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Ultrasonic bath assisted extraction of total polyphenols and betalains from dragon fruit peel: Optimization by Box–Behnken design

Extracción de polifenoles y betalainas totales de la cáscara de pitahaya mediante baño ultrasónico: Optimización por diseño Box-Behnken.

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ABSTRACT

This study aimed to optimize the conditions for ultrasonic bath-assisted extraction of betalains, polyphenols, and antioxidant compounds from dragon fruit peel (*Hylocereus undatus*). A Box-Behnken design was employed to evaluate the factors of sonication time, temperature, and ethanol percentage used as a solvent. The results showed that the optimal conditions for betalain extraction were a sonication time of 24 min, a temperature of 26°C, and 48 % ethanol. For total polyphenols, the optimal time was 80 min, the temperature was 53°C, and the ethanol percentage was 54 %. Antioxidant compounds exhibited optimal extraction conditions with a time range of 72 to 80 min, a temperature of 70°C, and an ethanol percentage of 54 % to 56 %. The maximum extraction yields were 1197.7 ± 32.7 µg/g of betalains, 5765.3 ± 148.4 µg/g of polyphenols, 2191.6 ± 180.1 µg Trolox/g by DPPH, and 1503.5 ± 25.8 µg Trolox/g by ABTS. No significant differences ($p \leq 0.05$) were observed between the optimal experimental values and the theoretical ones, indicating that the optimized extraction factors reliably predict extraction yields. These findings demonstrate the potential of dragon fruit peel as a sustainable source of natural antioxidants and pigments, supporting its application in the formulation of functional foods and contributing to the valorization of this underutilized agro-industrial by-product.

Keywords: Antioxidant activity; Betacyanins; Betaxanthins; *Hylocereus undatus*; Response surface.

RESUMEN

El objetivo del presente trabajo es optimizar las condiciones de extracción asistida por baño ultrasónico de betalaínas, polifenoles y compuestos antioxidantes a partir de la cáscara de pitahaya (*Hylocereus undatus*). Para su realización, se utilizó un diseño Box Becken en el que se evaluaron los factores: tiempo de sonicación, temperatura y el porcentaje de etanol utilizado como solvente. Los resultados mostraron que las condiciones óptimas para la extracción de betalainas fueron un tiempo de 24 minutos, una temperatura de 26 °C y un porcentaje de etanol del 48 %. En el caso de los polifenoles totales el tiempo óptimo fue de 80 min, la temperatura de 53 °C y el porcentaje de etanol del 54 %. Los compuestos antioxidantes tuvieron un tiempo de extracción óptimo entre los 72 y los 80 min, una temperatura de 70 °C y un porcentaje de etanol del 54 al 56 %. Los valores máximos de extracción fueron 1197.7 ± 32.7 µg/g de betalainas, 5765.3 ± 148.4 µg/g de polifenoles, 2191.6 ± 180.1 µg de trolox/g por DPPH y 1503.5 ± 25.8 µg de trolox/g por ABTS. No hubo diferencias significativas ($p \leq 0.05$) entre los valores experimentales y teóricos, pudiéndose inferir que los factores de extracción optimizados predicen con fiabilidad el rendimiento de la extracción. El presente trabajo demuestra el potencial de la cáscara de pitahaya como una fuente sostenible de antioxidantes y pigmentos naturales, respaldando su aplicación en la formulación de alimentos funcionales y contribuyendo a la valorización de este subproducto agroindustrial subutilizado.

Palabras clave: Actividad antioxidante; Betacianinas; Betaxantinas; *Hylocereus undatus*; Superficie de respuesta.

INTRODUCTION

The pitahaya, also known as dragon fruit, is an exotic fruit obtained from a perennial cactus species of the Hylocereus genus. It is native to the tropical and subtropical regions of the Americas and is considered a crop of global importance (Chen *et al*., 2021). Its significance lies in its recent surge in popularity due to its economic value and potential health benefits (Oney-Montalvo *et al*., 2023). Additionally, it can serve as a raw material in the food industry for the production of beverages, flours, yogurts, and jams (Tarte *et al*., 2023).

Unfortunately, not all parts of the fruit are utilized in industrial processes, and some by-products, such as the peel, are discarded (Jiang *et al*., 2021). The peel constitutes approximately one-third of the fruit and is often treated as waste from consumption or as a by-product of pulp processing. However, it is rich in nutrients and bioactive compounds (Jiang *et al*., 2021). Among these bioactive compounds, betalains and polyphenols stand out, which can be used to

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develop functional foods or as antioxidants to extend the shelf life of food products (Cunha *et al*., 2018).

Betalains are water-soluble pigments that, compared to other natural pigments such as anthocyanins, carotenoids, and chlorophylls, have not been extensively studied (Slimen *et al*., 2017). They are classified into two types: betacyanins and betaxanthins, both of which exhibit antioxidant, anticancer, antilipidemic, and antimicrobial activities (Oney-Montalvo *et al*., 2023). Betalains have been approved by the European Union for use as a food additive, offering the advantage of greater stability against pH and temperature changes, allowing them to be used in a wide variety of food products (Thirugnanasambandham and Sivakumar, 2017). On the other hand, polyphenols are plant secondary metabolites of significant interest to the food and pharmaceutical industries due to their biological properties (Rasouli *et al*., 2017). Studies have shown that a diet rich in these compounds helps prevent chronic degenerative conditions, such as cardiovascular diseases and type 2 diabetes (Williamson, 2017). For this reason, they are widely used in the formulation of functional foods.

