \quad (1)$$

2.7. Response Surface Methodology (RSM) Analysis

After extracting the experimental phenolic compounds (soluble phenols and flavonoids), an RSM was used to determine the best conditions for extracting soluble phenols and flavonoids from native Mexican pigmented corn kernel powder using ultrasound-assisted extraction. Thus, a second-order polynomial (Equation (2)) that includes all terms was used to predict the response.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (2)$$

where

Y = predicted response (total soluble phenols or total flavonoids).
X = independent variables (X_{ET}, X_{PC}, and X_{UP}).
b_1, b_2, and b_{3v} = coefficients of linear effect terms.
b_{11}, b_{22}, and b_{33} = quadratic effect terms.
b_{12}, b_{13}, and b_{23} = interaction effect coefficients.

Furthermore, model adequacy was assessed using the F-ratio. Significant interactions in the model were identified using a lack-of-fit test, and the R-square and R-adjusted were assessed at a 99% confidence level [30].

To assess the accuracy of the model, experimental verification was conducted on the optimal conditions derived from RSM analysis for the extraction of soluble phenols and flavonoids from native Mexican pigmented corn kernel powder using UAE. The soluble phenols, flavonoid contents, and their antioxidant capacity were measured.

2.8. Antioxidant (AOX) Capacity

The AOX capacity of the corn kernel extracts was assessed through ABTS, DPPH, and FRAP assays. Regarding the ABTS test, an extract sample (35 µL) was reacted with ABTS radical solution at 7 mM (265 µL), and after shaking in darkness (7 min), absorbance (517 nm) was recorded [31]. In the DPPH assay, an extract sample (40 µL) was mixed with DPPH solution at 190 mM (190 µL), and after shaking in darkness (10 min), absorbance (734 nm) was measured [32]. For the FRAP assay, a mixture of extract sample (36 µL), FRAP solution (264 µL), and distilled water (9 µL) was prepared, and after shaking in darkness (30 min), absorbance (595 nm) was recorded [33]. The FRAP solution encompassed sodium acetate buffer at 0.3 M and pH 3.6, ferric chloride hexahydrate at 20 mM, and 2,4,6-tripyridil-s-triazine at 10 mM. These reagents were mixed at 10:1:1 $v/v/v$ ratio.

The absorbance for all antioxidant capacity assays was recorded in a 96-well microplate reader, and the results are presented as millimole Trolox equivalent (mmol TE/g dry sample), calculated with high accuracy using a Trolox standard curve (R^2 = 0.992, 0.999, 0.997, respectively).

2.9. Statistical Analysis

RSM analysis was employed using STATISTICA software (v. 12.5 Statsoft®, Tulsa, OK, USA). Data are expressed as mean ± standard deviation (n = 9). Data were examined by ANOVA ($p < 0.01$), and the means were analyzed by the Tukey test ($\alpha = 0.01$). Furthermore, the Student *t*-test ($p < 0.01$) was used to study the differences in antioxidant activity response among optimal ultrasound-assisted extraction conditions. All experiments and determinations were performed in triplicate.