To harness the advantages of the compounds described above, methods for the extraction of betalains and polyphenols have been proposed (Eyshi *et al*., 2024). One of the most widely used techniques is ultrasound-assisted extraction, which is characterized by reducing extraction time and improving yield (Tabio-García *et al*., 2021). To maximize extraction yield, an optimization process must be performed, evaluating the effect of factors through a response surface methodology experimental design (Laqui-Vilca *et al*., 2018). Among these designs, the Box-Behnken design is one of the most commonly employed, as it has demonstrated greater efficiency compared to central composite designs and full factorial designs with three levels (Ferreira *et al*., 2007). The optimization of betalain extraction from pitahaya peel using a Box–Behnken design has already been established through other methods, such as microwave-assisted extraction (Thirugnanasambandham and Sivakumar, 2017). Although the ultrasonic extraction of betalains and polyphenols from the pulp of red pitaya (*Hylocereus polyrhizus*) has been previously optimized, these studies did not consider the fruit peel or include antioxidant compounds as part of the optimization (Vieira *et al*. 2024).

Given the aforementioned context, this study aimed to optimize ultrasonic bath-assisted extraction of betalains, polyphenols, and antioxidant compounds from dragon fruit (*Hylocereus undatus*) peel using a Box-Behnken experimental design. The goal was to utilize the obtained extract for the formulation of functional foods. In this way, the study sought to add value to dragon fruit, particularly its peel, which is currently considered a by-product and is discarded during industrial processes involving this fruit.

MATERIAL AND METHODS

Raw Material Procurement

The dragon fruit peels were collected in the community of Calkiní, Campeche, Mexico. The drying process was carried out in a convection oven at 45°C for 72 h. Afterward, the peels were ground using an electric grinder and sieved through a #40 mesh sieve with a particle size of 0.45 µm was obtained. Finally, the resulting powders were stored in 50 mL Falcon tubes at - 4°C and protected from light until analysis.

Ultrasonic extraction

An ultrasonic bath (Creworks, USA) with a 3L capacity, sonication energy of 120 W, and a frequency of 40 kHz was used. The extraction process involved weighing 100 mg of the sample and adding 4 mL of a water-ethanol mixture as the solvent. The extraction conditions included sonication times ranging from 10 to 80 min, temperatures between 20°C and 70°C, and ethanol concentrations in the solvent ranging from 0 % to 60 %. After extraction, the samples were centrifuged at 4500 rpm for 30 min, and the supernatant was filtered through a nylon membrane filter with a pore size of 45 µm.

Box Becken Experimental Design

The optimal conditions for the extraction of betalains, total polyphenols, and antioxidant compounds from dragon fruit peel were determined by analyzing the effect of the following factors: (1) sonication time, (2) temperature, and (3) ethanol percentage used as the extraction solvent. The factors selected for optimization, along with their respective levels, were defined based on the studies conducted by Viera *et al*. (2024) and Tabio-García *et al*. (2021). Table 1 presents the evaluated levels of these three independent variables for optimizing ultrasonic bath-assisted extraction using the Box-Behnken design.

Table 1. Conditions for optimizing ultrasonic bath-assisted extraction of betalains, polyphenols, and antioxidant compounds.
Tabla 1. Condiciones para optimizar la extracción asistida por ultrasonidos de betalaínas, polifenoles y compuestos antioxidantes.

Independent variables	Levels		
	-1	0	1
Time (min)	10	45	80
Temperature (°C)	20	45	70
Solvent (% Ethanol)	0	30	60

The relationship between the independent variables and the response values was constructed using a second-order polynomial response surface model. Each response variable was fitted to the following regression equation:

Where Yi represents the response variable, specifically the concentration of betalains, total polyphenols, and antioxidant activity. Meanwhile, β0, βi, βii and βij are the coefficients representing the regression model, while xi and xj are the coded variables that influence the response.

The accuracy of the mathematical model was determined using the R^2 coefficient. The analysis was performed with STATGRAPHICS Centurion XIX (Statgraphics Technologies Inc., The Plains, Virginia, USA). Overlays of contour plots and three-dimensional response surface plots were generated, and the optimal extraction conditions were selected based on the response surfaces of all variables.

Determination of Total Betalains

For the determination of total betalains, the extracts were analyzed spectrophotometrically at 538 nm and 480 nm for betacyanins and betaxanthins, respectively. This process was conducted using a UV-Vis spectrometer (PerkinElmer®) following the methodology reported by Shakir and Simone (2024). The absorbance readings obtained were used to calculate the betalain concentration in each sample using the formula shown below:

Betacyanins or betaxanthins (µg/mL) = (A)(DF)(MW)/(e)(l)

A: Absorbance.
DF: Dilution factor.
MW: Molecular weight.
e: Molar extinction coefficient.
l: cell length (1 cm).
Where molar extinction coefficients are:
Betaxanthins (MW=550 g/mol; e=60.000 L/mol cm in H_2O).
Betaxanthins (MW=308 g/mol; e=48.000 L/mol cm in H_2O).

Determination of Total Polyphenols

Total polyphenols were quantified using the Folin-Ciocalteu colorimetric method described by Singleton *et al*. (1999), with some modifications. A volume of 50 µL of extract was mixed with 3 mL of distilled water and 250 µL of Folin-Ciocalteu reagent. The mixture was homogenized and left to rest in the dark for 8 min. Subsequently, 750 µL of 20 % Na_2CO_3 and 950 µL of distilled water were added, the solution was homogenized, and it was left to rest at room temperature for 2 hours.

Following this procedure, the absorbance of the extracts was measured using a UV-Vis spectrophotometer (PerkinElmer®) at a wavelength of 765 nm. Gallic acid at various concentrations (5, 10, 15, 20, 25, 30, 40, 60, 80, and 100 mg/L) was used as an external standard to quantify the total polyphenols in the samples.

Determination of Antioxidant Activity by DPPH

The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, following the methodology described by Brand-Williams *et al*. (1995), with some modifications. In 15 mL Falcon tubes, 3.8 mL of DPPH solution were added, followed by 200 µL of the extract, and the mixture homogenized using a vortex. The solution was left to rest for 60 min before being analyzed with a UV-Vis spectrophotometer (PerkinElmer®) at 515 nm. The percentage of DPPH inhibition for each type of sample was calculated using the following formula:

Where At0 is the absorbance at 0 min, and At60 is the absorbance at 60 min. Trolox at different concentrations (100, 150, 200, 300, 400, 500, 600, 700, and 800 µM) was used as an external standard to express the antioxidant activity in the samples using the DPPH method.

Determination of Antioxidant Activity by ABTS

The determination of antioxidant activity was performed by adding 2.97 mL of the ABTS radical solution into a 15 mL Falcon tube. Subsequently, 30 µL of the extract was added, and the mixture was homogenized using a vortex. The solution was left to rest for 8 min, and the absorbance was measured using a UV-Vis spectrophotometer (PerkinElmer®) at 734 nm. The percentage of ABTS inhibition for each sample was calculated using the formula shown below:

Where At0 is the absorbance at 0 min, and At8 is the absorbance at 8 min. Trolox at different concentrations (100, 150, 200, 300, 400, 500, 600, 700, and 800 µM) was used as an external standard to express the antioxidant activity in the samples using the ABTS method.

RESULTS AND DISCUSSION

Effect of extraction parameters on betalains, polyphenols, and antioxidant compounds

Table 2 presents the results obtained from the optimization of ultrasound-assisted extraction of betalains, total polyphenols, and antioxidant compounds. The response variables showed a high correlation, with a value of 80 % for total betalains and 60 % for total polyphenols. Regarding antioxidant activity, a high correlation (92.8 %) was observed for the DPPH and the ABTS (92.5 %) methods.

For total betalains, treatment 5, which involved an extraction time of 10 min, an ultrasonic bath temperature of 45°C, and 0 % ethanol, yielded the highest betalain concentration (1236.6 µg/g). Conversely, treatment 12, conducted with an extraction time of 45 min, a temperature of 70°C, and 60 % ethanol, resulted in the lowest extraction yield (431.5 µg/g).

Regarding total polyphenols, treatment 12 achieved the highest extraction yield from dragon fruit peel (5830.4 µg/g) using an extraction time of 45 min, a temperature of 70°C, and 60 % ethanol. On the other hand, treatment 9 resulted in the lowest extraction yield (3547.8 µg/g), performed with an extraction time of 45 min, a temperature of 20°C, and 0 % ethanol.

The antioxidant activity showed the best results in treatment 4 (80 min, 70°C, and 30 % ethanol) and 12 (45 min, 70°C, and 60 % ethanol) as measured by the DPPH and ABTS methods, respectively, yielding values of 1809.4 µg Trolox/g for DPPH and 1401.3 µg Trolox/g for ABTS. Conversely, the lowest antioxidant activity values were determined in treatment 5 (10 min, 45°C, and 0 % ethanol) using the DPPH method and in treatment 6 (80 min, 45°C, and 0 % ethanol) using the ABTS method.

Optimization of parameters

Table 3 presents the analysis of variance (ANOVA) for optimizing total betalain extraction using the ultrasonic bath method. In this analysis, the significance of each effect was evaluated by comparing mean squares to an estimate of experimental error. The results show that none of the factors had p-values below 0.05, indicating no statistically significant effects at the 95 % confidence level. The coefficient of determination (R^2) indicates that the model explains 79.59 % of the variation in betalain content. The standard error of the estimate is 218.177, representing the standard deviation

Table 2. Experimental design for the ultrasound extraction of betalains, polyphenols, and antioxidant activity.
Tabla 2. Diseño experimental para la extracción ultrasónica de betalainas, polifenoles y actividad antioxidante.

Exp	Time (min)	Temperature (°C)	Ethanol (%)	Total betalains (µg/g)	Total polyphenols (µg/g)	DPPH (µg de trolox/g)	ABTS (µg de trolox/g)
1	10	20	30	1147.2	5227.5	1253.8	1340.7
2	80	20	30	1127.4	5226.2	1120.7	1326.3
3	10	70	30	445.5	4099.1	1356.6	1217.9
4	80	70	30	553.3	4958.3	1809.4	1321.7
5	10	45	0	1236.6	4927.4	730.7	960.7
6	80	45	0	923.6	4978.9	741.4	474.8
7	10	45	60	996.5	4186.7	1424.2	1289.0
8	80	45	60	671.2	5469.7	1594.1	1281.3
9	45	20	0	585.4	3547.8	844.7	870.8
10	45	70	0	1054.3	4762.5	918.6	792.8
11	45	20	60	1074.5	4953.2	1795.9	1160.3
12	45	70	60	431.5	5830.4	1651.3	1401.3
13	45	45	30	1178.2	5598.5	1397.5	1365.3
14	45	45	30	1090.5	5375.7	1671.2	1233.4
15	45	45	30	1158.1	5463.3	1464.3	1226.9

of the residuals. Additionally, the mean absolute error (MAE) is 104.567, showing the average size of the differences between observed and predicted values.

Figure 1 illustrates the response surface plots for the extraction of total betalains from dragon fruit peel, evaluating the effects of extraction time, ultrasonic bath temperature, and ethanol percentage used as the solvent. The temperature-ethanol interaction plot (Figure 1a) shows the highest extraction yield at low temperatures across a wide range of ethanol concentrations. Meanwhile, in Figure 1b, which represents the response surface of the time-ethanol interaction, the maximum extraction is observed at low ethanol concentrations and short extraction times. Finally, Figure 1c, depicting the time-temperature interaction, reveals that the greatest extraction yield occurs at short extraction times combined with low temperatures.