3. Results and Discussion

3.1. Ultrasound-Assisted Extraction of Soluble Phenols and Flavonoids from Pigmented Corn Powder

To investigate the effect of independent variables [X_{ET} = extraction time (2, 4, and 6 min), X_{PC} = pulse cycle (1:1, 2:1, and 3:1 s on/off), and X_{UP} = ultrasonic power (80, 90, and 100%)] on the extraction of soluble phenols (TSP) and flavonoids (FLA) by UAE from the native Mexican pigmented corn powder, fifteen experiments were performed using a three-factor and three-level Box–Behnken design, and to avoid systematic errors, all experiments were conducted in a randomly ordered manner. The results are shown in Table 1, which includes the experimental and predicted values, error rate, and extraction yield. Statistical differences were detected among treatments for TSPs and FLAs in an experimental-conditions-dependent manner ($p < 0.01$). Results show that the yield ranged from 5.54 to 6.97% for TSPs and 3.31 to 5.45% for FLAs, similar to those reported in other plant materials when applying UAE for extracting TSPs [34]. The highest TSP content was obtained at 4 min of extraction time and 100% of ultrasonic power, considering a 1:1 s on/off (34.87 mg GAE/g DW) or 3:1 s on/off (34.69 mg/g) pulse cycle. Furthermore, the highest FLA content was obtained at 80% of ultrasonic power at an extraction time of 4 min and 3:1 s on/off pulse cycle (27.49 mg CE/g DW) or 80% of ultrasonic power, 6 min extraction time, and 2:1 s on/off pulse cycle (27.94 mg/g). The lowest TSP and FLA contents were found under the experimental conditions of 2 min extraction time, pulse cycle of 3:1 s on/off, and ultrasonic power of 90% (27.72 mg/g and 16.59 mg/g, respectively). These values are higher than those reported in blue (TSP of 3.07 mg/g) and purple (TSP of 7 mg/g) native pigmented corn kernel powders by traditional extraction methods, including maceration and magnetic stirring [35,36]. UAE has been successful in extracting bioactive molecules from plant tissues [37–39]. This success can be attributed to the fact that ultrasound waves, through the cavitation phenomenon, improve solvent penetration into the plant material, increasing the contact surface area between liquid and solid phases, which facilitates the extraction of phenolic compounds [11,16]. Additionally, during the UAE, the temperature is controlled at 25 °C (±2 °C), which reduces polyphenol degradation and increases the extraction yield; nonetheless, the temperature control during UAE is vital to enhance the cavitation effects [40].

3.2. Response Surface Methodology (RSM) Analysis for the TSPs and FLAs from Pigmented Corn Powder

An RSM analysis determined the optimal UAE conditions for TSPs and FLAs from native Mexican pigmented corn kernel powder. The statistically significant influence of the independent factors (X_{ET}, X_{PC}, and X_{UP}) on the UAE of the TSPs and FLAs was verified by the analysis of variance (ANOVA, $p < 0.01$), as listed in Table 2. Regarding TSPs, the ANOVA showed that most of the experimental factors and their interactions were significant ($p < 0.01$), except for X_{PC}, X_{UP}, X_{ET}*X_{PC}, and X_{ET}*X_{PC}^2, and similar trends were observed for FLAs (except for X_{PC}^2 and X_{ET}*X_{UP}; $p > 0.01$). Rashad et al. [41] reported that some factors (time) and their interactions (solvent*time) were not significant. Similar trends were observed during the UAE of phenolic compounds from *Myrciaria cauliflora* fruit; the extraction temperature, sonication amplitude, pulse cycle, and their interactions were not significant ($p > 0.01$) [42].

Table 2. ANOVA for the quadratic model using ultrasound-assisted extraction conditions for native Mexican pigmented corn kernel powder.

Response	Source	SS	DF	MS	F-Value	*p*-Value	Lack of Fit (*p*-Value)	R-Square	R-Adjust
TSP	X_{ET}	1.19	1	1.19	0.98	0.3286 $^{++}$	0.4120 $^{++}$	0.8260	0.788
	X_{ET}^2	16.13	1	16.13	13.25	0.0009 $^{+}$			
	X_{PC}	7.43	1	7.43	6.10	0.019 $^{++}$			
	X_{PC}^2	32.19	1	32.19	26.43	<0.0001 $^{+}$			
	X_{UP}	0.36	1	0.36	0.29	0.5903 $^{++}$			
	X_{UP}^2	24.18	1	24.18	19.85	<0.0001 $^{+}$			
	X_{ET}*X_{PC}	3.32	1	3.32	2.73	0.1080 $^{++}$			
	X_{ET}*X_{PC}^2	0.006	1	0.006	0.005	0.9414 $^{++}$			
	X_{ET}^2*X_{PC}	24.18	1	24.18	19.85	<0.0001 $^{+}$			
	X_{ET}*X_{UP}	9.18	1	9.18	7.53	0.0098 $^{+}$			
	X_{ET}^2*X_{UP}	112.19	1	112.19	92.12	<0.0001 $^{+}$			
	X_{PC}*X_{UP}	12.18	1	12.18	10.08	0.0034 $^{+}$			
	Error	38.97	32	1.21					
FLA	X_{ET}	29.89	1	29.89	66.32	<0.0001 $^{+}$	0.0551 $^{++}$	0.9648	0.9540
	X_{ET}^2	13.26	1	13.26	29.42	<0.0001 $^{+}$			
	X_{PC}	61.84	1	61.84	137.21	<0.0001 $^{+}$			
	X_{PC}^2	1.80	1	1.80	4.01	0.0536 $^{++}$			
	X_{UP}	39.47	1	39.47	87.57	<0.0001 $^{+}$			
	X_{UP}^2	280.82	1	280.82	623.07	<0.0001 $^{+}$			
	X_{ET}*X_{PC}	9.14	1	9.14	20.28	<0.0001 $^{+}$			
	X_{ET}*X_{PC}^2	17.48	1	17.48	38.80	<0.0001 $^{+}$			
	X_{ET}*X_{UP}	1.05	1	1.05	2.33	0.1359 $^{++}$			
	X_{ET}^2*X_{PC}	16.38	1	16.38	36.36	<0.0001 $^{+}$			
	X_{ET}^2*X_{UP}	13.24	1	13.24	29.29	<0.0001 $^{+}$			
	X_{PC}*X_{UP}	12.17	1	12.17	27.01				
	Error	14.42	32	0.45					