The aforementioned observations indicate that betalains are better extracted during short periods (less than 45 min), which could be attributed to the degradation of these compounds. This behavior has been reported by Maran *et al.* (2015), where extraction times exceeding 45 min resulted in a reduction of total betalains. This factor is crucial to consider in ultrasonic extractions, as using very short extraction periods may be insufficient to recover all analytes present in the matrix, while excessively long extraction times could degrade the target compounds (Righi *et al*., 2018).

In the case of temperature, it was observed that below 45°C yielded the highest extraction efficiency. This phenomenon could also be attributed to the fact that high temperatures promote the degradation of betalains. This is supported by the work of Maran *et al*. (2015), who noted that ultrasonic extraction of pigments is influenced by temperature, specifically observing a decrease in betalain extraction as the temperature increases (Righi *et al*., 2018).

On the other hand, ethanol percentage demonstrated good extraction yields across a wide range, showing interaction with temperature (Figure 1A). When interacting with extraction time, the best yields were observed at ethanol percentages below 30 %. These results align with the findings of Roriz *et al*. (2017), who reported that ethanol concentra-

Table 3. ANOVA of the effect of time, temperature, and ethanol factors on the extraction of total betalains by ultrasonic bath.
Tabla 3. ANOVA del efecto del tiempo, la temperatura y la concentración de etanol en la extracción de betalaínas totales mediante baño ultrasónico.

Source	Sum of Squares	Df	**Mean Square**	**F-ratio**	**p-value**
A: Time	37853	1	37853	0.80	0.4134
B: Temperature	262821	1	262821	5.52	0.0656
C: % Ethanol	49031	1	49031	1.03	0.3567
AA	21705	1	21705	0.46	0.5295
AB	4065	1	4064	0.09	0.7819
AC	37	1	37	0.00	0.9786
BB	225684	1	225684	4.74	0.0814
BC	309023	1	309023	6.49	0.0514
CC	43534	1	43534	0.91	0.3828
Total error	238005	5	47601		
Total (corr.)	$1.16x10^6$	14			
Quadratic regression model equation					
Total betalains (µg/g) = 1142.23 - 68.7875A - 181.253B - 78.2877C - 76.671A^2 + 31.876AB - 3.071AC - 247.23B^2 - 277.949BC - 108.584C^2					

tions above 20 % decrease betalain extraction yields. This is attributed to the fact that betalains have a higher affinity for high polarity solvents, such as water.

The analysis of variance (ANOVA) evaluating the partitioning of variability in total polyphenol content is presented in Table 4. None of the evaluated effects exhibited *p*-values lower than 0.05, indicating the absence of statistically significant differences at the 95 % confidence level. The coefficient of determination (R^2) showed that the fitted model explains 53.37 % of the observed variability in total polyphenol content. The standard error of the estimate was 707.325, representing the standard deviation of the residuals. Likewise, the mean absolute error (MAE) was 339.238, reflecting the average magnitude of the absolute deviations between the experimental values and those predicted by the model.

Figure 2 shows the response surface plots obtained from the analysis using the Box-Behnken design for the optimization of ultrasonic bath-assisted extraction of total polyphenols from dragon fruit peel. In Figure 2a, the highest extraction yield occurs at approximately 45 °C with ethanol concentrations above 30 %. In Figure 2b, the maximum yield is achieved at extraction times exceeding 45 min, also with ethanol concentrations above 30 %. For the time-temperature interaction (Figure 2c), the optimal conditions are observed at times longer than 45 min combined with temperatures above 45 °C.

Unlike betalains, polyphenols exhibited better extraction yields within a temperature range of 45 to 70°C and longer extraction times, from 45 to 80 min. This demonstrates that polyphenols have greater stability at higher temperatures compared to betalains. This is supported by the work of Lombardelli *et al*. (2021), who reported an acceleration in betalain degradation at temperatures above 40°C, whereas polyphenols, as reported by Antony and Farid (2022) begin to degrade at temperatures starting from 90°C.

For ethanol percentage, a higher amount of polyphenols was extracted within the range of 30 % to 60 %. This behavior may be attributed to the fact that some polyphenols are more soluble in organic solvents such as ethanol compared to water. A mixture of ethanol and water allows for the extraction of a broader range of polyphenols, including those with high solubility in highly polar solutions (phenolic acids) and those more soluble in organic solvents (flavonoids).

The analysis of variance (ANOVA) evaluating the partitioning of variability in antioxidant activity, as determined by the DPPH assay, is presented in Table 5. Two of the evaluated effects exhibited *p*-values below 0.05, indicating that they are significantly different from zero at the 95 % confidence level. The coefficient of determination (R^2) showed that the fitted model explains 92.81 % of the observed variability in DPPH activity, while the adjusted R^2, which is more suitable for comparing models with different numbers of independent variables, was 79.88 %. The standard error of the estimate was 166.394, representing the standard deviation of the residuals. Likewise, the mean absolute error (MAE) was 83.474, reflecting the average magnitude of the absolute deviations between the experimental and predicted values.

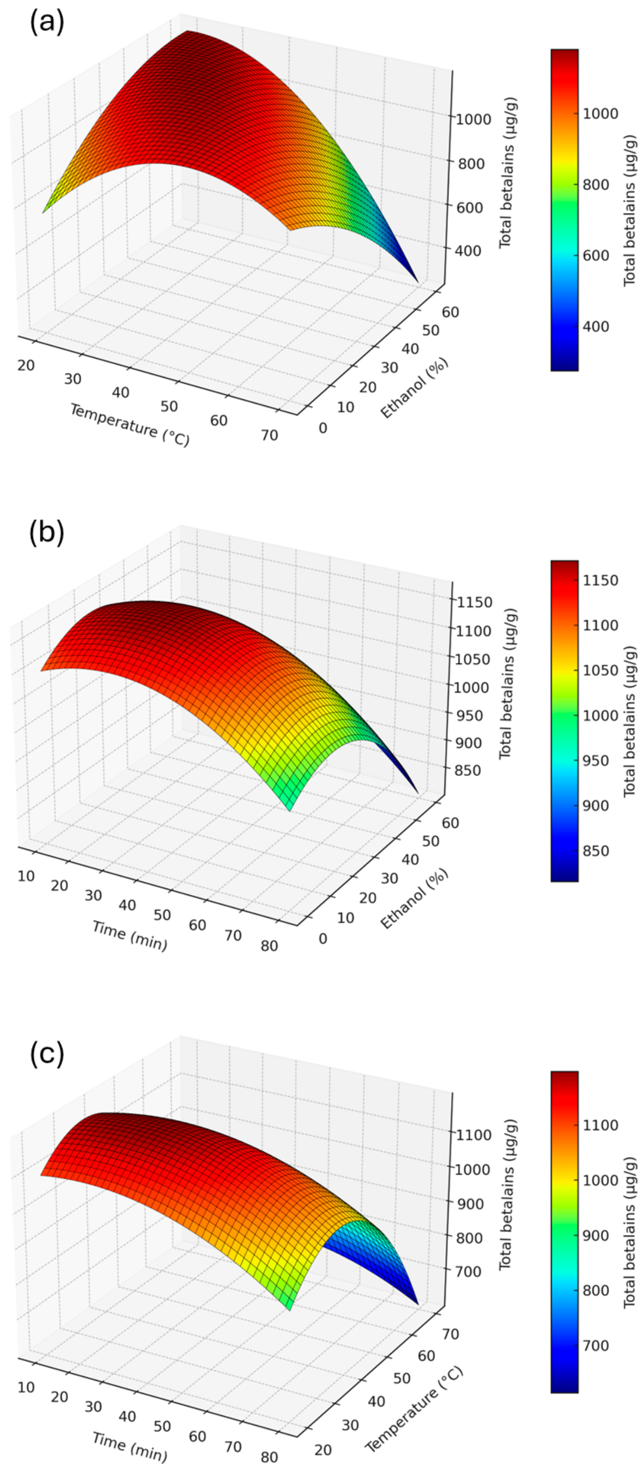

Fig. 1. Response surface plot for ultrasonic bath-assisted extraction of total betalains from dragon fruit peel. (a) effect of temperature and ethanol, (b) the effect of time and ethanol, (c) the effect of time and temperature.
Fig. 1. Diagrama de superficie de respuesta para la extracción ultrasónica asistida por baño de betalinas totales de cáscara de fruta de dragón. (a) efecto de la temperatura y el etanol, (b) el efecto del tiempo y el etanol, (c) el efecto del tiempo y la temperatura.

Table 4. ANOVA of the effect of time, temperature, and ethanol factors on the extraction of total polyphenols by ultrasonic bath.
Tabla 4. ANOVA del efecto del tiempo, la temperatura y la concentración de etanol en la extracción de polifenoles totales mediante baño ultrasónico.

Source	Sum of Squares	Df	Mean Square	F-ratio	p-value
A: Time	600833	1	600833	1.20	0.3231
B: Temperature	60481	1	60481	0.12	0.7422
C: % Ethanol	617898	1	617898	1.24	0.3170
AA	216348	1	216348	0.43	0.5399
AB	185105	1	185105	0.37	0.5696
AC	379123	1	379123	0.76	0.4238
BB	476619	1	476619	0.95	0.3739
BC	28475	1	28475	0.06	0.8209
CC	443056	1	443056	0.89	0.3899
Total error	2.50×10^6	5	500308		
Total (corr.)	2.36×10^6	14			
Quadratic regression model equation					
Total polyphenols (µg/g) = 5479.14 + 274.051A + 86.9493B + 277.916C - 242.063A^2 + 215.119AB + 307.865AC - 359.283B^2 - 84.373BC - 346.402C^2					

Table 6 presents the analysis of variance (ANOVA) for antioxidant activity determined using the ABTS assay, partitioning the observed variability into individual components associated with each of the evaluated effects. In this analysis, two effects exhibited *p*-values lower than 0.05, indicating that they are significantly different from zero at the 95 % confidence level. The coefficient of determination (R^2) showed that the fitted model explains 92.4571 % of the variability in antioxidant activity measured by ABTS, while the adjusted R^2, which is more suitable for comparing models with different numbers of independent variables, was 78.8798 %. The standard error of the estimate was 120.209, representing the standard deviation of the residuals. Likewise, the mean absolute error (MAE) was 60.5674, reflecting the average magnitude of the absolute deviations between the experimental and predicted values.

Figures 3 and 4 depict the response surface plots for the ultrasonic bath-assisted extraction of antioxidant compounds from dragon fruit peel, measured using the ABTS and DPPH methods, respectively. In both techniques, an increase in the ethanol percentage during extraction corresponded to an increase in the yield of extracted antioxidant compounds (Figures 3a and 4a). This may be attributed to the fact that some antioxidant compounds, such as certain polyphenols, have a higher affinity for organic solvents with intermediate polarity. Consequently, ethanol-water mixtures at various proportions provide optimal conditions for extracting a broader profile of antioxidant compounds, resulting in an extract with greater antioxidant capacity (Sun *et al*., 2015).