Extraction time (X_{ET}); pulse cycle (X_{PC}); ultrasonic power (X_{UP}); $^{+}$ significant ($p < 0.01$), $^{++}$ non-significant ($p > 0.01$).

Table 3 shows the β-coefficients of the fitted regression models for the TSP and FLA contents of UAE of native Mexican pigmented corn kernel powder. Most of the β-coefficients were significant ($p < 0.01$) for TSP, except X_{PC}, X_{PC}^2, and X_{ET}^2*X_{PC}, and similar trends were observed for FLA, where X_{PC}^2 and X_{ET}^2*X_{UP} were nonsignificant ($p > 0.01$). In both cases, the X_{PC} and X_{ET}*X_{PC} exhibited a positive effect during extraction. The pulse cycle has been reported to influence bubble formation during cavitation, improving/affecting the UAE [34]. From this information, a second-order polynomial equation was obtained for each response variable, excluding coefficients that were not significant ($p > 0.01$) aimed to improve the prediction ability of the model [38]. These mathematical models can predict the effect of the evaluated factors on the TSP and FLA contents (Equations (3) and (4)). Moreover, TSPs and FLAs showed an advisable relation with the predicted value and the experimental data ($R^2 = 0.8260$ and $R^2 = 0.9648$) for the estimated model. The nonsignificant lack of fit ($p > 0.01$) values imply good prediction of the model, indicating the effectiveness of the model [43], but also the R^2 is relevant to verify the model's suitability [44]. According to Fernández-Barbero et al. [42], a low value of R^2 during the UAE of phenolic compounds from a vegetable matrix can be associated with the type of bioactive compound extracted, considering its polarity and size, as demonstrated during the UAE of phenolic compounds from *Myrciaria cauliflora* fruit (R^2 of 0.706). Several authors have reported R^2 values ranging from 0.96 to 0.99 when applying UAE methods for corn-based materials and adequate adjustment of the experimental data to the models (lack of fit, $p > 0.05$ or $p > 0.01$) [14–16]. Moreover, it has also been reported that an R^2 of 0.78 to 0.85 in polynomial equations obtained from UAE processes exhibited adequate adjustment between the experimental and predicted data [45–47]. In this study, all experimental conditions exhibited similar values of TSP and FLA contents between experimental and predicted data when UAE was

used; nonetheless, their relative error was less than 10% (Table 1), similar to errors reported by other authors during the optimization of UAE using BBD and RSM [48,49].

$$\text{Total soluble phenols (mg GAE/g)} = 272.82 - 81.39X_{ET} + 10.58X_{ET}^2 - 3.97\,X_{UP} + 0.01X_{PC}^2 + 4.76X_{ET}{*}X_{PC} - 0.56X_{ET}^2{*}X_{PC} + 0.82X_{ET}{*}X_{UP} - 0.10X_{ET}^2{*}X_{UP} - 0.10X_{PC}{*}X_{UP} \quad (3)$$

$$\text{Total flavonoids (mg CE/g)} = 419.79 - 3.07X_{ET} + 0.81X_{ET}^2 - 8.89X_{UP} + 0.05X_{UP}^2 + 2.81X_{ET}{*}X_{PC} - 4.12X_{ET}^2{*}X_{PC} - 0.002X_{ET}^2{*}X_{UP} - 0.10X_{PC}{*}X_{UP} \quad (4)$$

Table 3. Regression coefficients for total soluble phenol and total flavonoid contents extracted by ultrasound from native Mexican pigmented corn kernel powder.

Source	Total Soluble Phenols		Source	Total Flavonoids	
	β-Coefficient	*p*-Value		β-Coefficient	*p*-Value
Mean/Interc	256.32	<0.0001 $^{+}$	Mean/Interc	449.70	<0.0001 $^{+}$
X_{ET}	–81.21	<0.0001 $^{+}$	X_{ET}	–25.47	<0.0001 $^{+}$
X_{ET}^2	10.55	<0.0001 $^{+}$	X_{ET}^2	3.88	<0.0001 $^{+}$
X_{PC}	6.46	0.2133 $^{++}$	X_{PC}	18.85	<0.0001 $^{+}$
X_{PC}^2	–1.63	0.0975 $^{++}$	X_{PC}^2	–3.81	0.0851 $^{++}$
X_{UP}	–3.73	<0.0001 $^{+}$	X_{UP}	–9.35	<0.0001 $^{+}$
X_{UP}^2	0.01	<0.0001 $^{+}$	X_{UP}^2	0.049	<0.0001 $^{+}$
$X_{ET}{*}X_{PC}$	8.48	<0.0001 $^{+}$	$X_{ET}{*}X_{PC}$	–0.54	0.4900 $^{++}$
$X_{ET}{*}X_{PC}^2$	–0.01	0.9419 $^{++}$	$X_{ET}{*}X_{PC}^2$	0.85	<0.0001 $^{+}$
$X_{ET}^2{*}X_{PC}$	–0.56	<0.0001 $^{+}$	$X_{ET}^2{*}X_{PC}$	–4.13	<0.0001 $^{+}$
$X_{ET}{*}X_{UP}$	0.82	<0.0001 $^{+}$	$X_{ET}{*}X_{UP}$	0.28	<0.0001 $^{+}$
$X_{ET}^2{*}X_{UP}$	–0.10	<0.0001 $^{+}$	$X_{ET}^2{*}X_{UP}$	–0.03	<0.0001 $^{+}$
$X_{PC}{*}X_{UP}$	–0.10	0.0034 $^{+}$	$X_{PC}{*}X_{UP}$	–0.10	<0.0001 $^{+}$

Extraction time (X_{ET}); pulse cycle (X_{PC}); ultrasonic power (X_{UP}); $^{+}$ significant ($p < 0.01$), $^{++}$ non-significant ($p > 0.01$).

The three-dimensional response surface plots display the significant interaction effects ($p < 0.01$) of UAE parameters on the TSP (Figure 2a,c,e) and FLA (Figure 2b,d,f) contents in pigmented corn powder. Nonetheless, from these 3D plots, it is possible to visually identify the region that presented the highest UAE of TSP and FLA contents [50]. The results suggest that TSP and FLA extraction from native Mexican pigmented corn kernel powder under UAE occurred under all extraction conditions, regardless of the extraction time, pulse cycle, or ultrasound power. Nonetheless, the elliptical shape of the plots indicates a significant interaction of the independent variables [45]. In the case of a 1:1 s on/off pulse cycle, 100% ultrasound power for 4 min is required for the extraction of a high content of TSPs (Figure 2a), while for FLAs, 100% ultrasound power for at least 6 min is necessary for a high content of FLAs (Figure 2b). Nevertheless, with a 2:1 s on/off pulse cycle, the highest STP content can be achieved at 100% or 80% ultrasound power for 4 or 6 min extraction time, respectively (Figure 2c). In contrast, for FLAs, 80% of ultrasound power for 6 min is necessary (Figure 2d). On the other hand, under a 3:1 s on/off pulse cycle, 100% ultrasound power for 3 min is required for the extraction of a high content of TSPs (Figure 2e), while 80% ultrasound power for 6 min is needed for a high FLA content (Figure 2f).