In the case of temperature interacting with ethanol percentage, no significant changes were observed in the concentration of extracted antioxidants across the evaluated temperature range. However, when interacting with time, as seen in the DPPH method (Figure 4c), high temperatures and extended extraction times promoted the extraction of this type of compound. This is because antioxidant compounds,

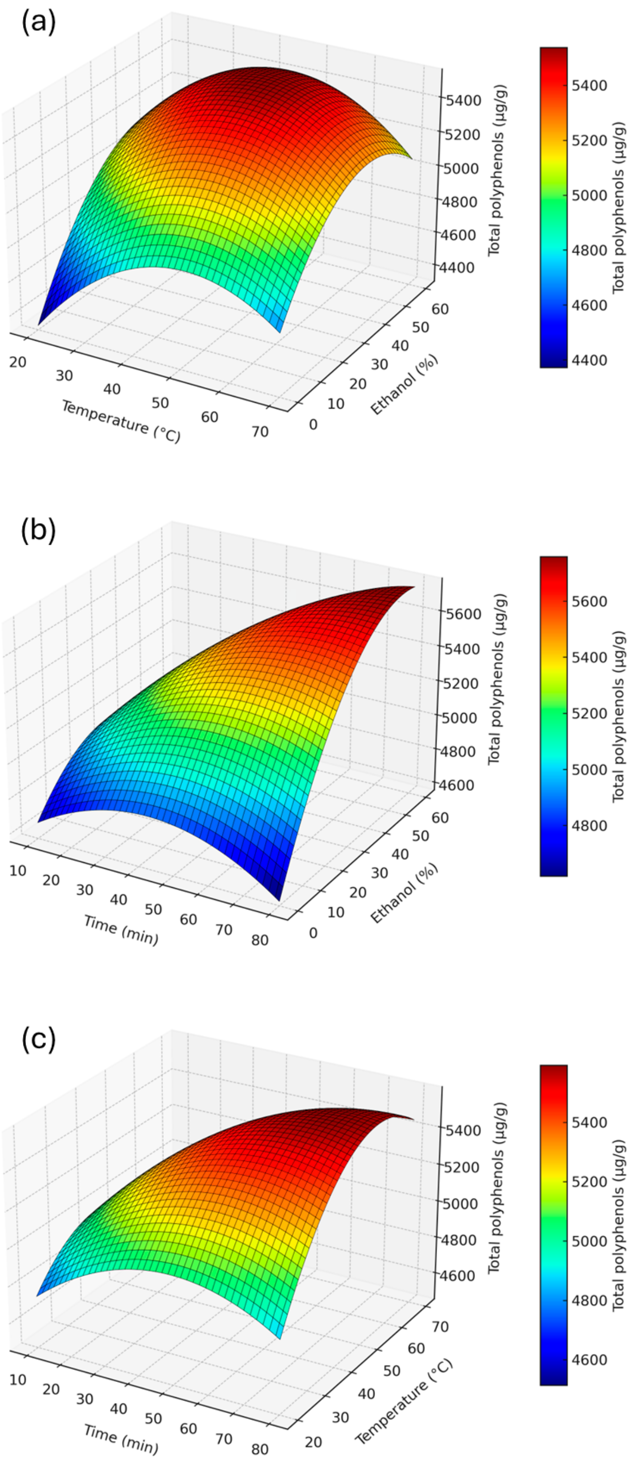

Fig. 2. Response surface plot for ultrasonic bath-assisted extraction of total polyphenols from dragon fruit peel. (a) effect of temperature and ethanol, (b) the effect of time and ethanol, (c) the effect of time and temperature.
Fig. 2. Diagrama de superficie de respuesta para la extracción ultrasónica asistida por baño de polifenoles totales de cáscara de fruta del dragón. (a) efecto de la temperatura y el etanol, (b) el efecto del tiempo y el etanol, (c) el efecto del tiempo y la temperatura.

Table 5. ANOVA of the effect of time, temperature, and ethanol factors on the extraction of antioxidant compounds (DPPH) by ultrasonic bath.
Tabla 5. ANOVA del efecto del tiempo, la temperatura y la concentración de etanol en la extracción de compuestos antioxidantes (DPPH) mediante baño ultrasónico.

Source	Sum of Squares	Df	Mean Square	F-ratio	p-value
A: Time	31302	1	31302	1.13	0.3363
B: Temperature	64951	1	64951	2.35	0.1862
C: % Ethanol	1.30×10^6	1	1.30×10^6	47.10	0.0010
AA	86363	1	86363	3.12	0.1376
AB	85792	1	85792	3.10	0.1387
AC	6340	1	6340	0.23	0.6524
BB	2706	1	2706	0.10	0.7672
BC	11928	1	11928	0.43	0.5406
CC	204686	1	204686	7.39	0.0418
Total error	138434	5	27686		
Total (corr.)	1.92×10^6	14			
Quadratic regression model equation					
Antioxidant activity by DPPH (µg de trolox/g) = $1511.0 + 62.5522A + 90.105B + 403.76C - 152.938A^2 + 146.452AB + 39.815AC + 27.0729B^2 - 54.6091BC - 235.448C^2$					

Table 6. ANOVA of the effect of time, temperature, and ethanol factors on the extraction of antioxidant compounds (ABTS) by ultrasonic bath.
Tabla 6. ANOVA del efecto del tiempo, la temperatura y la concentración de etanol en la extracción de compuestos antioxidantes (ABTS) mediante baño ultrasónico.

Source	Sum of Squares	Df	Mean Square	F-ratio	p-value
A: Time	20427	1	20427	1.41	0.2878
B: Temperature	158	1	158	0.01	0.9207
C: % Ethanol	516526	1	516526	35.75	0.0019
AA	747	1	747	0.05	0.8291
AB	3493	1	3493	0.24	0.6438
AC	57180	1	57180	3.96	0.1034
BB	6107	1	6107	0.42	0.5443
BC	25441	1	25441	1.76	0.2419
CC	248720	1	248720	17.21	0.0089
Total error	72250	5	14450		
Total (corr.)	957861	14			
Quadratic regression model equation					
Antioxidant activity by ABTS (µg de trolox/g) = $1275.2 - 50.5317A + 4.45087B + 254.098C - 14.2296A^2 + 29.5513AB + 119.562AC + 40.6702B^2 + 79.752BC - 259.542C^2$					

such as polyphenols, undergo thermal degradation at high temperatures (Antony and Farid, 2022).