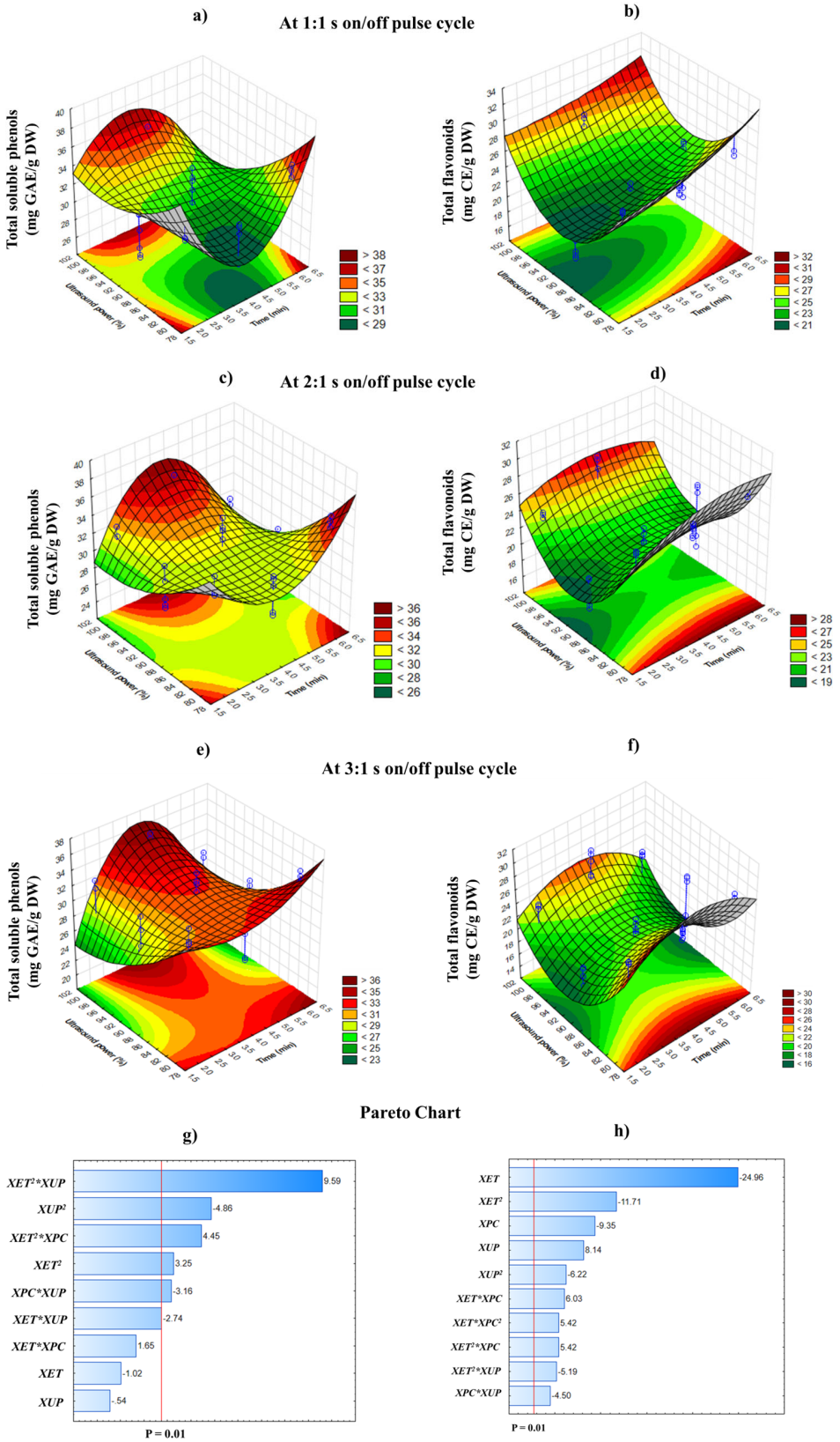

Figure 2. Response surface plots and Pareto Chart indicating the effect of ultrasound-assisted extraction on the total soluble phenols (**a**,**c**,**e**,**g**) and total flavonoids (**b**,**d**,**f**,**h**) contents from native Mexican pigmented corn kernel powder at 1:1, 2:1, and 3:1 second on/off pulse cycle. Extraction time (X_{ET}); pulse cycle (X_{PC}); ultrasonic power (X_{UP}).