In the case of extraction time, an increasing trend in the amount of extracted antioxidant compounds was observed as this factor increased. These conditions differ from those obtained for betalain extraction but show certain similarities with the results for total polyphenol extraction. This suggests that the antioxidant properties of dragon fruit peel may be primarily attributed to the polyphenols present.

Summary of experimental evaluation of optimal conditions

Table 3 presents the optimal conditions for the extraction of betalains, polyphenols, and antioxidant compounds. The

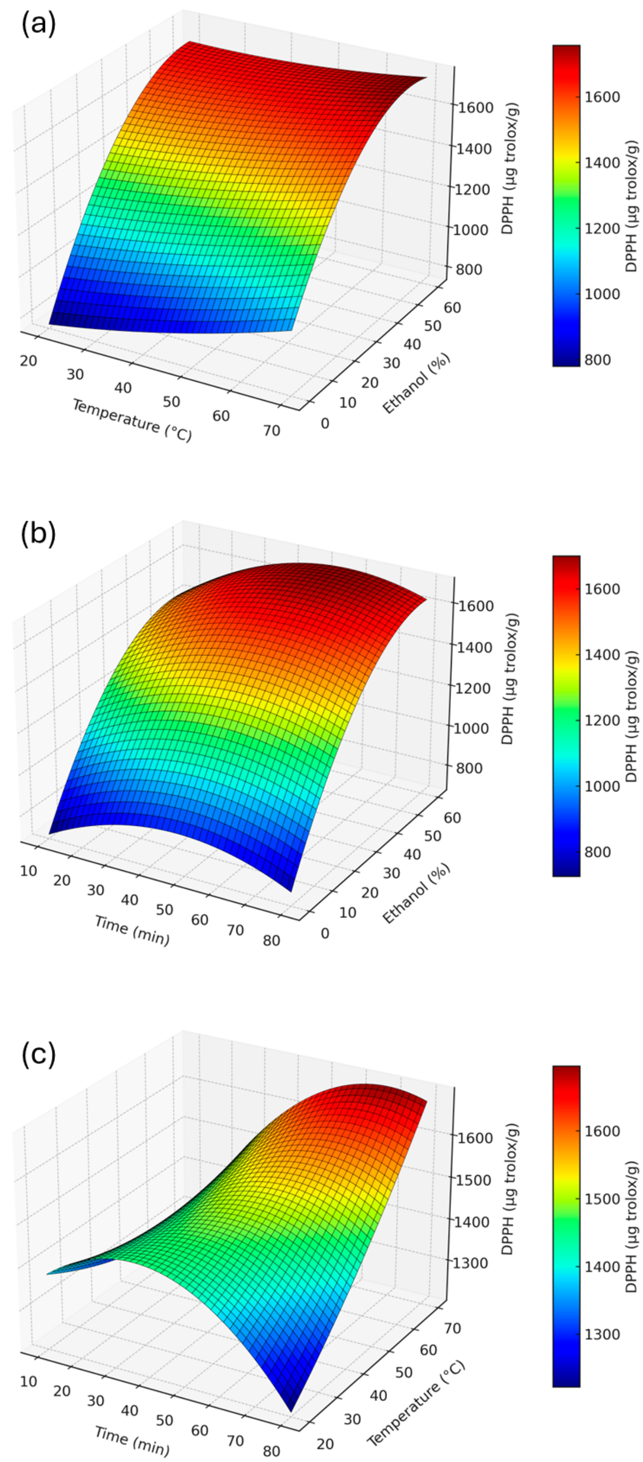

Fig. 3. Response surface plot for ultrasonic bath-assisted extraction of antioxidant compounds from dragon fruit peel, measured using the ABTS method. (a) effect of temperature and ethanol, (b) the effect of time and ethanol, (c) the effect of time and temperature.
Fig. 3. Diagrama de superficie de respuesta para la extracción ultrasónica asistida por baño de compuestos antioxidantes de la cáscara del fruto del dragón medida utilizando el método ABTS. (a) efecto de la temperatura y el etanol, (b) el efecto del tiempo y el etanol, (c) el efecto del tiempo y la temperatura.

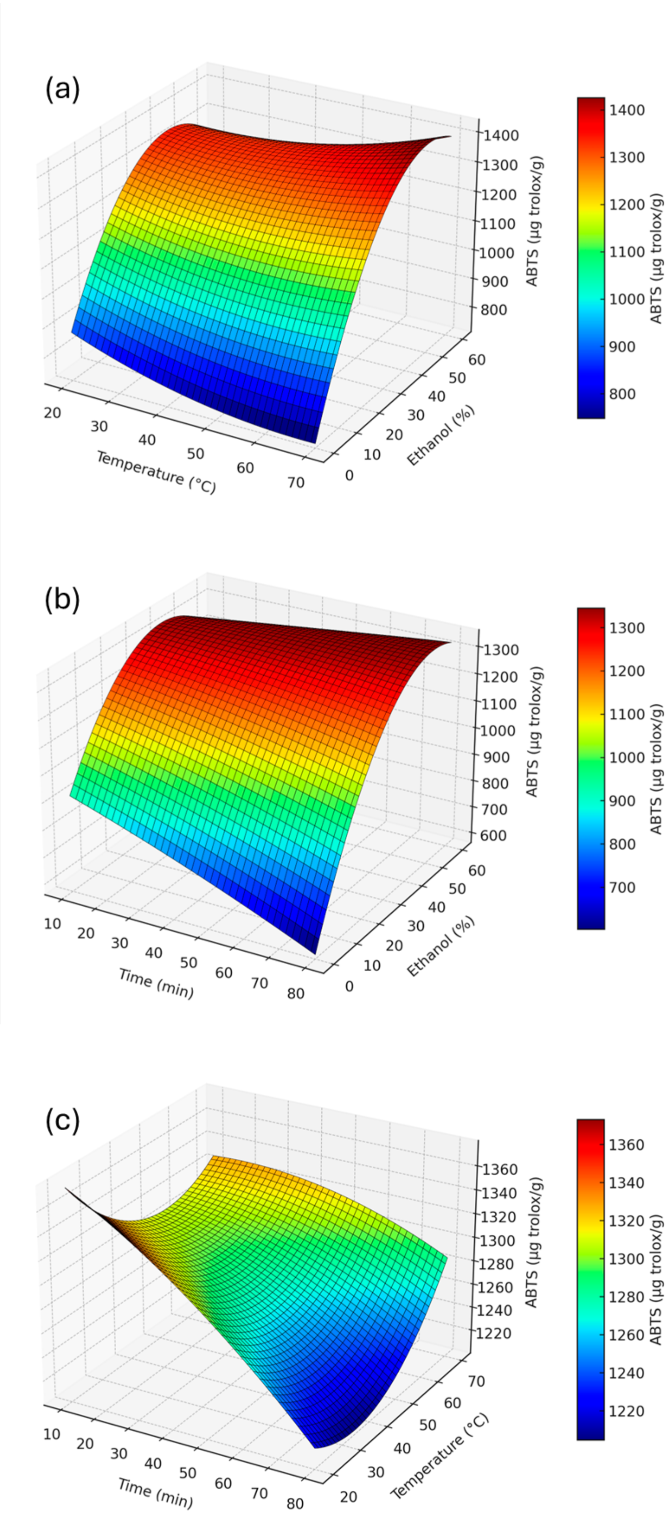

Fig. 4. Response surface plot for ultrasonic bath-assisted extraction of antioxidant compounds from dragon fruit peel, measured using the DPPH method. (a) effect of temperature and ethanol, (b) the effect of time and ethanol, (c) the effect of time and temperature.
Fig. 4. Diagrama de superficie de respuesta para la extracción ultrasónica asistida por baño de compuestos antioxidantes de la cáscara del fruto del dragón medida utilizando el método DPPH. (a) efecto de la temperatura y el etanol, (b) el efecto del tiempo y el etanol, (c) el efecto del tiempo y la temperatura.

optimal conditions for the extraction of antioxidant compounds, as evaluated by both techniques, showed that the optimal extraction time was between 72 and 80 min, with a temperature of 70°C and an ethanol percentage of 54-56 %. Polyphenols had similar extraction conditions to those of antioxidant compounds, coinciding in extraction time (80 min) and ethanol percentage (54 %), while the optimal extraction temperature was 53°C.

In the case of betalains, these compounds exhibited different optimal extraction conditions compared to the other groups of compounds analyzed. Their optimal conditions were an extraction time of 24 min, a temperature of 26°C, and an ethanol percentage of 48 %. These results indicate that betalains require shorter extraction times, lower ethanol percentages, and lower temperatures than the other compounds analyzed in this study to achieve optimal yields.

Table 4 shows that there were no significant differences ($p \leq 0.05$) between the optimal experimental values and the theoretical ones. This suggests that the extraction factors optimized through the response surface methodology reliably predict the extraction yields of betalains, polyphenols, and antioxidant compounds using ultrasonic bath-assisted extraction.

Tabla 7. Condiciones óptimas para extraer betalinas y polifenoles de pitahaya (Hylocereus undatus) por baño ultrasónico (valores predictivos).
Table 7. Optimal conditions for extracting betalins and polyphenols from pitahaya (*Hylocereus undatus*) by ultrasonic bath (predictive values).

	Time (min)	Temperature (°C)	Ethanol (%)
Total betalains	24	26	48
Total polyphenols	80	53	54
DPPH	73	70	54
ABTS	80	70	56

Table 8. Comparison of prediction and experiment for optimal conditions in ultrasound extraction.
Tabla 8. Comparación de la predicción y el experimento para las condiciones óptimas en la extracción de ultrasonido.

	Predict value (µg/g)	Experimental value (µg/g)	Adjustment percentage (%)
Total betalains	1207.3	1197.7 ± 32.7	99.2
Total polyphenols	5796.4	5765.3 ± 148.4	99.4
DPPH	1851.7	2191.6 ± 180.1	84.5
ABTS	1483.1	1503.5 ± 25.8	98.6

CONCLUSIONS

Using a Box-Behnken response surface design, the conditions for ultrasonic bath-assisted extraction of betalains, polyphenols, and antioxidant compounds from dragon fruit (*Hylocereus undatus*) peel were optimized. The results showed that extraction parameters significantly affected the yield of each compound class, with betalains requiring milder conditions (24 min, 26 °C, 48 % ethanol), while polyphenols and antioxidant compounds were best extracted under longer times and higher temperatures (up to 80 min, 70 °C, and 54–56 % ethanol). The strong correlation between experimental and predicted values ($p \leq 0.05$) confirmed the reliability of the optimization model. These findings highlight the potential of dragon fruit peel (an agro-industrial by-product typically discarded) as a valuable source of natural pigments and antioxidants. The advances in this research will support the use of these valuable compounds in the agro-industrial sector, especially for developing functional foods. This paves the way for creating new food products based on dragon fruit peel extracts, adding value to the fruit by utilizing a by-product usually discarded during industrial processing. Future research should focus on scaling up the process, testing the stability of the extracts in food matrices, and assessing their bioavailability in vivo.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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