Additionally, the Pareto Chart reveals the effect of independent variables in the polynomial equation on extracting TSPs (Figure 2f) and FLAs (Figure 2g) at a confidence level of 99%. The most important parameter during the UAE of TSPs was the interaction between extraction time and ultrasound power ($X_{ET}{}^2{*}X_{UP}$). It has been reported that ultrasonic power significantly influenced the effectiveness of ultrasound extraction; the increase in released compounds is attributable to acoustic cavitation by increasing mass transfer [51]. However, the extraction time is an important parameter because long extraction times can promote the degradation of bioactive compounds. Albarri and Sahin [52] reported that the recovery yield of phenolics from *Moringa oleifera* leaves using UAE was significantly affected by extraction time and ultrasound power and suggested that an equilibrium extraction time should be found. Conversely, the most critical parameter for extracting FLAs by ultrasound was extraction time (X_{ET}), followed by pulse cycle (X_{PC}). Adequate extraction times and pulse cycle conditions may improve bubble formation during cavitation, enhancing cell damage and the release of compounds; moreover, the pulse mode facilitates the extraction of phenolic compounds from plant materials when applying ultrasound, enabling control of temperature [48].

3.3. Optimization of the Extraction Process and Validation of the Models

Optimizations based on numerical values were applied as suggested to optimize the UAE variables, resulting in the optimal response [29]. The optimal UAE conditions for extracting TSPs and FLAs from native Mexican pigmented corn kernel powder are shown in Figure 3. The best-optimized conditions for TSPs were a 3.15 min extraction time, a 1.58 s on/off pulse cycle, and 100% ultrasound power (Figure 3a); these new experimental conditions did not match any run in the original experimental trial. It must be noted that higher extraction times and pulse cycles and low ultrasound power produced lower extraction yields of TSPs [53]. On the other hand, an extraction time of 4.18 min, a 3 s on/off pulse cycle, and 80% ultrasound power were the best experimental conditions for FLAs (Figure 3b). These experimental conditions matched with the eight-standard order in the original experimental trial. Ozcan and Daman [49] reported that during the UAE of phenolic compounds from spinach roots, the optimized conditions obtained from response surface methodology can match some runs of the original experimental trial. In general, the UAE of FLAs required more extraction time than TSPs, and these results agree with those reported by Sanou et al. [53], who explained that FLAs needed a higher extraction time during UAE due to the electrosensitivity of these molecules.

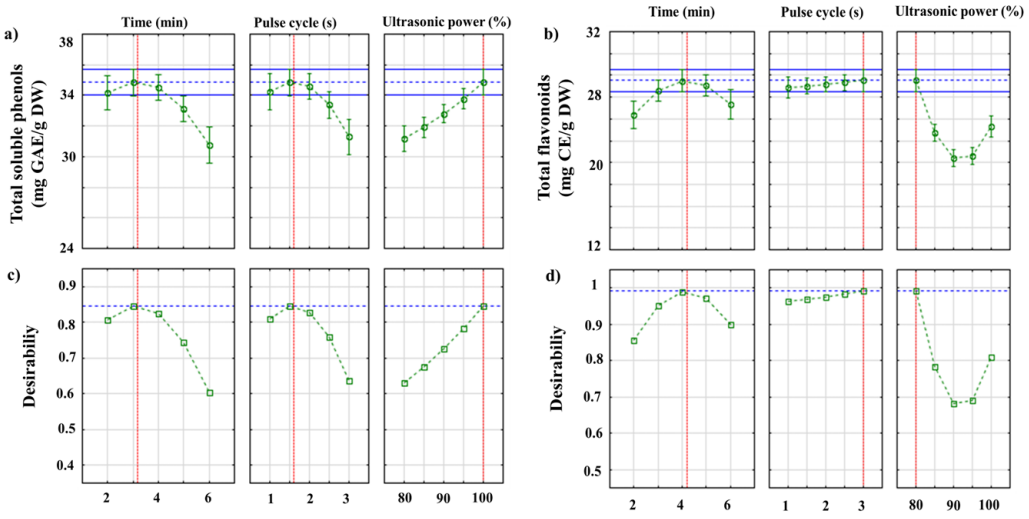

Figure 3. Optimization and desirability profile plots for total soluble phenol (**a**,**c**) and total flavonoid (**b**,**d**) contents from native Mexican pigmented corn kernel powder.

Additionally, the UAE of TSPs and FLAs from native Mexican pigmented corn kernel powder exhibited desirability (D) values of 0.86 and 0.98 (Figure 3c,d), suggesting a good prediction performance. In the scale of desirability function, D values ranging from 1 to 0.8 are classified as "very good" [49]. Furthermore, the desirability function for optimizing the UAE of bioactive compounds from vegetable matrices has been widely used [49,54]. On the other hand, these new experimental conditions produced predicted optimal responses of 35.07 mg GAE/g for TSPs and 27.51 mg CE/g for FLAs (Table 4).

Table 4. Predicted TSP and FLA contents and antioxidant activity of native Mexican native pigmented corn kernel powder at optimal ultrasound-assisted extraction conditions.

Parameter	Total Soluble Phenols (mg GAE/g)		Total Flavonoids (mg CE/g)	
	Predicted	Experimental	Predicted	Experimental
Extraction time (min)	3.158	3.158	4.188	4.188
Pulse cycle (s)	1.58	2 ♦	3	3
Ultrasound power (%)	100	100	80	80
Optimal response	35.07	34.06	27.51	27.04
−99% confidence level	33.98	33.57	26.52	26.61
+99% confidence level	36.17	34.54	28.52	27.47
Antioxidant activity				
DPPH (mmol TE/g)	80. 86 ± 0.16 ^{aA}		79.77 ± 0.33 ^{aA}	
ABTS (mmol TE/g)	43.78 ± 0.28 ^{cA}		31.31 ± 0.19 ^{cB}	
FRAP (mmol TE/g)	59.43 ± 0.32 ^{bA}		54.65 ± 0.60 ^{bB}	

GAE: gallic acid equivalent; CE: catechin equivalent; TE: trolox equivalent. ♦ Due to the ultrasound characteristics, the pulse cycle was used at 2:1 s on/off conditions. Lowercase letters indicate significant statistical differences between antioxidant activities ($p < 0.01$). Capital letters indicate significant statistical differences ($p < 0.01$) between ultrasound-assisted extraction conditions.

The predictive empirical models were validated through verification experiments conducted under optimal conditions. Table 4 shows the predicted and experimental values of TSPs and FLAs under optimal conditions by response surface methodology analysis. The experimental TSP (34.06 mg GAE/g) and FLA (27.04 mg CE/g) results were very close to the predicted values (35.07 mg GAE/g and 27.51 mg CE/g, respectively), demonstrating the adequacy and reliability of the fitted model for TSP and FLA responses.

Additionally, the methanolic extracts obtained from the native Mexican pigmented corn kernel powder under optimal ultrasound-assisted extraction conditions for TSPs (extraction time of 3.158 min, ultrasound power 100%, and pulse cycle of 2 s on/off) and FLAs (extraction time of 3.188 min, ultrasound power 80%, and pulse cycle of 3 s on/off) exhibited antioxidant properties through DPPH, ABTS, and FRAP methods. Under the optimal conditions (Table 4), the antioxidant capacity of the pigmented corn kernel extract for both TSP and FLA extraction conditions was DPPH > FRAP > ABTS ($p < 0.01$). Furthermore, no differences ($p > 0.01$) were observed between TSP and FLA extraction conditions for DPPH (80.86 and 79.77 mmol TE/g). However, for the ABTS (43.78 and 31.31 mmol TE/g) and FRAP (59.43 and 54.65 mmol TE/g) methods, the extraction conditions for TSPs resulted in higher antioxidant properties ($p < 0.01$). These findings could be related to the recovered phenols and how they neutralize radicals (donating electrons, chelating metals, or transferring electrons) [55]. Furthermore, these results indicate that phenolic compounds from native Mexican pigmented corn kernel powder could be utilized in various industrial applications.

4. Conclusions

The variation in ultrasound-assisted extraction (UAE) parameters exhibited a positive impact on soluble phenols (TSP) and flavonoids (FLA) extracted from native Mexican pigmented corn kernel powder. By using response surface methodology, the UAE conditions were successfully optimized. The results showed that the combination of extraction time and pulse cycle had the most significant influence on the extraction of TSP. In contrast,

extraction time was critical for FLA extraction. Additionally, the ultrasound power significantly affected the recovery yield; 100% ultrasound power was required for TSP extraction, while 80% ultrasound power was sufficient for FLA extraction. This study highlights that employing statistical tools along with ultrasound can enhance the extraction of compounds with antioxidant properties from a native Mexican pigmented corn kernel powder, which could be potentially used in functional or nutraceutical foods and beverages, as well as in pharmaceutical and cosmetics applications. Further studies are needed to evaluate the potential biological activities of native pigmented corn kernel extracts, as well as to evaluate their toxicological effects. Furthermore, the impact of different solvents and solid–liquid ratios in extracting bioactive from this or other native colored corn materials could be assessed.

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